Mastercycler® ep *realplex* *

Instruction Manual

* New! Modified Slip Cover
(light transmissive cover for optical openings in heated platen assembly, US patent pending).

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Which document do you refer to? Product: _____ Document ID: _____ (found on the second or last page of the manual, e.g. MixMate / B 5353 900.015-01/0506)		
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Certificate

Licensed Real-Time Thermal Cycler

This instrument, Serial No. , is an Authorized Thermal Cycler.

Its purchase price includes the up-front fee component of a license under United States Patent Nos. 4.683,195, 4,683,202 and 4,965,188, owned by Roche Molecular Systems, Inc., and under corresponding claims in patents outside the United States, owned by F. Hoffmann-La Roche Ltd., covering the Polymerase Chain Reaction ("PCR") process to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This Instrument is also an Licensed Real-Time Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA.

No rights are conveyed expressly, by implication or estoppel to any patents on real-lime methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit.

Applied Biosystems does not guarantee the performance of this instrument.

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1 Safety precautions

Please read the operating manual carefully before using the Mastercycler ep *realplex*. The Mastercycler ep *realplex* may only be used to carry out real-time PCR and related methods by qualified specialists at the research lab.

Note: If the device is not used according to the manufacturer's operating manual, this will result in any guarantee claims or liability for resulting damage becoming null and void.

The following instructions must be unconditionally observed:

- The safety regulations applicable to the lab must always be observed when working with the device. To avoid damage from condensation, the device should only be switched on 12 hours after setup. This also applies when the location of the device is changed.
- Choose an installation site where the device will not be exposed to major temperature fluctuations and will be free from direct sunlight.
- The mains voltage must match the information on the identification plate. The device must be connected to a grounded socket. The device must be switched off and the plug removed before maintenance and cleaning are carried out. Wait for the block to cool down. The connections must be checked for correctness before startup.
- The Mastercycler ep *realplex* must stand properly on a stable work surface. There must be enough space available to prevent blocking of the front and rear ventilation slots and to allow cooling air to pass beneath the device. The distance between the rear ventilation slots and the wall should be at least 10 cm. Make sure that the ventilation slots remain freely accessible and the inlets and outlets are not blocked. There should be no paper or other objects under the device that might block ventilation.
- No objects should be placed on the heated lid.
- If the lid is in the cleaning position, components with sharp edges are exposed. When cleaning the heated lid remember that there is a risk of injury from the sharp edges of the roll flex cable and the guide rail.
- Do not insert your fingers between the lid and the housing of the device when opening or closing the lid or when opening to the cleaning position as they may get caught. Do not reach beneath the heated lid when open.
- Sample material may not be filled directly into the thermoblock.
- Damage to the block and the heated lid may be caused if sample tubes other than those recommended are used. Unsuitable tubes can be damaged so severely that sample material may escape. This is especially important when working with infectious materials.
- Make sure that the PCR plates are correctly positioned in the block or damage to the block and the lid may result and the escape of sample material.
- If tubes are heated in the block without using the heated lid, they may burst if the temperature is too high, causing sample material to escape.

The thermoblock, the inner side of the heated lid and micro test tubes / PCR plates can reach temperatures of over 50 °C very quickly. There is a risk of burns!

The heated lid should be kept closed until temperatures of approx. 30 °C or less are reached. Do not use materials (tubes, PCR plates, caps, foils, mats) that are not sufficiently temperature-resistant (to 120 °C).

▼▲ Caution: Hot Surface
Gradient

Symbol on the thermoblock:
Warns of a potential hot thermoblock (▼)or a hot inner side of the heated lid (▲).
▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ indicates that gradient operation may result in the temperature being hotter on the right side than on the left.

- Explosive, flammable and volatile materials may not be heated in the Mastercycler ep *realplex*. The device may not be operated in rooms with a risk of explosion.
- The appropriate safety regulations should be observed when working with pathogenic material, radioactive materials or other materials presenting a risk to health.
- Make sure that no liquids enter the device.
- Make sure that the instrument is warmed up for at least 15 min before starting an assay or a calibration.

1 Safety precautions

- Repairs should only be carried out by an Eppendorf AG authorized specialist. Such authorization can only be obtained by participating in a certified course of training.
- **Transfer**
  If the device is passed on to someone else, please include the instruction manual.
- **Disposal**
  In case the product is to be disposed of, the relevant legal regulations are to be observed.
- **Information on the disposal of electrical and electronic devices in the European Community**
  The disposal of electrical devices is regulated within the European Community by national regulations based on EU Directive 2002/96/EC on waste electrical and electronic equipment (WEEE).

  According to these regulations, any devices supplied after 13.08.05 in the business-to-business sphere, to which this product is assigned, may no longer be disposed of in municipal or domestic waste. They are marked with the following symbol to indicate this.

  As disposal regulations within the EU may vary from country to country, please contact your supplier if necessary.

1.1 Sicherheitshinweise

Vor Gebrauch des Mastercycler ep *realplex* lesen Sie bitte die Bedienungsanleitung vollständig durch. Der Mastercycler ep *realplex* darf nur zur Durchführung der real-time PCR und verwandter Methoden von qualifiziertem Fachpersonal im Forschungslabor eingesetzt werden.

Hinweis: Wird das Gerät nicht entsprechend der Bedienungsanleitung des Herstellers benutzt, ist jede Gewährleistung und Haftung für dadurch entstandene Schäden ist ausgeschlossen.

Die folgenden Bestimmungen sind unbedingt zu beachten:

- Bei den Arbeiten mit dem Gerät sind stets die für das Labor geltenden Sicherheitsbestimmungen einzuhalten. Um Schäden durch Kondensat zu vermeiden, darf das Gerät erst 12 Stunden nach dem Aufstellen eingeschaltet werden. Dies gilt ebenso wenn der Standort des Gerätes gewechselt wird.
- Der Aufstellungsort sollte so gewählt werden, dass das Gerät keinen nennenswerten Temperaturschwankungen ausgesetzt ist. Direkte Sonneneinstrahlung muss vermieden werden.
- Die Netzspannung muss mit den Angaben auf dem Typenschild übereinstimmen. Das Gerät muss an eine geerdete Steckdose angeschlossen werden. Bei Wartungsarbeiten und Reinigung muss das Gerät vorher ausgeschaltet und der Netzstecker gezogen werden. Abkühlen des Blockes abwarten. Vor der Inbetriebnahme müssen die Anschlüsse auf Richtigkeit überprüft werden.
- Der Mastercycler ep *realplex* muss komplett auf der stabilen Arbeitsfläche stehen und einen sicheren Stand haben. Es muss so viel Platz vorhanden sein, dass die vorderen und hinteren Lüftungsschlitze nicht verdeckt werden, und Luft zur Kühlung unter das Gerät gelangen kann. Der Abstand von den hinteren Lüftungsschlitzen zur Wand muss mindestens 10 cm betragen. Es ist darauf zu achten, dass die Lüftungsschlitze frei sind und die Zu- und Abluftwege nicht blockiert werden. Es dürfen keine Papiere oder Ähnliches unter das Gerät gelangen, da es zu einer Blockierung der Lüftung kommen kann.
- Es dürfen keine Gegenstände auf dem Heizdeckel gestellt werden.
- Befindet sich der Deckel in der Reinigungsposition, sind scharfkantige Bauteile zugänglich. Es ist darauf zu achten, dass bei der Reinigung des Heizdeckels die scharfen Kanten des Rollflexkabels und der Führungsschiene zu Verletzungen führen können.
- Beim Öffnen und Verschließen des Deckels sowie beim Aufklappen in die Reinigungsposition die Finger nicht zwischen den Deckel und das Gehäuse des Gerätes legen, da sonst die Gefahr des Einklemmens besteht. Bei geöffnetem Heizdeckel nicht unter den Deckel fassen.
- Es darf kein Probenmaterial direkt in den Thermoblock gefüllt werden.
- Bei dem Einsatz anderer als der empfohlenen Probengefäße kann es zu Schäden des Blockes und des Heizdeckels kommen. Ungeeignete Gefäße können so stark beschädigt werden, dass Probenmaterial freigesetzt wird. Darauf ist insbesondere bei dem Arbeiten mit infektiösem Material zu achten.
- Es ist darauf zu achten, dass die PCR Platten richtig im Block sitzen, da es sonst zu Schäden des Blockes und des Deckels sowie zum Freisetzen des Probenmaterials kommen kann.
- Werden Gefäße in dem Block ohne die Nutzung des Heizdeckels temperiert, können diese bei zu hohen Temperaturen bersten und das Probenmaterial freigesetzt werden.

Thermoblock, Heizdeckelinnenseite und Reaktionsgefäße / PCR-Platten erreichen sehr schnell Temperaturen oberhalb 50 °C. Es besteht Verbrennungsgefahr!

Heizdeckel geschlossen halten, bis Temperaturen um 30 °C oder weniger erreicht sind. Keine Materialien (Gefäße, PCR-Platten, Verschlüsse, Folien, Matten) verwenden, die nicht ausreichend temperaturbeständig (bis 120 °C) sind.

▼▲ Caution: Hot Surface
Gradient

Symbol auf dem Thermoblock:
Es warnt vor einem evtl. heißen Thermoblock (▼) oder einer heißen Deckelinnenseite (▲).
zeigt, dass bei Gradientenbetrieb die Temperatur auf der rechten Seite heißer ist als auf der linken Seite.

- Explosive, brennbare und heftig reagierende Stoffe dürfen nicht im Mastercycler ep *realplex* temperiert werden. Das Gerät darf nicht in explosionsgefährdeten Räumen betrieben werden.
- Beim Umgang mit pathogenem Material, radioaktiven Stoffen oder anderen gesundheitsgefährdenden Stoffen sind die entsprechenden Sicherheitsvorschriften zu beachten.
- Es ist sicherzustellen, dass keine Flüssigkeit in das Gerät eindringt.
- Es ist sicherzustellen, dass eine Aufwärmzeit von min. 15 Minuten vor dem Start eines Assays oder einer Kalibration erreicht wird.

1.1 Sicherheitshinweise

- Reparatur ist nur einem von der Eppendorf AG autorisierten Fachmann vorbehalten. Die Autorisierung kann nur durch eine zertifizierte Schulung erlangt werden.
- **Weitergabe**
  Bei einer eventuellen Weitergabe des Produktes bitten wir Sie, diese Bedienungsanleitung beizufügen.
- **Entsorgung**
  Im Falle einer Entsorgung des Produktes sind die jeweiligen gesetzlichen Vorschriften zu beachten.
- **Information zur Entsorgung von elektrischen und elektronischen Geräten in der Europäischen Gemeinschaft**
  Innerhalb der Europäischen Gemeinschaft wird für elektrisch betriebene Geräte die Entsorgung durch nationale Regelungen, die auf der EU-Richtlinie 2002/96/EC über Elektro- und Elektronik-Altgeräte (WEEE) basieren, vorgegeben.

  Danach dürfen alle nach dem 13.08.2005 gelieferten Geräte im Business-to-Business-Bereich, in den dieses Produkt eingeordnet ist, nicht mehr mit dem kommunalen oder Hausmüll entsorgt werden. Um dies zu dokumentieren sind sie mit folgendem Kennzeichen ausgestattet.

  Da die Entsorgungsvorschriften innerhalb der EU von Land zu Land unterschiedlich sein können, bitten wir Sie im Bedarfsfall Ihren Lieferanten anzusprechen.

  In Deutschland gilt diese Kennzeichnungspflicht ab dem 23.03.2006. Ab diesem Termin hat der Hersteller für alle ab dem 13.08.2005 gelieferten Geräte, eine angemessene Möglichkeit der Rücknahme anzubieten. Für alle vor dem 13.08.2005 gelieferten Geräte ist der Letztverwender für die ordnungsgemäße Entsorgung zuständig.

1.2 Consignes de sécurité

Avant utilisation du Mastercycler ep *realplex*, veuillez lire complètement le manuel d'utilisation. Le Mastercycler ep *realplex* peut être utilisé uniquement pour réaliser des réactions PCR en temps réel et des méthodes de même nature par un personnel spécialisé qualifié au sein d'un laboratoire de recherches.

Remarque: si l'appareil n'est pas utilisé conformément au manuel d'utilisation du fabricant, toute garantie et responsabilité pour les dommages en résultant sont exclues.

Les réglementations suivantes doivent être absolument respectées:

- En travaillant avec l'appareil, les réglementations de sécurité valables pour le laboratoire doivent être toujours respectées. Afin d'éviter tout endommagement en raison du condensat, l'appareil ne peut être mis en service que 12 heures après avoir été installé. Ceci s'applique aussi lorsque l'emplacement de l'appareil a été modifié.
- Choisir un lieu d'installation où l'appareil ne sera pas exposé à des variations importantes de température. Eviter une exposition directe de l'appareil aux rayons solaires.
- La tension du secteur doit correspondre aux indications sur la plaque signalétique. L'appareil doit être raccordé à une prise de courant reliée à la terre. Lors des travaux de maintenance et de réparation, l'appareil doit être coupé et la fiche secteur doit être débranchée. Laissez refroidir le bloc. Avant de mettre en service l'appareil, l'exactitude des branchements doit être vérifiée.
- Le Mastercycler ep *realplex* doit être posé entièrement sur une surface de travail stable et doit être installé sûrement. L'appareil doit être posé à distance suffisante des parois pour que les fentes d'aération avant et arrière ne soient pas cachées et que l'air puisse passer sous l'appareil pour le refroidir. Il convient de laisser au minimum 10 cm entre les fentes d'aération arrière et le mur. Les fentes d'aération doivent être dégagées et l'arrivée ainsi que la sortie d'air ne doivent pas être obturées. Aucun papier ou autre objet ne doit se trouver sous l'appareil afin de ne pas bloquer la ventilation.
- Aucun objet ne doit être posé sur le couvercle chauffant.
- Si le couvercle se trouve en position de nettoyage, des arêtes vives sont accessibles. Lors du nettoyage du couvercle chauffant, les arêtes vives du câble Rollflex et du rail de guidage peuvent entraîner des blessures.
- Lors de l'ouverture et de la fermeture du couvercle et aussi lors du positionnement sur la position de nettoyage, ne placez pas les doigts entre le couvercle et le boîtier de l'appareil pour ne pas risquer de coincer vos doigts. Lorsque le couvercle chauffant est ouvert, ne le saisissez pas par en dessous.
- Aucune partie de l'échantillon ne peut être versée directement dans le Thermobloc.
- En utilisant des récipients d'échantillon autres que ceux recommandés, le bloc et le couvercle chauffant peuvent être endommagés. Des récipients inadéquats peuvent être fortement endommagés et laisser s'échapper une partie de l'échantillon. Il convient donc de faire très attention en travaillant avec des échantillons infectieux.
- Il faut veiller à ce que les plaques PCR soient placées correctement dans le bloc pour ne pas provoquer d'endommagement du couvercle et du bloc et de fuite d'une partie de l'échantillon.
- Si les récipients dans le bloc sont équilibrés sur le plan de la température sans utiliser le couvercle chauffant, ils peuvent éclater en cas de température trop élevée et une partie de l'échantillon peut s'échapper.

Le Thermobloc, l'intérieur du couvercle chauffant et les récipients de réactif / les plaques PCR atteignent très rapidement des températures supérieures à 50 °C. Un risque de brûlure existe !

Le couvercle chauffant doit rester fermé jusqu'à obtention de température égale ou inférieure à 30 °C. N'utilisez pas de matériaux (récipients, plaques PCR, bouchons, films, nattes) qui ne résistent pas suffisamment à la température (jusqu'à 120 °C).

▼▲ Caution: Hot Surface

Gradient

Symbole sur le Thermobloc:
Indique que le Thermobloc est éventuellement chaud (▼) ou que l'intérieur du couvercle est chaud (▲). Indique que la température est plus élevée sur le côté droit que sur le côté gauche en mode gradient.

- Les matières explosives, inflammables et réagissant violemment dans le Mastercycler ep *realplex* ne doivent pas être ajustées sur le plan de la température. L'appareil ne peut pas fonctionner dans des zones exposées aux explosions..
- En cas de maniement de matières pathogènes, de substances radioactives ou autres substances dangereuses pour la santé, les directives de sécurité correspondantes doivent être respectées.
- Aucun liquide ne doit pénétrer dans l'appareil.
- S'assurer que l'instrument a été chauffé pendant au moins 15 min avant de démarrer l'essai ou le calibrage.

1.2 Consignes de sécurité

- Les réparations doivent être effectuées uniquement par un spécialiste agréé par la société Eppendorf AG. L'agrément ne peut être obtenu qu'à travers une formation validée.
- **Remise du produit à un tiers**
  Si vous mettez ce produit à disposition d'un tiers, veillez à y joindre le présent mode d'emploi.
- **Elimination du produit**
  Il convient de se conformer aux réglementations légales applicables pour l'élimination du produit.
- **Informations concernant la mise au rebut des appareils électriques et électroniques dans l'Union européenne**
  Au sein de l'Union européenne, les appareils électriques sont régis par des réglementations nationales, basées sur la directive européenne 2002/96/CE relative aux déchets d'équipements électriques et électroniques (DEEE).

  En vertu de cette directive, tout appareil livré après le 13.08.2005 dans le secteur Business-to-Business, dont ce produit fait partie, ne pourra plus être mis au rebut avec les ordures ménagères ou par les services municipaux. Pour faciliter leur identification, ces appareils seront pourvus du symbole suivant:

  Etant donné que les réglementations relatives à l'élimination des déchets au sein de l'UE peuvent varier d'un pays à l'autre, nous vous invitons à contacter vos fournisseurs au besoin.

1.3 Avvertenze di sicurezza

Prima di utilizzare il Mastercycler ep *realplex*, leggere integralmente le istruzioni per l'uso. Il Mastercycler ep *realplex* può essere utilizzato esclusivamente da personale qualificato per eseguire la PCR real-time e metodi affini nei laboratori di ricerca.

Avvertenza: se l'apparecchio non fosse utilizzato conformemente alle istruzioni per l'uso del produttore, è esclusa qualsiasi garanzia e responsabilità per i danni che potrebbero derivarne.

Osservare scrupolosamente le seguenti disposizioni:

- Durante l'utilizzo dell'apparecchio rispettare sempre le disposizioni di sicurezza valide per il laboratorio.
  Per evitare danni dovuti alla condensa, l'apparecchio può essere acceso soltanto 12 ore dopo il posizionamento. Questo vale anche nel caso in cui l'apparecchio cambi collocazione.
- Scegliere un luogo di posa tale che l'apparecchio non sia sottoposto a sbalzi di temperatura. Evitare l'esposizione diretta alla luce solare.
- La tensione di rete deve corrispondere alle indicazioni riportare sulla targhetta d'identificazione. L'apparecchio deve essere collegato ad una presa con messa a terra. Prima di eseguire interventi di manutenzione e pulizia l'apparecchio deve essere spento e la spina staccata. Attendere il raffreddamento del blocco.Prima della messa in funzione controllare che i collegamenti siano corretti.
- Il Mastercycler ep *realplex* deve poggiare completamente su una superficie di lavoro stabile e rimanere ben fermo. Deve essere presente spazio a sufficienza affinché le fessure di ventilazione anteriori e posteriori non siano coperte e l'aria per il raffreddamento possa giungere sotto l'apparecchio. La distanza delle fessure di ventilazione posteriori dalla parete deve essere di almeno 10 cm. Fare attenzione che le fessure di ventilazione non siano ostruite e che le vie d'entrata e d'uscita dell'aria non siano bloccate. Non collocare carta o materiale simile sotto l'apparecchio, poiché potrebbe bloccare la ventilazione.
- Non collocare alcun oggetto sul coperchio riscaldato.
- Con il coperchio in posizione di pulizia sono accessibili i componenti con spigoli vivi. Procedere con attenzione alla pulizia del coperchio riscaldato, poiché i bordi affilati del cavo Rollflex e della guida possono causare lesioni.
- Durante l'apertura e la chiusura del coperchio così come durante il suo sollevamento nella posizione di pulizia, non infilare le dita tra il coperchio e l'alloggiamento dell'apparecchio; in caso contrario sussiste il pericolo di schiacciamento. Non infilare le mani sotto il coperchio qualora il coperchio sia riscaldato ed aperto.
- Non inserire alcun materiale campione direttamente nel termoblocco.
- Se si utilizzano provette diverse da quelle consigliate, il blocco ed il coperchio riscaldato potrebbero danneggiarsi. Provette non appropriate potrebbero essere danneggiate al punto da rilasciare il materiale campione. Prestare attenzione soprattutto in caso d'utilizzo con materiale infettivo.
- Fare in modo che le piastre PCR siano alloggiate correttamente nel blocco, in caso contrario il blocco e il coperchio potrebbero danneggiarsi con conseguente fuoriuscita di materiale campione.
- Qualora le provette fossero termostate nel blocco senza l'utilizzo del coperchio riscaldato, a temperature eccessive potrebbero scoppiare, con conseguente fuoriuscita di materiale campione.

Il termoblocco, il lato interno del coperchio riscaldato e le provette/piastre PCR raggiungono molto rapidamente temperature superiori a 50 °C. Pericolo d'ustione!

Tenere chiuso il coperchio riscaldato fino al raggiungimento di temperature intorno a 30 °C o inferiori.
Non utilizzare materiali (provette, piastre PCR, chiusure, film, feltri) prive di resistenza termica sufficiente (fino a 120 °C).

▼▲ Caution: Hot Surface
Gradient

Simbolo sul termoblocco:
Indica che il termoblocco (▼) o la parte interna del coperchio potrebbe essere caldi (▲).
▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ indica che nella modalità operativa "gradiente" la temperatura sul lato destro è più elevata rispetto alla temperatura sul lato sinistro.

- Non termostatare sostanze esplosive, combustibili e fortemente reagenti nel Mastercycler ep *realplex*. L'apparecchio non può essere utilizzato in locali a rischio d'esplosione.
- In caso di manipolazione di materiale patogeno, sostanze radioattive o altre sostanze pericolose per la salute, osservare le relative norme di sicurezza.
- Fare in modo che nessun liquido penetri nell'apparecchio.

1.3 Avvertenze di sicurezza

- Prima di inziare un assay o una calibrazione, accertarsi che sia trascorso un tempo di riscaldamento dello strumento di almeno 15 minuti.
- Gli interventi di riparazione devono essere eseguiti esclusivamente da personale esperto autorizzato da Eppendorf AG. L'autorizzazione può essere conseguita esclusivamente mediante un corso di formazione certificato.
- **Cessione**
  In caso di cessione del prodotto vogliate allegare le presenti istruzioni d'uso.
- **Smaltimento**
  Per lo smaltimento del prodotto attenersi alle disposizioni giuridiche in materia.
- **Informazioni sullo smaltimento degli apparecchi elettrici ed elettronici nella Comunità Europea**
  All'interno della Comunità Europea lo smaltimento è regolamentato da disposizioni nazionali basate sulla Direttiva UE 2002/96/CE sui rifiuti di apparecchiature elettriche ed elettroniche (WEEE).

  In base a tale disposizione tutti gli apparecchi forniti dopo il 13.08.2005 nel settore Business-to-Business, di cui fa parte il presente prodotto, non possono più essere smaltiti con i rifiuti comunali o domestici. A scopo identificativo sono contrassegnati con i seguenti simboli.

  Poiché la normativa in materia di smaltimento in ambito UE può cambiare a seconda del Paese, vogliate contattare i Vostri fornitori in caso di necessità.

1.4 Instrucciones de seguridad

Antes de utilizar el Mastercycler ep *realplex*, por favor, lea atentamente las instrucciones de manejo.
El Mastercycler ep *realplex* debe ser utilizado solamente en el laboratorio de investigación por personal especializado calificado para la realización del real-time PCR y métodos similares.

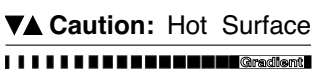
Advertencia: Si el aparato no se utiliza siguiendo las instrucciones de manejo del fabricante,queda excluida toda garantía y responsabilidad por daños que de ello se deriven.

Es imprescindible tener en cuenta las siguientes instrucciones:

- Al trabajar con el aparato hay que observar siempre las disposiciones de seguridad vigentes en el laboratorio. Para evitar daños producidos por la condensación, el aparato no debe ser conectado hasta transcurridas 12 horas de su instalación. Esto es también aplicable cuando el aparato es trasladado a otra ubicación.
- A la hora de instalar el equipo, se debe escoger un lugar en el que no se den grandes variaciones de temperatura. Evítese exponer el equipo directamente a la luz solar.
- La tensión de la red debe coincidir con los datos de la placa de características. El aparado debe ser enchufado a una toma de corriente conectada a tierra. Antes de proceder a trabajos de mantenimiento y limpieza, el aparato debe ser desactivado y desenchufado de la red eléctrica. Esperar a que se enfríe el bloque. Antes de la puesta en marcha se debe comprobar la idoneidad de las conexiones.
- El Mastercycler ep *realplex* debe estar completamente apoyado sobre una superficie de trabajo estable y en una posición segura. Debe haber espacio suficiente como para que las ranuras de ventilación anteriores y posteriores no queden tapadas, y pueda llegar debajo del aparato aire para su ventilación. La distancia entre las ranuras de ventilación posteriores y la pared debe ser como mínimo de 10 cm. Se debe tener cuidado de que las ranuras de ventilación estén libres y no se bloqueen las vías de aire de entrada y salida. No debe haber papeles u objetos semejantes debajo del aparato, ya que eso puede bloquear la ventilación.
- No deben depositarse objetos sobre la tapa de la calefacción.
- Si la tapa se encuentra en la posición de limpieza, puede accederse a elementos de cantos afilados. Se debe tener en cuenta que durante la limpieza de la tapa de calefacción los cantos afilados del cable rollflex y de los rieles de guía pueden provocar lesiones.
- Al abrir y cerrar la tapa, así como al levantarla en posición de limpieza, hay que tener cuidado de no colocar los dedos entre la tapa y la carcasa del aparato, porque de hacerlo existe el riesgo de que queden atrapados. Cuando está abierta la tapa de calefacción no se debe sujetar por debajo.
- No se debe introducir ningún material de muestra directamente en el termobloque.
- Utilizando recipientes de muestra distintos de los recomendados se pueden producir daños en el bloque y la tapa de calefacción Recipientes no adecuados pueden resultar tan dañados que se derrame el material de muestra, lo que hay que tener especialmente en cuenta al trabajar con material infeccioso.
- Hay que comprobar que las placas PCR están adecuadamente colocadas en el bloque, ya que de lo contrario pueden producirse daños en éste y en la tapa, así como también el derrame del material de muestra.
- Si se templan tubos de reacción sin utilizar la tapa de calefacción, pueden estallar a temperaturas demasiado altas y derramarse el material de muestra.

El termobloque, el lado interior de la tapa de calefacción y los tubos de reacción / las placas PCR alcanzan muy rápidamente temperaturas superiores a 50 °C. ¡Existe riesgo de quemadura!

Mantener la tapa de calefacción cerrada hasta que las temperaturas bajen a 30 °C o menos. No utilizar materiales (recipientes, placas PCR, cierres, láminas, alfombrillas) que no sean suficientemente estables térmicamente (hasta 120 °C).



Símbolo sobre el termobloque:
Avisa de un termobloque eventualmente caliente (▼) o de una parte interior de la tapa caliente (▲). ▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ indica que con un funcionamiento de gradiente, en el lado derecho la temperatura es más alta que en la izquierda.

- Las sustancias explosivas, inflamables y que reaccionan violentamente no deben ser templadas en el Mastercycler ep *realplex*. El aparato no debe funcionar en locales con riesgo de explosión.
- Al manipular material patógeno, sustancias radioactivas u otros materiales que supongan un riesgo para la salud se deben observar las correspondientes normativas de seguridad.

1.4 Instrucciones de seguridad

- Hay que asegurarse de que no penetra ningún líquido en el aparato.
- Asegúrese de calentar el instrumento durante al menos 15 minutos antes de iniciar el ensayo o el calibrado.
- Las reparaciones deben ser realizadas exclusivamente por un técnico autorizado por Eppendorf AG. La autorización puede obtenerse solamente mediante la correspondiente formación certificada.
- **Entrega a terceros**
  Si entrega este producto a terceras personas, le rogamos que incluya con el mismo estas instrucciones de uso.
- **Eliminación**
  En caso de que necesite eliminar este producto, asegúrese de respetar las disposiciones legales aplicables al respecto.
- **Información para la eliminación de aparatos electrónicos en la Comunidad Europea**
  Dentro de la Comunidad Europea, la eliminación de aparatos eléctricos y electrónicos está regida por disposiciones nacionales, basadas en la directiva UE 2002/96/CE sobre residuos de aparatos eléctricos y electrónicos (WEEE).

  Según ella, todos los aparatos suministrados después del 13.08.2005, en el ámbito del business-to-business en el que están clasificados esos aparatos, no deben ser ya eliminados con la basura municipal o doméstica.
  Para documentarlo irán provistos de los siguientes indicadores.

Como las disposiciones de eliminación pueden variar en los distintos países de la Unión Europea, le rogamos que en caso necesario se dirija a su proveedor.

1.5 Avisos de segurança

Antes de utilizar o Mastercycler ep *realplex*, leia o manual de instruções cuidadosamente. O Mastercycler ep *realplex* só deverá ser empreguado para real time PCR e métodos similares em laboratórios de investigação por pessoal técnico qualificado.

Nota: Se o aparelho não for usado de acordo com o disposto no manual de instruções do fabricante, cessam todas as garantias e responsabilidades no que se refere aos danos resultantes.

As seguintes instruções devem ser respeitadas impreterivelmente:

- Durante os trabalhos com o aparelho, têm que ser sempre cumpridas as prescrições de segurança aplicáveis para o laboratório. A fim de prevenir danos devido à condensação, o aparelho só deve ser ligado 12 horas após a sua instalação. O mesmo aplica-se quando o aparelho muda de local.
- Aquando da escolha do local de instalação é preciso ter em conta que o aparelho não poderá estar sujeito a variações térmicas consideráveis. É de evitar toda e qualquer exposição directa à radiação solar.
- A tensão de rede tem de coincidir com os dados na placa de identificação. O aparelho deve ser conectado a uma tomada com ligação à terra. Em caso de trabalhos de manutenção e limpeza, o aparelho tem que ser previamente desligado e a tomada removida da rede. Aguarde até o bloco resfriar. Antes da colocação em funcionamento, deve verificar se as ligações estão corretas.
- O Mastercycler ep *realplex* tem que ser instalado sobre uma superfície de trabalho estável e segura. Tem que haver espaço suficiente para as ranhuras de ventilação dianteiras e traseiras permanecerem desimpedidas e para permitir que o ar para arrefecimento passe por baixo do aparelho. A distância entre as ranhuras de ventilação traseiras e a parede deve ser de, no mínimo, 10 cm. Certifique-se de que as ranhuras de ventilação se mantêm desimpedidas e que as vias de entrada e saída de ar não estão bloqueadas. Não deverá haver papéis ou objectos semelhantes sob o aparelho, caso contrário a ventilação pode ficar obstruída.
- Nunca deposite objectos sobre a tampa térmica.
- Se a tampa ficar em posição de limpeza, ficam acessíveis componentes com arestas vivas. Quando limpar a tampa térmica, não se esqueça de que as arestas vivas do cabo rollflex e da calha de guia podem causar ferimentos.
- Ao abrir, fechar ou colocar a tampa na posição de limpeza, não coloque os dedos entre a tampa e a caixa do aparelho, para não ficar preso. Não ponha a mão sob a tampa térmica quando esta estiver aberta.
- O bloco térmico não pode ser directamente preenchido com o material de amostra.
- Caso sejam usados outros recipientes de ensaio que não os recomendados, podem ocorrer danos no bloco e na tampa térmica. Os recipientes impróprios podem ser danificados ao ponto de liberarem o material de amostra. Deve atender especialmente a este risco quando trabalhar com material infeccioso.
- Assegure-se de que as placas PCR ficam bem assentes no bloco, para não danificar o bloco ou a tampa nem liberar o material de amostra.
- Se os recipientes forem aquecidos no bloco sem o uso da tampa térmica, podem rebentar devido a temperaturas excessivas e liberar o material de amostra.

O bloco térmico, o lado interior da tampa térmica e os micro tubos reativos / as placas PCR atingem muito rapidamente temperaturas acima dos 50 °C. Existe risco de queimaduras!

Mantenha a tampa térmica fechada até a temperatura atingir valores à volta de 30 °C ou menos. Não utilize materiais (tubos, placas PCR, fechos, películas, esteiras) sem a resistência térmica necessária (até 120 °C).

▼▲ Caution: Hot Surface

Símbolo no bloco térmico:
adverte para o eventual aquecimento do bloco térmico (▼) ou do lado interior da tampa (▲). ▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ indicando que o lado direito está mais quente do que o esquerdo durante o funcionamento com gradiente.

- As substâncias explosivas, inflamáveis ou voláteis não podem ser aquecidas no Mastercycler ep *realplex*. O aparelho não pode funcionar em salas sujeitas ao perigo de explosão.
- No manuseamento de material patogênico, substâncias radioativas ou outros materiais prejudiciais à saúde devem ser respeitadas as normas de segurança aplicáveis.
- Certifique-se de que nenhum líquido entre no aparelho.
- Certifique-se de que o instrumento é aquecido durante pelo menos 15 min. antes de iniciar um teste ou calibração.

1.5 Avisos de segurança

- A reparação só pode ser executada por um técnico autorizado pela Eppendorf AG. A autorização apenas pode ser obtida através de formação certificada..
- **Cedência**
  Na eventualidade de ceder o produto a terceiros, pedimos para juntar também o manual de instruções.
- **Eliminação**
  Para a eliminação do produto tem de ser respeitadas as prescrições legais aplicáveis.
- **Informação sobre a eliminação de aparelhos eléctricos e electrónicos na União Europeia**
  Na União Europeia, a eliminação está regulamentada por normas nacionais baseadas na Directiva 2002/96/CE relativa aos resíduos de equipamentos eléctricos e electrónicos (REEE).

  Por conseguinte, todos os aparelhos fornecidos depois de 13.08.2005 no âmbito de Business-to-Business, no qual este produto está inserido, deixam de poder ser eliminados juntamente com o lixo camarário ou doméstico. Como forma de documentação, estão identificados com os símbolos a seguir.

  Uma vez que as normas de eliminação dentro da EU podem diferir de um país para outro, solicitamos-lhe que, em caso de dúvida, se informe junto do seu fornecedor.

1.6 Veiligheidsvoorschriften

Voordat u de Mastercycler ep *realplex* in gebruik neemt, dient u de handleiding volledig door te nemen. De Mastercycler ep *realplex* mag uitsluitend worden gebruikt voor het uitvoeren van real-time PCR en aanverwante methoden door gediplomeerd vakpersoneel in een onderzoekslaboratorium.

Let op: Wanneer het apparaat niet wordt gebruikt in overeenstemming met de handleiding van de fabrikant, vervalt elke garantie en aansprakelijkheid voor daaruit voortvloeiende schade.

De volgende voorschriften dienen te allen tijde in acht te worden genomen:

- Als u met het apparaat werkt, dient u altijd de voor het laboratorium geldende veiligheidsvoorschriften in acht te nemen. Om schade door condensatie te voorkomen, mag het apparaat pas 12 uur na het opstellen worden ingeschakeld. Dit geldt ook wanneer het apparaat naar een andere locatie wordt gebracht.
- De opstellingsplaats moet zodanig worden gekozen dat het apparaat niet aan noemenswaardige temperatuurschommelingen is blootgesteld. Directe zonbestraling moet worden vermeden.
- De netspanning moet overeenkomen met de gegevens op het typeplaatje van het apparaat. Het apparaat moet worden aangesloten op een geaard stopcontact. Bij onderhoudswerkzaamheden en reiniging moet het apparaat eerst worden uitgeschakeld en moet de stekker uit het stopcontact worden gehaald. Wacht tot het blok is afgekoeld. Voorafgaand aan de ingebruikname moet worden gecontroleerd of alle aansluitingen correct zijn.
- De Mastercycler ep *realplex* moet volledig op een stabiel werkvlak en op een veilige plaats staan. Er moet zoveel ruimte beschikbaar zijn, dat de ventilatieopeningen aan de voor- en achterkant niet worden afgedekt en dat er lucht onder het apparaat kan komen ter afkoeling. De afstand van de ventilatieopeningen aan de achterkant tot de wand moet minstens 10 cm bedragen. Zorg ervoor dat de ventilatieopeningen vrij zijn en dat de luchtaanvoer- en afvoerwegen niet worden geblokkeerd. Er mag geen papier of iets dergelijks onder het apparaat komen, aangezien daardoor de ventilatie kan worden belemmerd.
- Er mogen geen voorwerpen op de hittedeksel worden geplaatst.
- Als de deksel zich in de reinigingspositie bevindt, zijn onderdelen met scherpe randen bereikbaar. Let erop dat bij reiniging van de hittedeksel de scherpe randen van de rollflexkabel en de geleidingsrail verwondingen kunnen veroorzaken.
- Do not insert your fingers between the lid and the housing of the device when opening or closing the lid or when Houd bij het openen en sluiten van de deksel, alsmede bij het opklappen van de deksel in de reinigingspositie, geen vingers tussen de deksel en de behuizing van het apparaat, omdat de vingers anders bekneld kunnen raken. Houd uw handen niet onder de hittedeksel wanneer deze is geopend.
- Proefmateriaal mag niet rechtstreeks in het thermoblok worden geplaatst.
- Gebruik van andere dan de aanbevolen proefreageerbuizen kan resulteren in schade aan het blok en de hittedeksel. Niet-geschikte reageerbuizen kunnen zo sterk beschadigd raken, dat er proefmateriaal vrijkomt. Hier dient met name op te worden gelet bij het werken met infectieus materiaal.
- Zorg ervoor dat de PCR-platen correct in het blok zijn geplaatst, aangezien anders het blok en de deksel beschadigd kunnen raken en er proefmateriaal kan vrijkomen.
- Als reageerbuizen in het blok zonder gebruik van de hittedeksel op de juiste temperatuur worden gebracht, kunnen deze bij te hoge temperaturen barsten en kan het proefmateriaal vrijkomen.

Het thermoblok, de binnenkant van de hittedeksel en de reageerbuizen / PCR-platen bereiken zeer snel temperaturen van meer dan 50 °C. Er bestaat gevaar voor verbranding!

Houd de hittedeksel gesloten tot een temperatuur van rond de 30 °C of lager is bereikt. Gebruik geen materialen (reageerbuizen, PCR-platen, afsluitingen, folie, matten) die niet voldoende bestand zijn tegen hoge temperaturen (tot 120 °C).

▼▲ Caution: Hot Surface
Gradient

Symbool op het thermoblok:
Dit symbool waarschuwt voor een evtl. heet thermoblok (▼) of een hete binnenkant van de deksel (▲). Geeft aan dat bij gradiëntwerking de temperatuur aan de rechterkant hoger is dan aan de linkerkant.

- Explosieve, brandbare en heftig reagerende stoffen mogen niet in de Mastercycler ep *realplex* op de juiste temperatuur worden gebracht. Het apparaat mag niet worden gebruikt in ruimtes waar explosiegevaar bestaat.
- Bij de omgang met pathogeen materiaal, radioactieve stoffen of andere stoffen die een gevaar voor de gezondheid kunnen opleveren, dienen de hiervoor geldende veiligheidsvoorschriften in acht te worden genomen.

1.6 Veiligheidsvoorschriften

- Voorkom dat er vloeistof het apparaat binnendringt.
- Zorg ervoor dat het instrument minstens 15 minuten is opgewarmd voor u een assay of kalibratie start.
- Reparaties mogen uitsluitend worden uitgevoerd door een vakman die hiertoe de bevoegdheid heeft gekregen van Eppendorf AG. Deze bevoegdheid kan uitsluitend worden verkregen door een erkende opleiding.
- **Het doorgeven**
  Indien het product wordt doorgegeven, verzoeken wij u deze gebruiksaanwijzing bij te sluiten.
- **Afvalverwijdering**
  Indien het product wordt verwijderd moeten de wettelijke voorschriften in acht worden genomen.
- **Informatie over de afvalverwijdering van elektrische en elektronische apparaten in de Europese Gemeenschap**
  Binnen de Europese Gemeenschap wordt de afvalverwijdering geregeld door nationale voorschriften die baseren op de EU-richtlijn 2002/96/EC inzake elektronische en oude elektronische apparaten (WEEE).

  Daarnaar mogen alle apparaten die na 13.08.2005 worden geleverd en voor zakelijk gebruik zijn ingeschaald niet meer met het huisvuil worden afgevoerd. Om dit te documenteren zijn ze van het volgende kenmerk voorzien.

  Omdat de milieuvoorschriften binnen de EU van land tot land kunnen verschillen, verzoeken wij u eventueel contact op te nemen met uw leverancier.

1.7 Sikkerhedshenvisninger

Inden De bruger Mastercycler ep *realplex* bedes De læse betjeningsvejledningen fuldstændigt igennem. Mastercycler ep *realplex* må kun anvendes af fagpersonale på et forskningslaboratorium til at udføre real-time PCR og lignende metoder.

Henvisning: Hvis apparatet ikke benyttes i overensstemmelse med producentens betjeningsvejledning, er enhver garanti og ethvert ansvar for derved opståede skader udelukket.

De følgende bestemmelser skal ubetinget overholdes:

- Ved arbejder med apparatet skal man altid overholde de sikkerhedsbestemmelser, som gælder for laboratoriet. For at undgå skader på grund af kondensvand må der først tændes for apparatet 12 timer efter opstilling. Det gælder også, hvis apparatet flyttes hen til et andet sted.
- Opstillingsstedet skal vælges sådan, at apparatet ikke udsættes for større temperatursvingninger. Undgå direkte sollys.
- Netspændingen skal stemme overens med angivelserne på typeskiltet. Apparatet skal tilsluttes til en jordet stikdåse. Der skal slukkes for apparatet, og stikket skal trækkes ud at stikkontakten inden vedligeholdelsesarbejde og rengøring. Vent, til blokken er afkølet. Kontroller inden ibrugtagning, at tilslutningerne er korrekte.
- Hele Mastercycler ep *realplex* skal stå sikkert på den stabile arbejdsflade. Der skal være så meget plads, at de forreste og de bageste udluftningsslidser ikke dækkes, og at der kan komme luft til afkøling ind under apparatet. Afstanden mellem de bageste udluftningsslidser og væggen skal være mindst 10 cm. Man skal sørge for, at udluftningsslidserne er frie, og at ind- og udluftningsvejene ikke blokeres. Der må ikke komme papir eller lignende ind under apparatet, da det kan medføre en blokering af udluftningen.
- Der må ikke stilles nogen genstande på varmelåget.
- Hvis låget er i rengøringsposition, er dele med skarpe kanter tilgængelige. Ved rengøring af varmelåget skal man være opmærksom på, at rulleflexkablets og føringsskinnens skarpe kanter kan medføre kvæstelser.
- Når låget åbnes og lukkes eller klappes op til rengøringsposition, må man ikke lægge fingrene ind mellem låget og apparatets hus, da der ellers er risiko for at få fingrene i klemme. Grib ikke ind under låget med åbent varmelåg.
- Der må ikke fyldes prøvemateriale direkte i termoblokken.
- Ved brug af andre prøvebeholdere end de anbefalede kan der opstå skader på blokken og varmelåget.
- Uegnede beholdere kan blive så stærkt beskadiget, at der frigøres prøvemateriale. Det skal man især være opmærksom på, hvis man arbejder med infektiøst materiale.
- Man skal sørge for, at PCR-pladerne sidder korrekt i blokken, da der ellers kan opstå skader på blokken og låget, og der kan frigøres prøvemateriale.
- Hvis beholdere tempereres i blokken uden at bruge varmelåget, kan disse briste ved for høje temperaturer og prøvemateriale frigøres.

Termoblokken, varmelågets indvendige side og reaktionsbeholderne / PCR-pladerne kommer meget hurtigt op på temperaturer over 50 °C. Der er fare for forbrænding!

Hold varmelåget lukket, til der er nået temperaturer på omkring 30 °C eller mindre. Benyt ingen materialer (beholdere, PCR-plader, lukninger, folier, måtter), som ikke er tilstrækkeligt temperaturbestandige (op til 120 °C).

▼▲ Caution: Hot Surface

Gradient

Symbol på termoblokken:
Advarer mod en evt. meget varm termoblok (▼) eller en meget varm indvendig side af låget (▲). viser, at temperaturen ved gradientdrift er varmere på højre side end på venstre side.

- Eksplosive, brændbare og voldsomt reagerende stoffer må ikke tempereres i Mastercycler ep *realplex*. Apparatet må ikke benyttes i eksplosionsfarlige rum.
- Ved håndtering med patogent materiale, radioaktive stoffer eller andre sundhedsfarlige stoffer skal de tilsvarende sikkerhedsbestemmelser overholdes.
- Man skal sikre sig, at der ikke trænger væske ind i apparatet.
- Sørg for at forvarme instrumentet i mindst 15 min. før start på et assay eller en kalibrering.

1.7 Sikkerhedshenvisninger

- Reparationer må kun udføres af en fagmand, som er autoriseret af Eppendorf AG. Autorisationen kan kun opnås ved en certificeret uddannelse.
- **Videregivelse**
  I tilfælde af, at produktet bliver videregivet, beder vi Dem vedlægge denne betjeningsvejledning.
- **Bortskaffelse**
  Hvis produktet skal bortskaffes, skal de tilsvarende lovbestemmesler overholdes.
- **Information om bortskaffelse af elektriske og elektroniske apparater i det Europæiske Fællesskab**
  Inden for det Europæiske Fællesskab er bortskaffelsen fastlagt af de nationale bestemmelser, som baserer på EU-direktiv 2002/96/EC vedrørende brugte elektro- og elektronik-apparater (WEEE).

  Ifølge disse bestemmelser må apparater, som er leveret efter 13.08.2005 på business-to-business-området, som dette produkt hører ind under, ikke længere bortskaffes sammen med kommunalt affald eller husholdningsaffald. For at dokumentere dette er de forsynet med følgende markering.

  Da reglerne om bortskaffelse kan være forskellige fra land til land inden for EU, bedes De efter behov spørge leverandøren.

1.8 Turvallisuusohjeet

Lue käyttöohje huolellisesti ennen Mastercycler ep *realplex* -laitteen käyttöä. Mastercycler ep *realplex* -laitetta saa käyttää ainoastaan real-time PCR ja muissa sille sukua olevissa menetelmissä. Laitetta saavat käyttää vain koulutuksen saaneet henkilöt tutkimuslaboratorioissa.

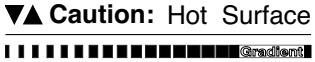
Huom: Jos laitetta ei käytetä valmistajan käyttöohjeessa kuvatulla tavalla, valmistaja ei ole vastuussa väärästä käytöstä aiheutuvista vahingoista eikä korvaa niitä.

Seuraavia määräyksiä on ehdottomasti noudatettava:

- Laitetta käytettäessä aina on noudatettava laboratoriossa voimassa olevia turvamääräyksiä. Laitteen saa käynnistää vasta 12 tunnin kuluttua sen pystytyksestä, jotta vältettäisiin lauhteesta aiheutuvat viat. Tämä on voimassa myös silloin, kun laitteen paikkaa vaihdetaan.
- Sijaintipaikka ei saa olla alttiina mainittaville lämpötilan vaihteluille, suoraa auringonpaistetta on vältettävä.
- Verkkojännitteen on vastattava tyyppikilpeen merkittyjä tietoja. Laite on liitettävä maadoitettuun pistorasiaan. Laite on kytkettävä pois päältä ja pistotulppa on irrotettava pistorasiasta ennen huoltotöitä ja puhdistusta. Laitteen on annettava jäähtyä. Liitännät on tarkistettava ennen laitteen käyttöönottoa.
- Mastercycler ep *realplex* on sijoitettava tukevan työskentelytason päälle ja sen on seisottava tukevasti. Tilaa on oltava niin paljon, että edessä ja takana olevat tuuletusraot eivät peity ja että ilmaa pääsee laitteen alle. Laitteen takana olevien tuuletusrakojen etäisyyden seinästä on oltava vähintään 10 cm. On tarkistettava, että tuuletusraot ovat vapaita ja tulo- ja poistoilman reittejä ei tukita. Laitteen alle ei saa joutua paperia tai vastaavaa, sillä se voi estää ilmanvaihdon.
- Lämmityskannen päälle ei saa asettaa mitään tavaroita.
- Kun kansi on puhdistusasennossa, päästään käsiksi teräväreunaisiin rakenneosiin. Muista aina, että lämmityskannen puhdistuksen yhteydessä Rollflex-kaapelin ja ohjainkiskon terävät reunat voivat johtaa loukkaantumisiin.
- Älä pistä sormiasi kannen ja laitteen rungon väliin kantta avatessasi ja sulkiessa sekä kääntäessäsi sitä puhdistusasentoon. Sormesi voivat muuten jäädä puristuksiin. Älä koske kannen alle lämmityskannen ollessa auki.
- Näytemateriaalia ei saa täyttää suoraan Thermoblock-kappaleeseen.
- Thermoblock-kappale ja lämmityskansi voivat vioittua, jos käytetään muita kuin suositeltuja näyteastioita. Tarkoitukseen soveltumattomat astiat voivat vioittua niin pahoin, että näytemateriaalia pääsee vapautumaan. Muista tämä erityisesti työskennellessäsi tarttuvan materiaalin kanssa.
- PCR-levyjen on istuttava oikein Thermoblock-kappaleessa, sillä kappale ja kansi voivat muuten vioittua ja näytemateriaalia pääsee vapautumaan.
- Jos astiat temperoidaan Thermoblock-kappaleessa ilman lämmityskannen käyttöä, ne voivat haljeta liian korkeissa lämpötiloissa ja näytemateriaalia pääsee vapautumaan.

Thermoblock-kappale, lämmityskannen sisäpuoli ja reaktioastiat / PCR-levyt saavuttavat erittäin nopeasti yli 50 °C lämpötilan. Palovammojen vaara!

Pidä lämmityskansi suljettuna, kunnes lämpötila on 30 °C tai sitä alhaisempi. Älä käytä materiaaleja (astioita, PCR-levyjä, sulkimia, kalvoja, mattoja), jotka eivät ole riittävän lämmönkestäviä (120 °C).



Thermoblock-kappaleessa pöeva symboli:
Varoittaa kuumasta Thermoblock-kappaleesta (▼) tai kannen kuumasta sisäpuolesta (▲).
▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ ilmoittaa, että gradienttikäytössä lämpötila on oikealla puolella kuumempi kuin vasemmalla puolella.

- Mastercycler ep *realplex* -laitteessa ei saa temperoida räjähtäviä, syttyviä tai voimakkaasti reagoivia aineita. Laitetta ei saa käyttää räjähdysvaarallisissa tiloissa.
- Käsiteltäessä patogeenista materaalia, radioaktiivisia aineita tai muita terveydelle vaarallisia aineita on huomioitava vastaavat turvamääräykset.
- Nesteiden pääsy laitteen sisään on estettävä.
- Instrumentin on annettava lämmetä vähintään 15 minuuttia ennen kuin koe tai kalibrointi aloitetaan.
- Ainoastaan Eppendorf AG:n valtuuttama ammattimies saa suorittaa korjaukset. Valtuudet on mahdollista saada ainoastaan sertifioidun koulutuksen kautta.

1.8 Turvallisuusohjeet

– **Tuotteen luovuttaminen toiselle**
Jos tuote luovutetaan jollekin toiselle henkilölle, on tämä käyttöohje annettava hänelle tuotteen mukana.

– **Hävittäminen**
Tuotteen käytöstäpoistossa ja hävittämisessä on noudatettava voimassa olevia lakisääteisiä määräyksiä.

– **Käytöstä poistettujen sähköteknisten ja elektronisten laitteiden hävittäminen EU-maissa**
Euroopan yhteisön sisällä hävityksen on tapahduttava sähkö- ja elektroniikkatuotteita koskevaan EU-direktiiviin 2002/96/EC(WEEE) pohjautuvien kansallisten säädösten mukaisesti.

Direktiivin mukaan 13.08.2005 jälkeen myyntiin tulleita business-to-business -laitteita, joihin myös oheinen laite luetaan, ei enää saa lajitella yhteiskunta- tai kotitalousjätteiden joukkoon. Kyseiset laitteet on varustettu seuraavalla tunnuksella.

Koska jätehuoltoa koskevat määräykset EU-maissa eroavat toisistaan, pyydämme tarvittaessa kääntymään laitteen toimittaneen liikkeen puoleen.

1.9 Wskazówki bezpieczeństwa

Przed użyciem urządzenia Mastercycler ep *realplex* należy w całości przeczytać instrukcję obsługi. Urzadzenie Mastercycler ep *realplex* może być używane wyłącznie do badań 'real-time PCR' i metodami pokrewnymi, przez odpowiednio przygotowany personel laboratorium badawczego.

Wskazówka: W przypadku korzystania z urządzenia w sposób niezgodny z instrukcją obsługi wydaną przez producenta, wszelkie zobowiązania gwarancyjne tracą ważność, a producent nie ponosi odpowiedzialności za poniesione z tej przyczyny szkody.

Należy bezwzględnie przestrzegać następujących zaleceń:

- Przy pracy z urządzeniem należy stale przestrzegać regulaminu bezpieczeństwa obowiązującego na terenie danego laboratorium. Aby uniknąć uszkodzeń na skutek osadzania się pary wodnej, urządzenie wolno włączyć dopiero po upływie 12 godzin od postawienia w pomieszczeniu. Obowiązuje to także w przypadku późniejszych zmian miejsca ustawienia urządzenia.
- Miejsce pracy nie powinno narażac instrumentu na duże wahania temperatury. Unikać bezpośredniego nasłonecznienia.
- Napięcie sieci elektrycznej musi odpowiadać danym na tabliczce znamionowej. Urządzenie musi być podłączone do uziemionego gniazdka elektrycznego. Na czas konserwacji i czyszczenia urządzenie należy uprzednio wyłączyć i wyjąć wtyczkę z gniazda sieci elektrycznej. Odczekać, aż blok urządzenia się schłodzi. Przed włączeniem urządzenia po raz pierwszy, należy sprawdzić prawidłowość połączeń.
- Mastercycler ep *realplex* musi stać w całości na stabilnym podłożu zapewniającym bezpieczną eksploatację. Dookoła należy pozostawić tyle miejsca, aby przednie i tylne otwory wentylacyjne nie były zakrywane, a pod urządzenie mogło swobodnie wpływać powietrze potrzebne do chłodzenia. Odstęp tylnych otworów wentylacyjnych od ściany musi wynosić co najmniej 10 cm. Należy uważać, aby otwory wentylacyjne pozostawały odkryte, a w okolicach wlotu i wylotu powietrza nie znajdowały się żadne przeszkody. Pod urządzenie nie mogą dostawać się żadne papiery itp. ponieważ może to utrudnić dopływ powietrza.
- Na pokrywie grzejnej nie wolno ustawiać żadnych przedmiotów.
- Gdy pokrywa jest otwarta do czyszczenia, dostępne stają się ostro zakończone części. Należy pamiętać, że podczas czyszczenia pokrywy grzejnej może dojść do skaleczenia o ostre krawędzie prowadnicy kablowej oraz szyny prowadzącej.
- Przy otwieraniu i zamykaniu pokrywy, a także przy odchylaniu jej do czyszczenia, nie wkładać palców między pokrywę a obudowę urządzenia, ponieważ grozi to ich skleszczeniem. Przy otwartej pokrywie grzejnej nie wkładać rąk pod pokrywę.
- Badany materiał nie może być napełniany bezpośrednio do bloku termicznego.
- Przy stosowaniu naczyń innych niż zalecane probówki może dojść do uszkodzenia bloku i pokrywy grzejnej. Nieodpowiednie naczynia mogą zostać uszkodzone do tego stopnia, że wydostanie się badany materiał. Należy o tym pamiętać szczególnie w przypadku badań na materiale stwarzającym ryzyko infekcji.
- Zwrócić uwagę, aby płytki PCR były umieszczone prawidłowo w bloku, ponieważ istnieje ryzyko uszkodzenia bloku i pokrywy, a także wydostania się badanego materiału.
- Gdy w bloku podgrzewane są naczynia bez użycia pokrywy grzejnej, może dojść do ich rozerwania pod wpływem zbyt wysokich temperatur i w konsekwencji do wydostania się badanego materiału.

Blok termiczny, wewnętrzna strona pokrywy grzejnej oraz naczynia reakcyjne / płytki PCR rozgrzewają się bardzo szybko do temperatur powyżej 50 °C. Możliwe oparzenia!

Pokrywa grzejna powinna pozostać zamknięta, dopóki temperatura spadnie poniżej 30 °C. Nie stosować materiałów (naczyń, płytek PCR, zamknięć, folii, mat), które nie są wystarczająco odporne na wysokie temperatury (do 120 °C).

▼▲ Caution: Hot Surface

Gradient

Znak na bloku termicznym:
Ostrzega przed możliwie gorącym blokiem termicznym (▼) lub gorącą stroną wewnętrzną pokrywy (▲). ▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮▮ pokazuje, że w trybie pomiaru gradientu temperatura po prawej stronie jest wyższa niż po lewej stronie.

- W urządzeniu Mastercycler ep *realplex* nie wolno podgrzewać substancji wybuchowych, palnych i silnie reagujących. Urządzenie nie może pracować w pomieszczeniach, w których występuje atmosfera grożąca wybuchem.
- Przy pracy z materiałem patogennym, substancjami radioaktywnymi oraz innymi substancjami szkodliwymi dla zdrowia należy przestrzegać odnośnych przepisów bezpieczeństwa.
- Należy zapewnić, aby do wnętrza urządzenia nie mogły przedostać się żadne płyny.

1.9 Wskazówki bezpieczeństwa

- Przed rozpoczęciem oznaczania lub kalibracji należy wygrzewać przyrząd przez co najmniej 15 minut.
- Naprawy mogą być wykonywane wyłącznie przez specjalistów posiadających autoryzację firmy Eppendorf AG. Wyłączną podstawą uzyskania autoryzacji jest ukończenie szkolenia potwierdzone odpowiednim ceryfikatem.
- **Sprzedaż produktu**
  W przypadku ewentualnej sprzedaży produktu, prosimy o przekazanie niniejszej instrukcji obsługi wraz z urządzeniem.
- **Utylizacja produktu**
  W przypadku utylizacji produktu należy przestrzegać odnośnych przepisów prawnych.
- **Informacja na temat utylizacji urządzeń elektrycznych i elektronicznych w krajach Unii Europejskiej**
  Na obszarze Wspólnoty Europejskiej kwestie utylizacji odpadów regulowane są przez ustawy prawa narodowego oparte na dyrektywie UE 2002/96/EC w sprawie odpadów pochodzących z urządzeń elektrycznych i elektronicznych (WEEE).
  W związku z tym wszystkie urządzenia dostarczone po dniu 13.08.2005r. na zasadzie Business-to-Business (sprzedaż między podmiotami gospodarczymi), do której zaklasyfikowany jest niniejszy wyrób, nie mogą być usuwane wraz z odpadami komunalnymi lub domowymi. Aby to udokumentować zostały one oznakowane w następujący sposób.

Przepisy dotyczące utylizacji na obszarze Unii Europejskiej mogą być różne w zależności od kraju, dlatego w razie potrzeby prosimy zwrócić się do dostawcy.

2 Installation

2.1 Delivery package

The delivery package includes the following parts:

1 mounting set for Mastercycler ep *realplex*
1 mains power cable
1 PC CAN bus cable
1 CAN bus cable
1 USB CAN adapter
1 Torx screw driver
1 Allen head key
1 CD with instruction manual and software
1 instruction manual in English (printed)
1 twin.tec PCR plate 96, skirted / semi-skirted
1 clip for rollflex cable
1 certificate of conformity

2.2 Setting up the device

To avoid damage caused by condensation, the device should only be switched on 12 hours after setup.
This also applies to a change of location.

The Mastercycler ep *realplex* must stand properly on a stable work surface.

Make sure that the Mastercycler ep *realplex* is not placed close to instruments that could cause vibrations (e.g. centrifuges).

There must be enough space available to prevent blocking of the front and rear ventilation slots and to allow cooling air to pass beneath the device.

The distance between the rear ventilation slots and the wall should be min. 10 cm.

Space requirement:	Width:	26.0 cm
	Depth:	41.0 cm
	Height:	39.6 cm

Mains connection: A safety plug socket for the thermo module.
The *realplex* module is supplied via the mains connection of the thermo module.

Make sure that the ventilation slots remain freely accessible and the inlets and outlets are not blocked.
There should be no paper or other objects under the device that might block ventilation.

The mains voltage must match the data on the identification plate.

The device must be connected to a grounded socket.

The device should be set up so it can be easily disconnected from the mains.

The delivery carton should be retained to return the device safely in the case of repair.

Note: Transport and storage must not exceed max. 50 °C.
The device should be set up to prevent direct exposure to sunlight.

2.3 Functional units

2.3.1 Mastercycler ep *realplex*

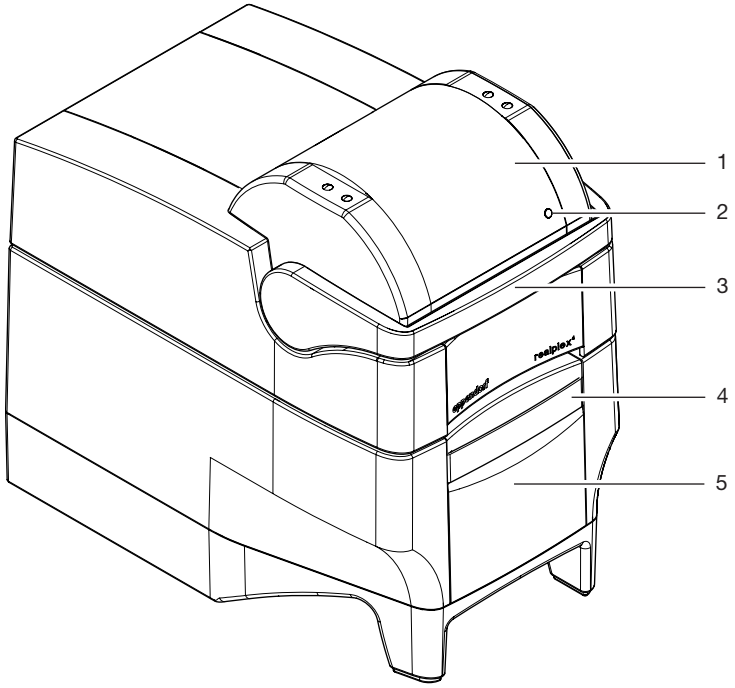


Figure 1: Front view, *realplex* module closed

1 *realplex* module
2 Status display
3 Sealing clamp (locked)
4 Holding clip for control panel (not necessary for Mastercycler ep realplex)
5 Thermo module

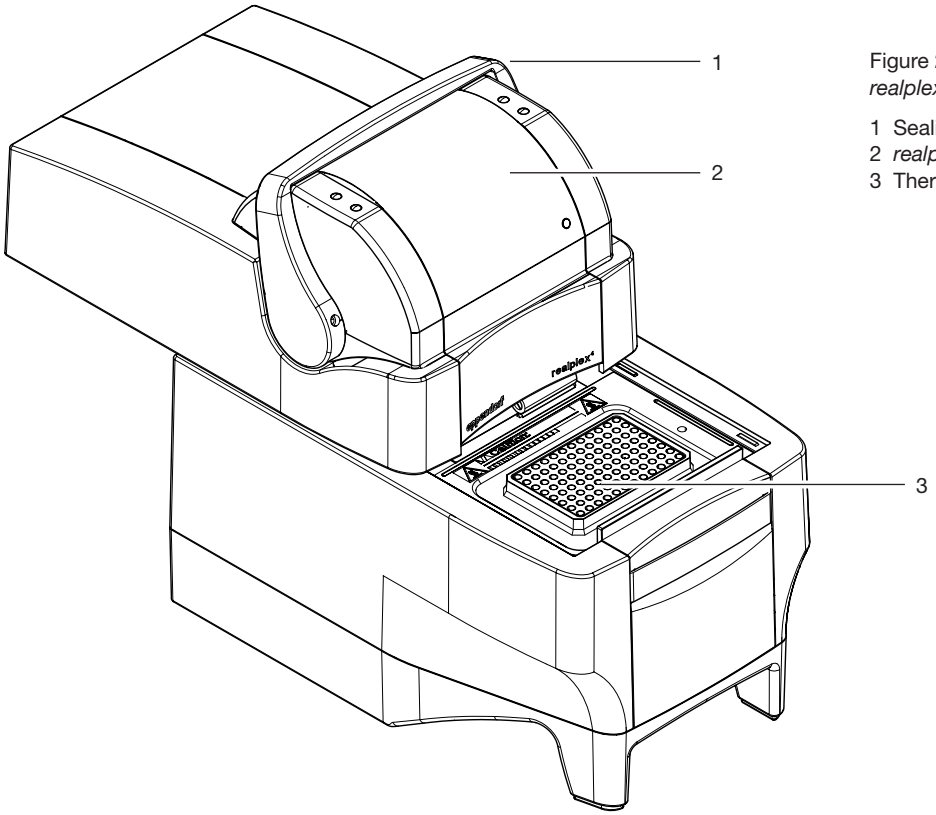


Figure 2: Front view, *realplex* module opened

1 Sealing clamp (opened)
2 *realplex* module
3 Thermoblock

After samples have been loaded onto equipment the *realplex* module is pulled to the front, over the inserted tubes or plate, closed with the clamp, and a program then started. When moving the sealing clamp into position, make sure you do not catch your fingers.

Do not reach beneath the lid when the *realplex* module is open.

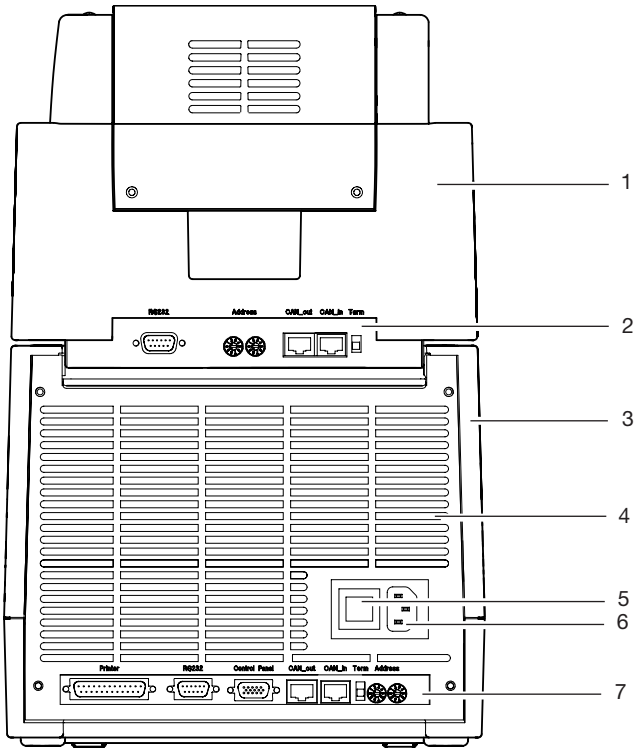


Figure 3: Rear view

1 *realplex* module
2 Connection strip of *realplex* module (see Fig. 4)
3 Thermo module
4 Ventilation slots
5 Mains power switch 0 = off / I = on
6 Mains power socket
7 Connection strip of thermo module (see Fig. 5)

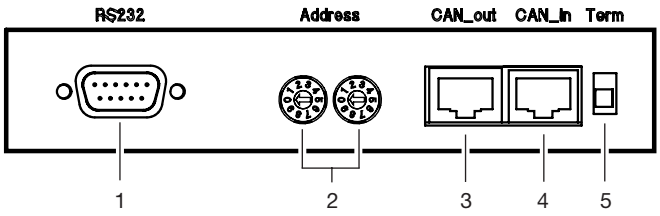


Figure 4: Connection strip of *realplex* module

1 RS232: serial interface
2 Address: rotary switch Address
3 CAN_out
4 CAN_in
5 Terminal resistance

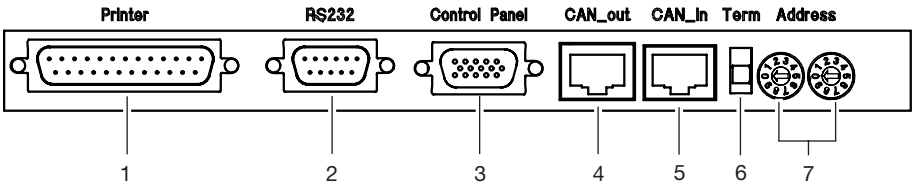


Figure 5: Connection strip of thermo module

1 Printer: parallel interface
2 RS232: serial interface
3 Control panel: connection of control panel (not necessary for Mastercycler ep *realplex*)
4 CAN_out
5 CAN_in
6 Terminal resistance
7 Address: rotary switch Address

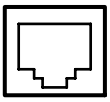
Note: Only devices complying with the standards EN/IEC 60950 and UL 60950A should be connected to the interfaces.

2 Installation

2.4 Startup

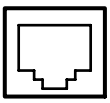
2.4.1 Connection of *realplex* module to thermo module

CAN_out



Connect the CAN bus connecting cable to the CAN_out connection of the thermo module.

CAN_in



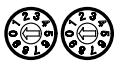
Connect the free end of the CAN bus connecting cable to the CAN_in connection of the *realplex* module.

Term

ON

Set the Term switch at the rear of the *realplex* module to ON and that of the thermo module to OFF.

Address



Move "Address" on the *realplex* module using the rotary switch address at the rear of the *realplex* module to the setting 11. The arrowheads of both rotary switches are each set to 1.
The "Address" on the thermo module is set to 02 (arrowhead of the left-hand rotary switch set to 0 and the arrowhead of the right-hand rotary switch set to 2).

Note: Make sure that the right addresses are allocated so that after switch-on of the devices they are detected automatically by the software and shown in the software display.

2.4.2 Connection of Mastercycler ep *realplex* to a computer

Computer hardware requirements:

AMD Athlon / Intel Pentium III @ 1.0 GHz, min. 512 MB RAM
4 GB hard-disk storage (up to 1 GB required for software and database)
USB 1.0/1.1 or USB 2.0 connector
CD-ROM writer + software for writing CDs (integrated or external)
CAN USB adapter, firmware 4.2 (standard accessories)

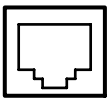
Requirement on operating system of computer:

Windows XP (Home Edition or higher)

Optional for a network environment:

Network card for connection to additional workstation computers
Installation of a TCP/IP network

CAN_in



Connect the PC CAN bus to the CAN_in connection of the thermo module.

Connect the free end of the PC CAN bus cable to the USB CAN adapter.

Connect the USB CAN adapter to the USB interface of the computer.

Before switching on the device check that the correct mains voltage is available for the device. Compare the data on the identification plate at the left hand side of the thermo module.

Connect the mains power cable to the thermo module and then connect to the mains power supply (see Fig. 3 Rear view). The mains power supply of the *realplex* module is via the connected thermo module.

After the user has switched on the device and started the *realplex* software, the connected Mastercycler ep *realplex* is detected automatically by the software and shown on the status display.

The computer must not be disconnected from the Mastercycler ep *realplex* while running.

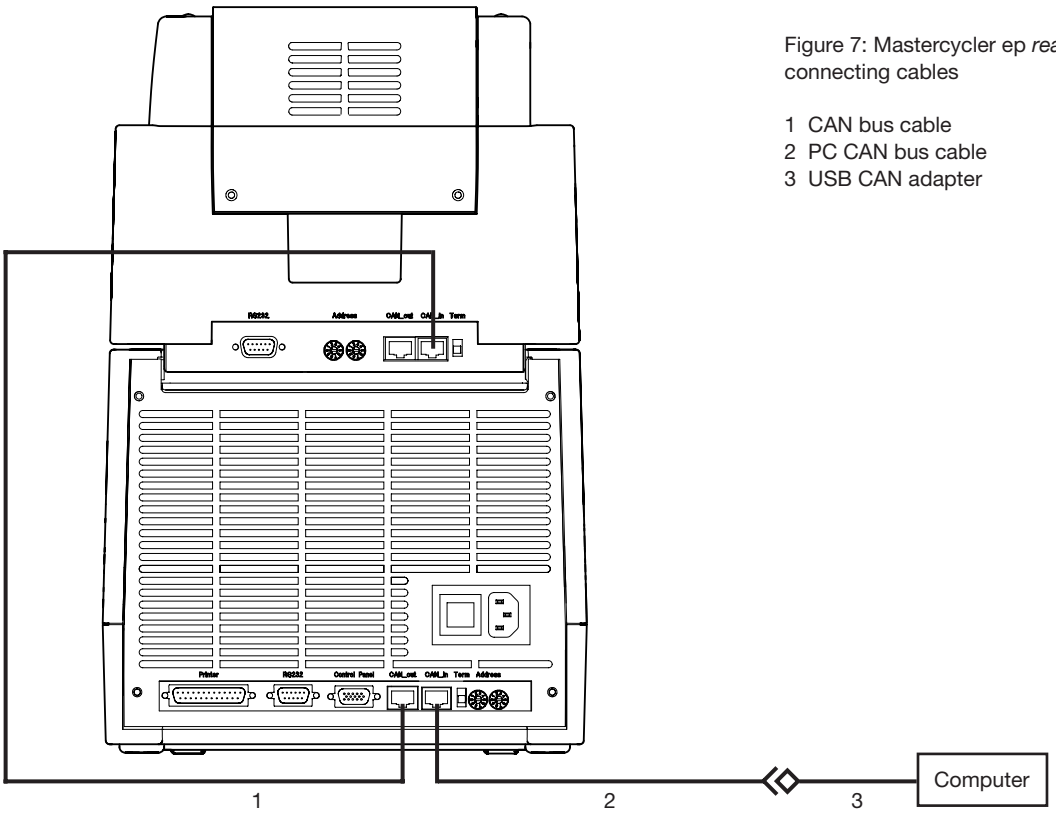


Figure 7: Mastercycler ep *realplex* with connecting cables

1 CAN bus cable
2 PC CAN bus cable
3 USB CAN adapter

2.4.3 Connection of other components

Note: Only devices complying with the standards EN/IEC 60950 and UL 60950A should be connected to the interfaces.

2.4.4 Installation of *realplex* software

2.4.4.1 Main installation procedure

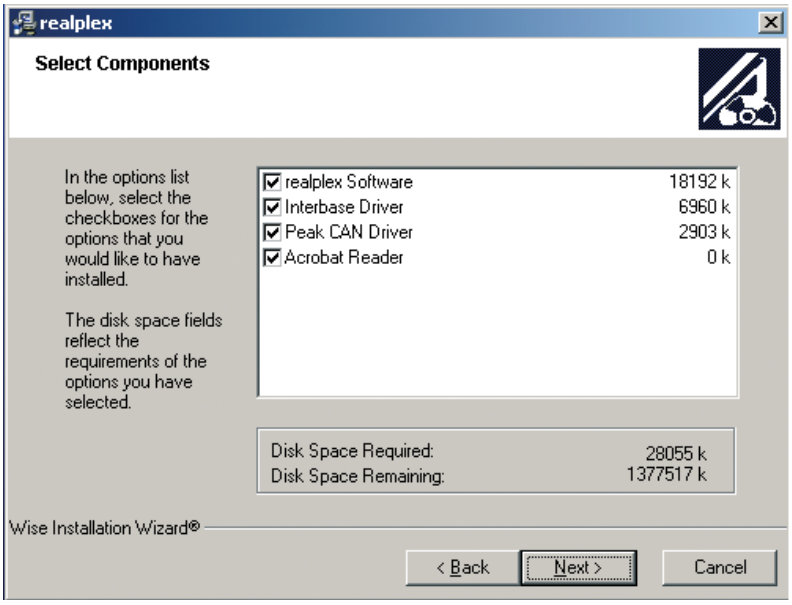
First close all programs that are currently running on your computer. Insert your USB CAN adapter into the USB port. A few seconds later a hardware installation wizard will be displayed.

Close Wizard with **Cancel.**

Note: Do not remove the USB CAN adapter until the installation is complete!

Attention: For correct installation of the *realplex* software computer administration rights are highly recommended!

Now insert the ***realplex*** CD-Rom, and start **setup.exe** in the CD-Rom directory (if Autorun is active, the setup will start automatically). Then the software will ask you for the components you wish to install:



Continue with **Next**. Several windows will pop up to the screen, accept the default settings with **Next**, **Yes**, **Install** and **Finish**, respectively. When the installation is complete, restart the computer.

Now you will find the new group Eppendorf in the startup menu, from where you can start the software you have installed. Additionally, the software automatically creates icons on the desktop of the computer.

2.4.4.2 Restore database

If the *realplex* software is installed the first time on startup, the software will recognize that no database is available and will automatically start the **Database Tool**. Just accept the default settings given on the sheet **Restore Database**, and press button **Start Restore**. When restore has finished, the *realplex* software can be started by clicking the button **Start realplex** in the upper right hand corner.

If *realplex* software has already been installed earlier, a restore of the database will not be necessary, because the existing database from the earlier installation can be used.

2.4.4.3 Start the *realplex* software

Switch on Mastercycler ep *realplex* and wait until the LED on the top switches to green. Now you are ready to start the *realplex* software.

You also may start the *realplex* software with the cycler switched off or even with no cycler hardware at all. In the latter case, an error message will be displayed. Ignore the message and continue.

Attention: Do not use any programs, which require major parts of the computer´s capacity (e.g. CD Creator, Media Player) during usage of the *realplex* software!

Never run the computer in battery mode! Change the settings for the power supply of the computer to be sure that it will not switch to the standby mode during an ongoing assay!

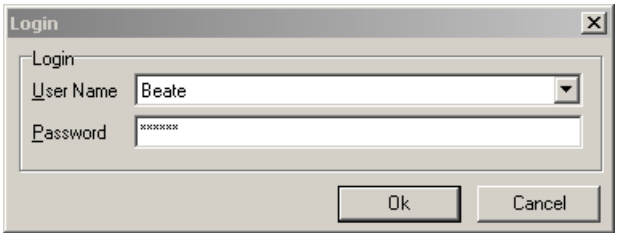
3 Operation

3.1 Login as administrator

When the *realplex* software is opened for the first time, a dialogue **Login** (see 3.2) is displayed, prompting the Administrator named Eppendorf to enter his password. This is factory-set and can be changed in the dialogue **User Administration** if required (see section 3.4.1). Whenever the program is opened again, not only the Administrator but also all users he has set up on the system can then log in (see section 3.2).

3.2 Login

To work with the Mastercycler ep *realplex* users have to log in when opening the program. All users known to the system are listed in a selection box. Select the required user and enter the relevant password.
New users are set up by the Administrator (see section 3.4.1.1).



If the wrong password has been entered accidentally, the display shows **Login failed**. After confirmation with the function key **OK**, the Login procedure is repeated.

If the dialogue is ended using the function key **Cancel**, the main screen is displayed (see section 3.3). However, all function keys are inactive. To work in the program, the user has to open the dialogue **Login** again (see section 3.2) and repeat the Login procedure.

Note: The logged-in user now has full read and write access to his folders and assays and can start his assays on the connected Mastercycler ep *realplex*. The folders and assays of other users can be viewed. To start and edit the assays of other users it is necessary to copy them to his own folder.

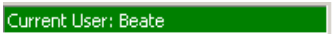
3.3 User Change

To log in to the system when another user is already logged on, proceed as follows:

Logout is performed by selecting the appropriate icon.

If the icon **Login** is selected, the dialogue **User Login** is displayed. The user can then log in as described in section 3.2.

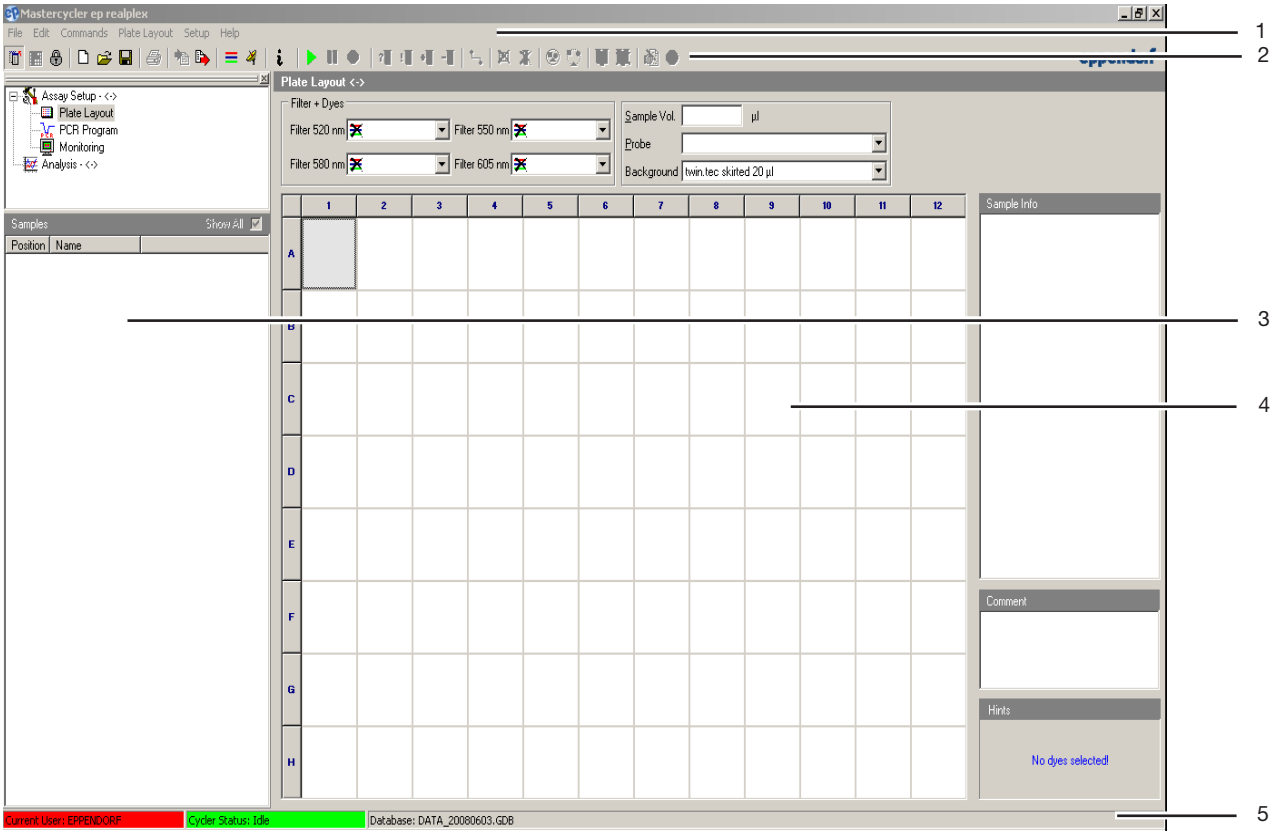
Alternatively these functions can also be accessed via the menu item **File**.
Only one user can ever be logged on. The login of a new user automatically results in logout of the existing user.



The current user is shown in the status display at the bottom of the screen.

The main screen appears after login as the Administrator (or user). The main screen is divided into Navigator, work area and status display. If individual program functions are selected in the navigation tree on the left, they will be displayed in the work area. The status display provides information about the status of the thermo module and the user currently logged on.

3 Operation



1 Menu bar
2 Tool bar
3 Navigator
4 Work area
5 Status display

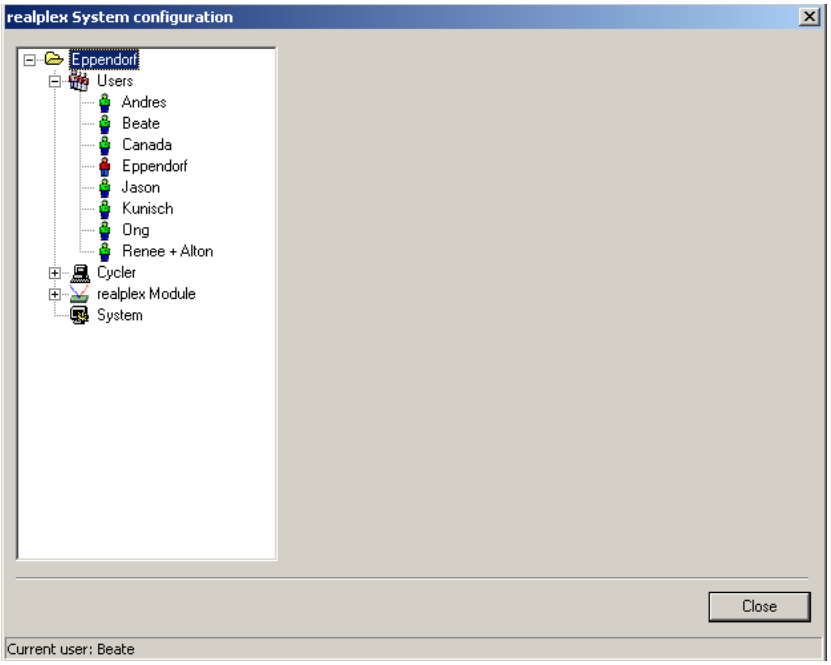
The Navigator can be closed or opened by selection of the icon **Show/Hide Navigator**. Alternatively this function can also be accessed via the menu item **Setup**.

Note: All actions which are available for the respective program functions and system configurations can be accessed via the tool bar or the menu bar. Selected actions are additionally accessible via the right mouse button.

3 Operation

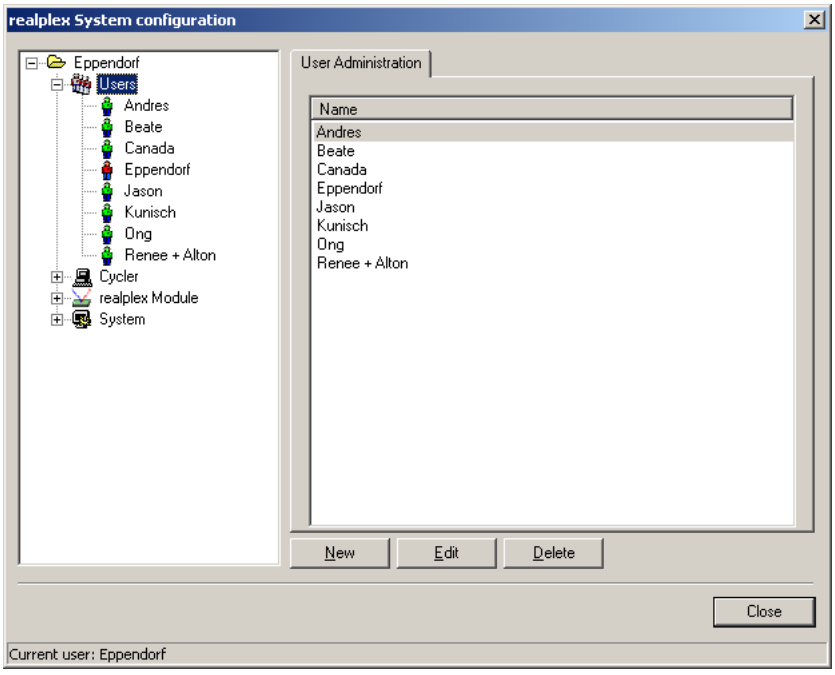
3.4 System configuration

In the menu bar the dialogue **realplex System configuration** can be opened under the menu item **Setup** by selecting **System Configuration**. This lists all users as well as the connected modules on the left. In addition, nodes for system settings and other functions are available. In the navigation tree different levels are indicated by symbols. The subordinate folders become visible if individual nodes are selected with the cursor. To close the nodes, reselect. If levels or subordinate folders are selected, the relevant window is displayed on the right of the dialogue.



3.4.1 User administration

If the node **Users** is selected, the tab sheet **User Administration** is displayed, listing all users. The logged-in Administrator can use this dialogue to set up new users and edit or delete users who are already set up.

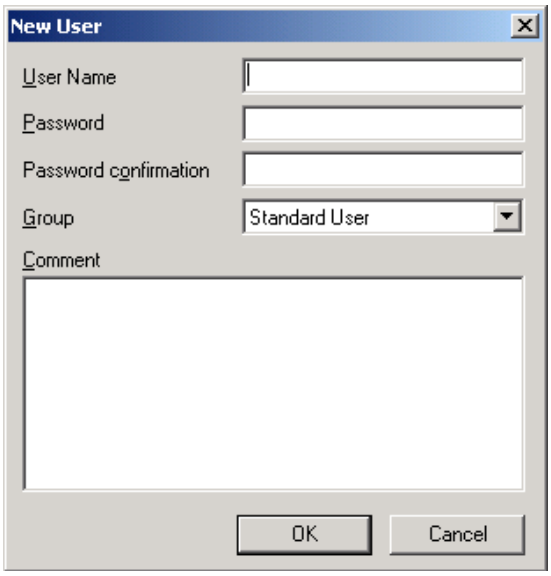


The function keys are only accessible for a logged-in Administrator. If a user is logged-in without Administrator rights, these function keys are then inactive.

3.4.1.1 Setting up new users

New users can only be set up by the Administrator when he is logged in. To do so, the dialogue **realplex System configuration** is opened under the menu item **Setup**.

If the node **Users** is selected, the tab sheet **User Administration** can be seen on the right of the dialogue.
The function key **New** is used to open another dialogue **New User**.



The name and password of the new user is entered in the relevant fields.

The individual access rights of a user are determined via the user group. There are two preset user groups.

Administrator: has access to all program and administration functions.
Standard User: have access to all program functions.

In addition, it is possible to enter a remark in the field Comment. After confirmation the dialogue is closed and the newly setup user displayed in the dialogue **realplex System configuration** under **Users**. Color-coding indicates the group to which a user belongs: administrators have red jackets and standard users green ones.
Fixed access rights are assigned to the two user groups and cannot be changed.

Note: If a user has forgotten his password, the logged-on Administrator can allocate a new password for the user (see section 3.4.1.2). The Administrator is not able to view user passwords.

3.4.1.2 Editing of users

In the dialogue **realplex System configuration** all data for an existing user can be edited by the Administrator. To do so, the user is first selected in the window **User Administration** and the dialogue **Edit User** then opened using the function key **Edit**. After the data have been changed, this is confirmed with **OK**.

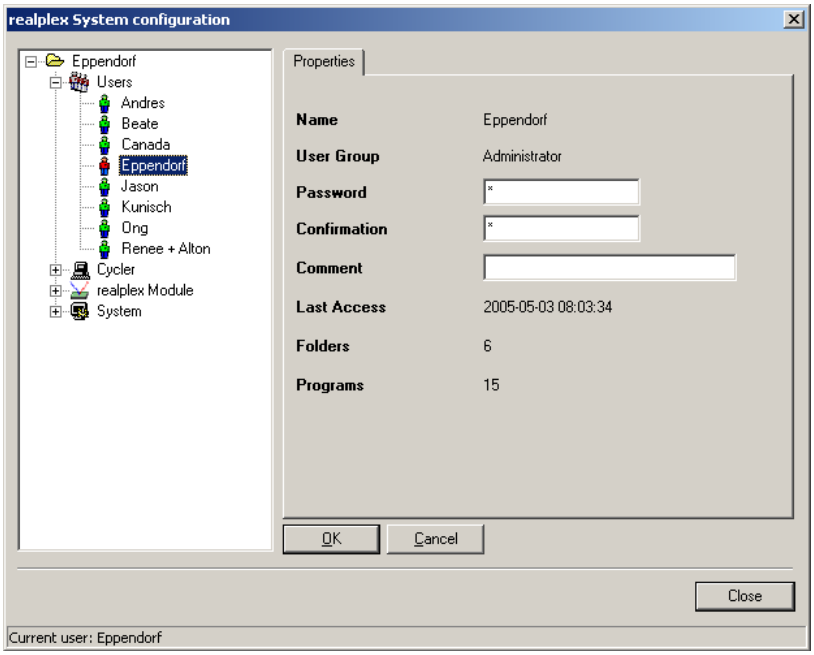
3.4.1.3 Deleting users

Users can only be deleted by the Administrator. To do so, he must log in as described in section 3.1 and open the dialogue **realplex System configuration**. After a user has been selected in the window **User Administration**, he is deleted via the function key **Delete**.

3 Operation

3.4.2 User levels

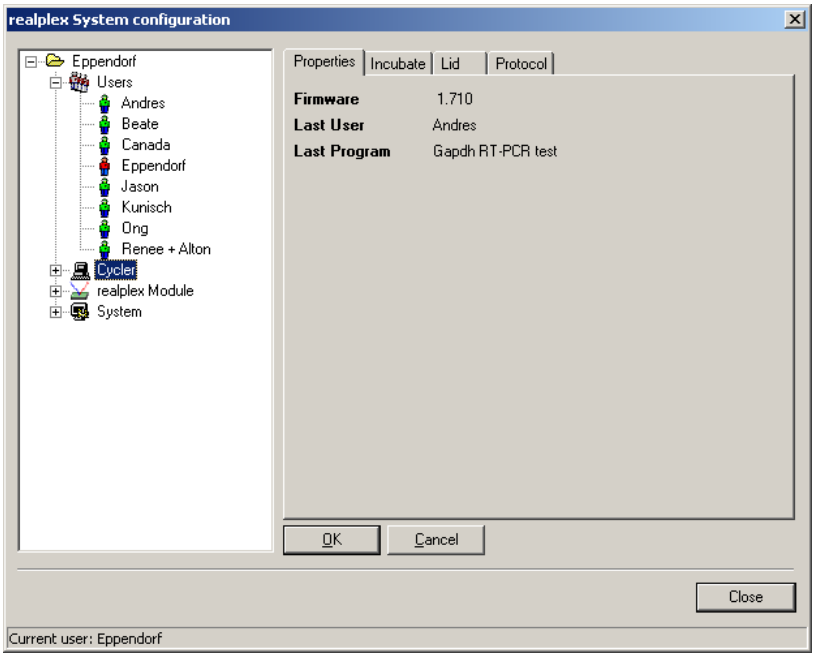
If individual users are selected under **realplex System configuration**, user-specific information is shown on the right side of the dialogue. Besides the user's name and his user group, this tab sheet also includes the number of folders and programs set up by the user. The time of last access is also shown. A logged-in user can change the comment and password here.



To change the password inputs have to be made, firstly in the field **Password** and secondly in the field **Confirmation**. The entries are confirmed with the function key **OK** and the dialogue ended with **Close**. The user's password cannot be viewed by the Administrator.

3.4.3 Cycler

All information available for the connected Cycler can be viewed in the Cycler node.

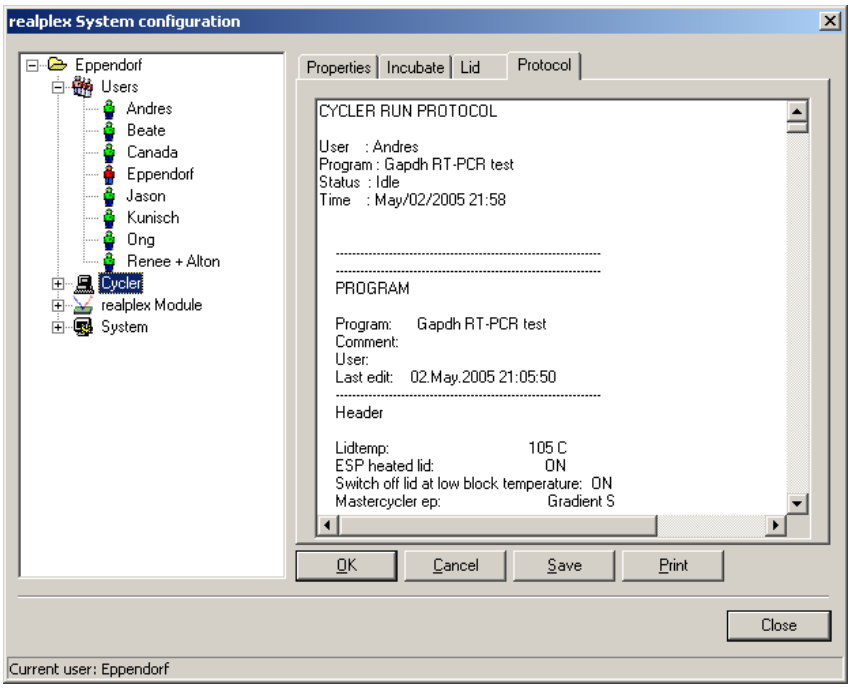


3 Operation

The device characteristics are listed under **Properties**. Besides data relating to the firmware the last user to be logged on is specified here, as well as the last program run.

The tab sheets **Incubate** and **Lid** can be used to bring the thermoblock and the heated lid to an adjustable temperature. To do so, enter the required value in the field **New temperature** and confirm by selecting the check box **Incubate**. To stop tempering, deactivate the checkbox **Incubate**.

The documentation of the PCR programs can be viewed in the tab sheet **Protocol**.



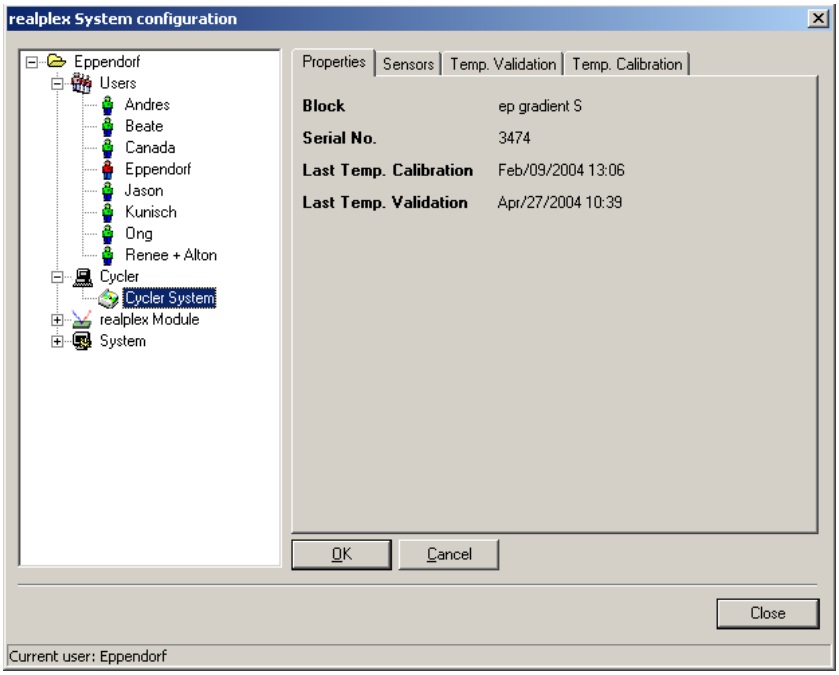
The header contains information about the logged-in user who started the PCR program as well as the name and start time of the PCR program. This is followed by data relating to properties and header settings of the program. In addition, information about the individual program steps is displayed. The protocol can not be deleted.

Save If this function key is selected, the protocol is saved as a TXT file.

Print This function key can be used to print out the protocol to a connected printer.

3 Operation

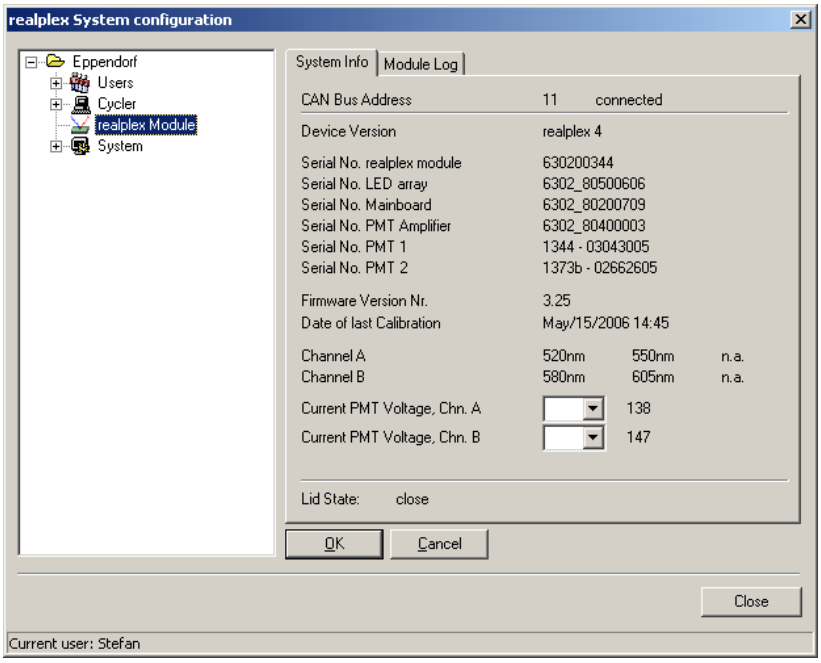
Double-clicking on the Cycler node, the node **System** is displayed with information about the block properties.



Properties:	The tab sheet **Properties** shows the block type and serial number of the thermo module as well as the data for the last validation and calibration of the thermoblock.
Sensors:	Display of current sensor temperatures.
Temp. Validation:	Testing of device temperature using a temperature sensor which is part of the temperature validation system (see Ordering information). The execution is described in the operating manual of the temperature validation system.
Temp. Calibration:	Adjustment of device temperature with the help of a temperature sensor (forms part of temperature validation system). Performance is described in the operating manual of the temperature validation system.

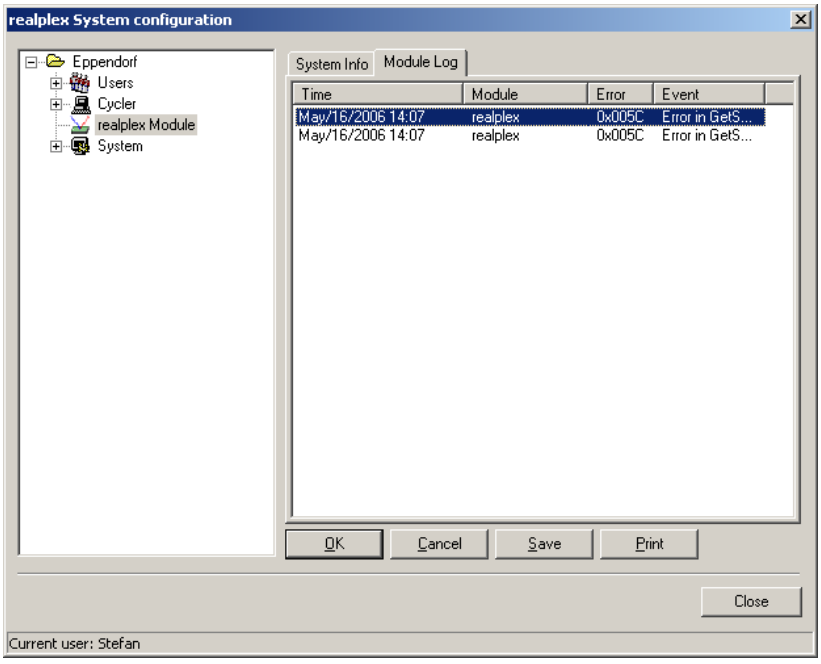
3.4.4 *realplex* Module

System-specific information for the *realplex* module is shown under the node **realplex Module**.



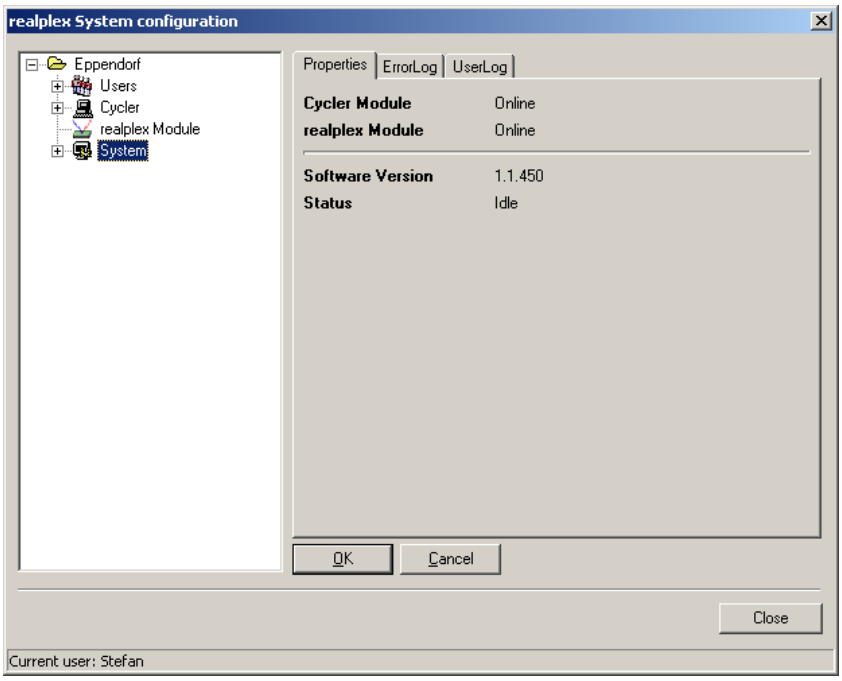
Besides information about the version of the Mastercycler ep *realplex* and the version of the device software, the serial numbers of certain assemblies are shown here. There is also data on the filter equipment and the photomultiplier voltage selected. In addition, **realplex Module** indicates which CAN bus address has to be set at the *realplex* module and whether the connection between the thermo and *realplex* module is via a CAN bus cable. **Cover State** shows the current position of the heated lid.

Double-clicking on the node **realplex Module** displays another node underneath. The directory **Module Log** documents all errors occurring during operation. The table can be saved as a TXT file using the function key **Save** or printed out to a connected printer with **Print**.



3.4.5 System level

The system node provides information about the current software version and includes UserLog and ErrorLog to monitor user activities and any errors.



Alternatively the dialogue **realplex System Info** can also be opened using the icon **Show System Info**.

3.4.5.1 Properties

Here the status displays of the thermo and *realplex* module can be viewed.

3.4.5.2 ErrorLog

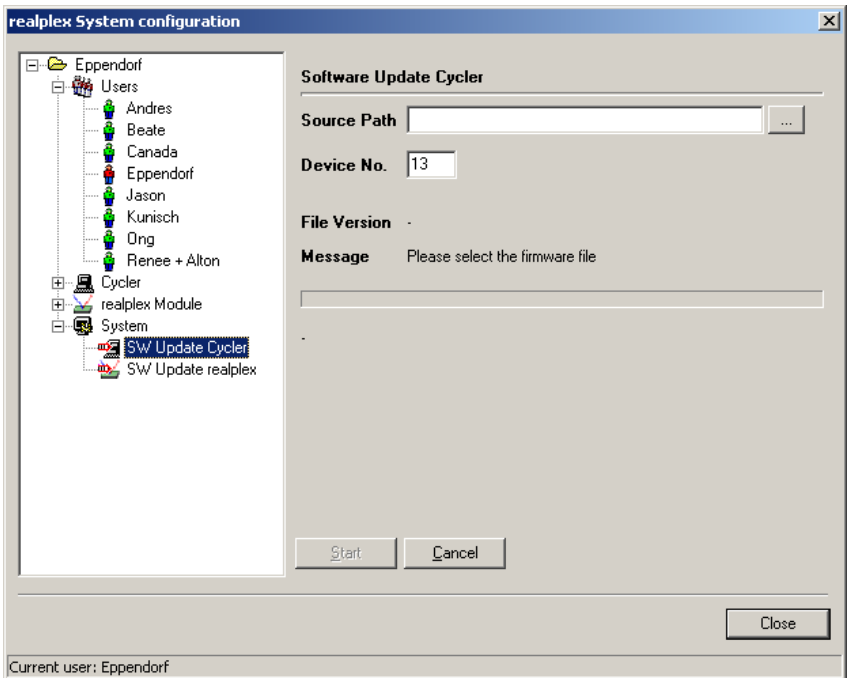
ErrorLog documents all error messages. This data can be saved using the function key **Save** or printed out to a connected printer with **Print**. The ErrorLog can not be deleted.

3.4.5.3 UserLog

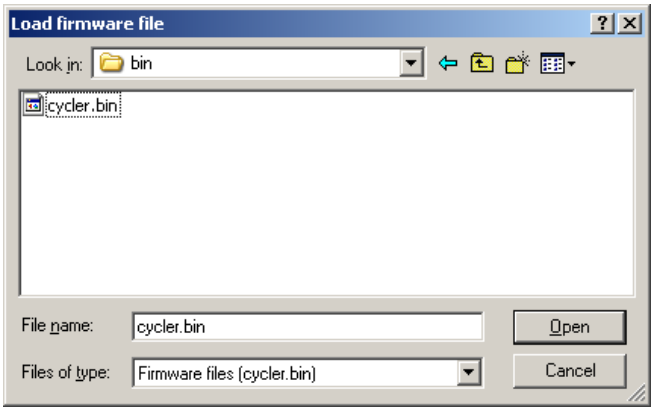
UserLog documents all actions carried out on the Mastercycler ep *realplex*. The system records all Login / Logout procedures and all actions which involve saving data, on a user-related basis. In addition, all program interactions such as start, pause or abort are documented. The UserLog can be saved or printed out to a connected printer. The UserLog can not be deleted. Whereas the tab sheet **UserLog** covers only the last approximately 5000 entries, all existing actions are listed in the **Archive**.

3.4.5.4 Software update

Double-clicking on the system node displays two other nodes. After selection of one of the nodes a software update can be carried out in the right-hand work area.



... If the icon **Browse** has been selected, the dialogue **Load firmware file** is displayed.



After selection of a software version, it is loaded with **Open** in the field **Source Path**. The software update is carried out by selecting the function key **Start**.

Note: Updating the *realplex* module from firmware 2.xx is not possible by the way described here. The **Firmware Update Tool** from the start menu has to be used instead.

3 Operation

3.5 Assay management

The following functions are available in the tool bar to manage assays:

1 New Assay
2 Open Assay
3 Save Assay
4 Print Report
5 Import Assay from File
6 Export Assay to File

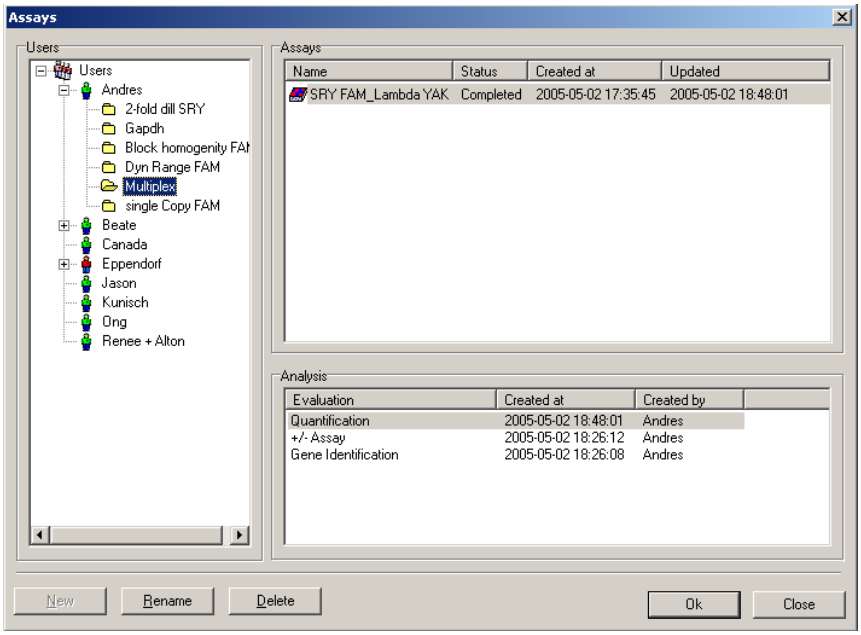
Note: Alternatively, these functions are also accessible via the menu item **File** or the right mouse button when the cursor is positioned in the navigation tree.

3.5.1 Creating assays

If the icon **New Assay** is selected, a blank plate layout and a standard PCR program are loaded in the work area.

3.5.2 Opening of assays

After selection of the icon **Open Assay**, the dialogue **Assays** is opened.



If a user or folder is highlighted, all assays stored there are shown on the right. In addition to the name, the date of creation and updating of the assay are specified in the panel **Assays**. Moreover, the table shows the status of the assay.

The assay marked **Setup** has not yet started. On opening it is loaded to **Assay Setup** as well as to **Analysis**. However, no data are available in the latter.

Assays which can be used as a template for the creation of new assays are marked **Template**.
See section 3.5.4 and 3.5.5 for further information about the creation and usage of templates.

In the case of assays marked **Complete** data acquisition has already taken place. If this assay is opened, the data are displayed under **Analysis** in the navigation tree. In addition, the assay appears in **Assay Setup** unless another assay is currently running (see section 6.1.1.1).
If individual assays are selected, all analyses of this assay saved to date are shown in the lower window **Analysis**. The date of creation and the name of the user who performed analysis are also listed here.

Assays which have been aborted e.g. due to a power outage or system failure are marked **Aborted**. They may also be analyzed if sufficient data were acquired before the assay was aborted.

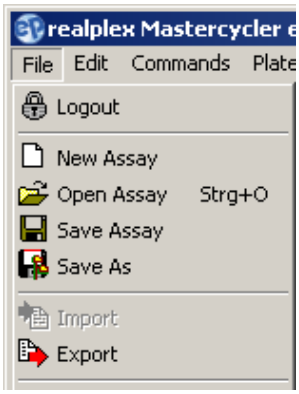
3 Operation

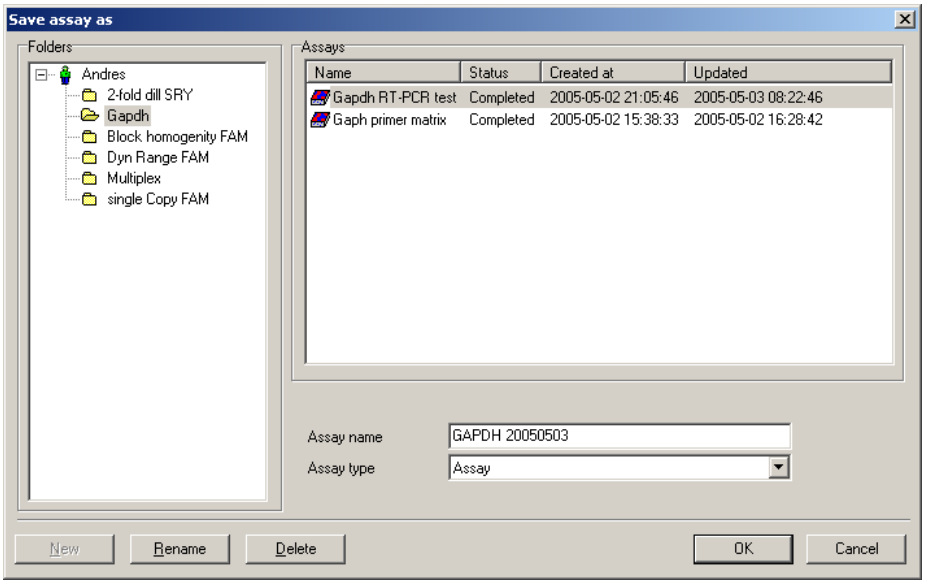
3.5.3 Saving assays

A logged on user can save assays under his node or in one of his folders. The editing functions for these programs are only accessible to the logged-in user. Other users are only able to view and copy these assays.

If the icon **Save Assay** is selected, the current assay is saved.

To save an assay under a name or to another folder, the relevant dialogue can be opened by selecting the function **Save as** under **File** in the menu.





After the inputting of the name, Assay is selected using the check box **Assay type** and confirmed with **OK**.

Note: Only the following characters are allowed for assay names:
alphanumerical characters, umlaut ßäÄöÖüÜ, blank, and .,-_#()=.

3.5.4 Saving templates

If an assay should be used more than once after creation, it is saved as a template. To do so, proceed as under **Saving assays**, selecting Template in the combo box **Assay type**.

3.5.5 Working with templates

Templates can be used for the simple creation of new assays. To do so, the template is loaded in **Assay Setup** using the function **Open Assay** and then saved under a new name and Assay as Assay type in the user's node.

3.5.6 Copying assays

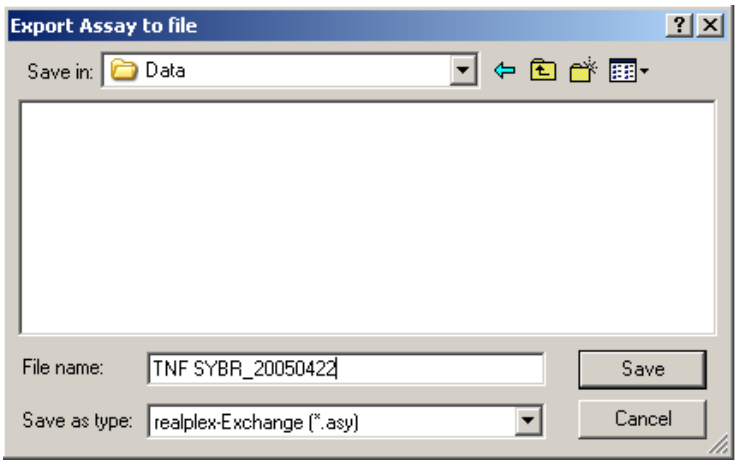
A user can copy assays and templates of other users to his own directory in order to start or edit them in his own folders. To do so, **Open Assay** is used with the dialogue **Assays** to select and open the relevant assay in the folder of another user. The user then saves the assay under his own user node via **Save as** (see chapter 3.5.3).

The copied assay can now be processed or started directly on a connected Mastercycler ep *realplex*.

3.5.7 Exporting of assays

The export and import functions are used to exchange assays between several workstations. Here the assays are exported or imported with the plate layout, PCR program and measured data.

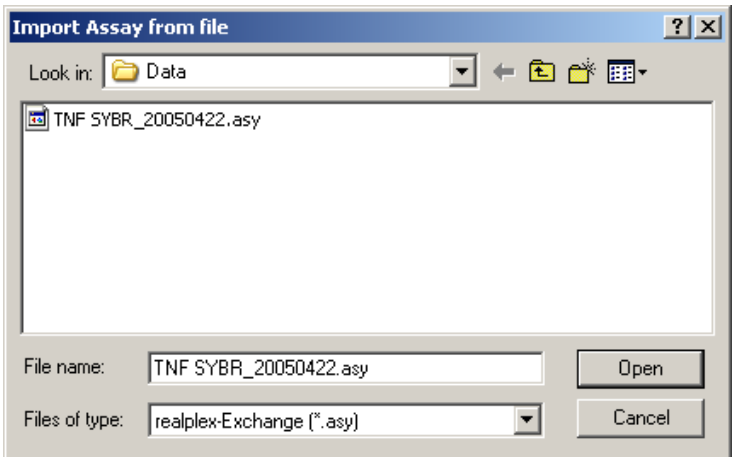
The icon **Export assay to file** can be used to save the current assay in ASY format to a computer at another workstation for further processing.



To save an assay the upper combo box is used to select a storage location, followed by entering the file name and selection of **realplex-Exchange (*.asy)** in the combo box **File type**.

3.5.8 Importing of assays

If the icon **Import assay from file** is selected, an assay in ASY format can be loaded to a blank Assay Setup. Therefore, a new assay has to be opened first.



To import an assay, the relevant storage location must be selected in the upper combo box followed by the format **realplex-Exchange (*.asy)** as the File type.

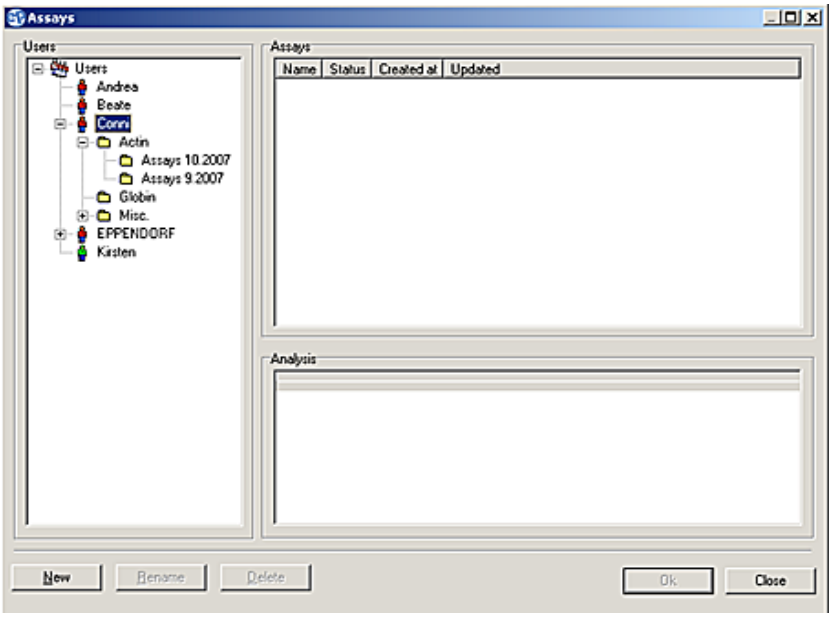
3.5.9 Creating folders

A logged-in user can create folders under his name (node) and file associated assays there so he can find them more easily later on. To create a new folder proceed as follows:

Open the dialogue **Assays** using the icon **Open Assay**.

New After selecting his own user node, the user adds a new folder with the function key **New**. After selecting a folder, subfolders can be created in the same way.

Rename The created folders can be renamed by highlighting and then selecting **Rename**. Also assays can be renamed by this function.



Folder and subfolders are arranged alphabetically.
Assays can now be copied or created in the generated folders (see section 3.5.4 and 3.5.2).

3.5.10 Deletion of assays and folders

Open the dialogue **Assays** using the icon **Open Assay.** The user can delete a selected assay from his own user node using the function key **Delete** and confirming with **OK**. The same procedure is used for folders.

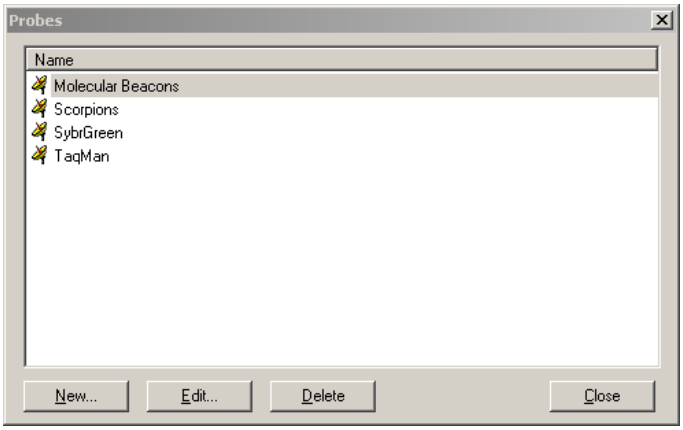
Delete After an assay or folder is selected, it can be deleted using the function key **Delete** and confirming with **Yes**.

3 Operation

3.6 Probe administration

Probe administration allows a probe type to be selected later on in the plate layout of an assay. This is solely for the purpose of documentation and has no influence on the PCR program. The measuring point necessary for data acquisition is determined on creation of the PCR program (see section 4.3.6).

If the icon **Probes** is selected, the relevant dialogue will open.



In this dialogue it is possible to manage the probes used which are available to every user under **Plate Layout**.

New

The name of the probe type is entered in the dialogue displayed.
Confirm with **OK**.

Edit

The name of an existing probe can be amended in the dialogue displayed. Confirm with **OK**.

Delete

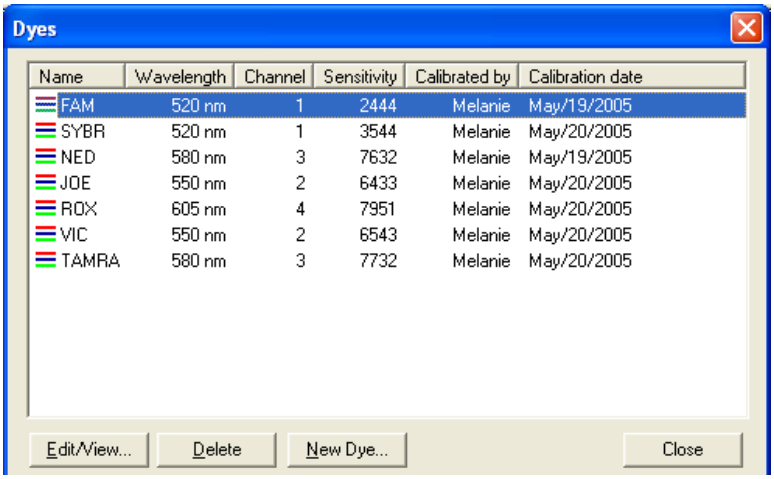
Confirm the deletion of a probe type using **Yes**.

3.7 Dye administration

If the icon **Dyes** is selected, the relevant dialogue is opened.

This dialogue is used for the management of the dyes which have to be specified for fluorescence measurement. Here it is the emission maximum of the respective dye that is important as the user has to select the appropriate filter (e.g. FAM and filter 520 nm (510 nm – 530 nm)). Please consult the documentation of the respective manufacturer for the emission maximum of the dye used.

Note: Before performing color calibration (see section 3.8.2) the relevant dyes have to be defined.



Name — Name of dye (max. 12 characters).

Wavelength — Wavelength of emission maximum of dye in nm.

Channel — Channel for detection of the dye. This is automatically preset by the software based on the wavelength specified.

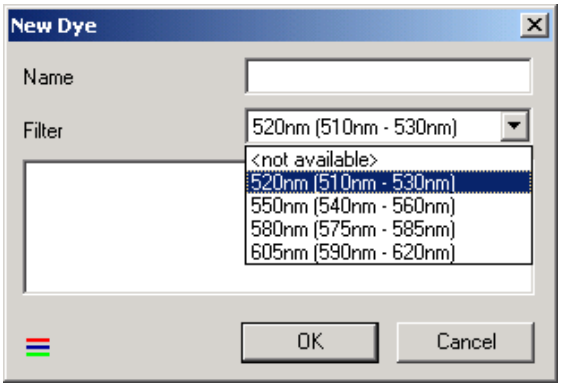
Sensitivity — Sensitivity level of the photo multiplier specified through dye calibration. This consists of a four-digit number code, with a number being specified for each of the four filters (1 = low and 9 = high sensitivity level). This number code has its seeds in the color calibration and is preset by the software. The sequence of numbers corresponds to the emission filters 520, 550, 580, and 605 nm (from left to right).

Calibrated by — User who performed the last dye calibration.

Calibration date — Date of last dye calibration.

3 Operation

New Dye In the dialogue displayed all data for a new dye can be entered.



This dialogue is used to enter the name and the filter in which the new dye can be detected. The filter must match the emission wavelength of the new dye.

Edit/View... In the dialogue displayed the comment for an existing dye can be amended. Confirm with **OK**.

Delete Confirm the deletion of a dye using **Yes**.

Attention: If the filter of the dye is changed, the calibration data of this dye will be lost.

3.8 Calibration

For fluorescence measurement with the Mastercycler ep *realplex* it is necessary to perform a background and a color calibration. Calibration is carried out with the same temperatures as for subsequent measurement.

Note: To ensure that there is no liquid on the sealing cover and no air bubbles in the liquid, the calibration plates have to be centrifuged prior to calibration. Make sure that no liquid has evaporated; otherwise use a new calibration plate. Every well of a PCR plate has to be filled with the same volume of liquid. If PCR tubes are used, every position of the thermoblock has to be loaded. Make sure that the instrument has been warmed up for at least 15 minutes before starting a calibration.

Attention: The software automatically determines the necessary sensitivity of the photomultiplier for every dye and filter. No other inputs are required from the user.

Background calibration must always be performed before color calibration!

3.8.1 Background calibration

If the menu item **Setup** is selected, the dialogue **Background calibration** can be opened, listing all background calibrations stored. This list also includes the date of calibration and the user who performed calibration.

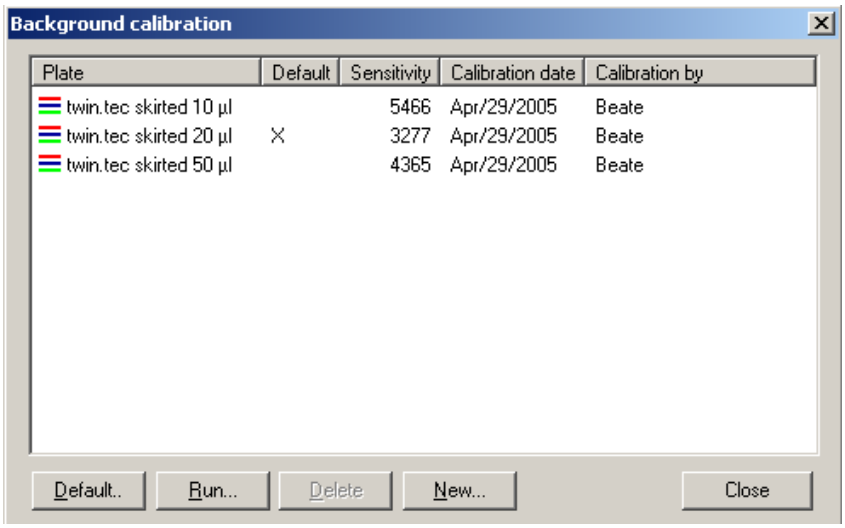


Plate — Name of plate used (max. 32 characters).

Default — The background calibration, which is preset as the default setting is marked with a cross.

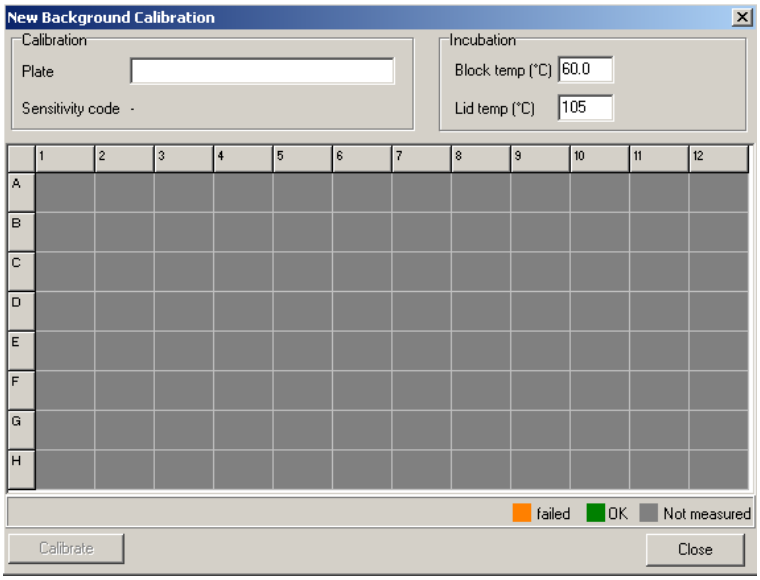
Sensitivity — Sensitivity level of the photomultiplier specified through dye calibration. This consists of a four-digit number code, with a number being specified for each of the four filters (1 = low and 9 = high sensitivity level). This number code is preset by the software.

Calibrated at — Date on which background calibration was performed.

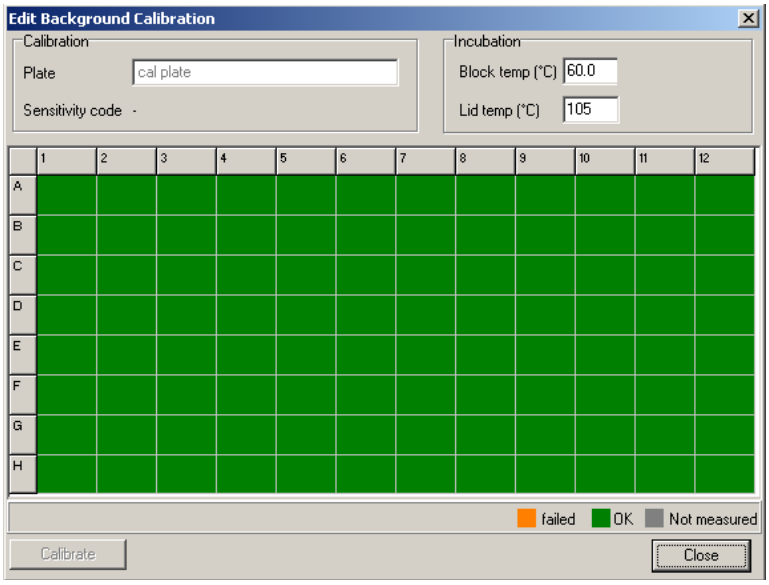
Calibrated by — User who performed background calibration.

New — A new background calibration process is performed in the dialogue displayed.

3 Operation



- After the dialogue **New** is opened, a name is entered for the background plate.
- The block and lid temperature are entered in the field **Incubation** in the dialogue on the right. The default settings are 60 °C for the block and 105 °C for the lid temperature.
- After insertion of the background plate the calibration is performed by the function key **Calibrate**. Confirm with **OK**.
- After heating and the first reading the software will prompt to turn the plate. Follow the software prompt to turn the plate and confirm with **OK**.
- If the background calibration is successfully performed, the plate layout shows 96 green positions of the background plate. The prompt **Calibration successfully finished** can be closed by confirming with **OK**.



If the background calibration failed in some positions, the calibration data can be discarded by selecting Cancel. In this case the former calibration data for this consumable will be used. By selecting Proceed, the background calibration data will be excepted anyhow.

View/Run... In the dialogue displayed all temperature data for an existing background calibration can be changed, and the calibration process then repeated. The data of the previous background calibration will be overwritten.

Default If the function **default** is selected, this calibration will be the default setting for all assays. This is shown with a cross in the list of background calibrations.

Delete Confirm deletion of a background calibration using **Yes**.

One reason for a failed background calibration could be a fluorescence contamination in some wells of the thermoblock. For cleaning of the thermoblock see 7.2.

3.8.2 Color calibration

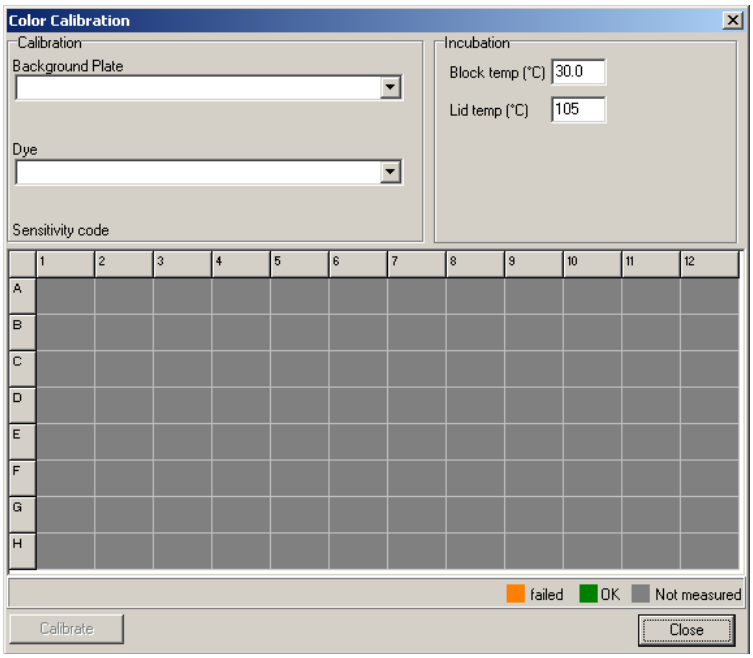
During color calibration the different sensitivity levels of the 96 light paths are adjusted.

In addition, color calibration is the prerequisite for the performance of multiplex assays, which are based on the detection of more than one dye. The emission spectrum of these fluorescence dyes is generally so wide that the light emitted can be detected in more than one filter. Color calibration results in calibration data which the software can use to calculate this crosstalk when performing multiplex assays.

Note: For color calibration it is generally possible to use all tubes and sealing covers which are suitable for real-time PCR. However, it should be remembered that background calibration which is used during color calibration must also be performed with the same consumables.

3.8.2.1 Color Calibration using commercially available calibration kits

Note: Only calibration kits in 96-well format are suitable for color calibration of the Mastercycler ep *realplex*.
These kits must include one plate per dye (e. g. ABI Prism 7000 SDS 96-well Spectral Calibration Kit).
If a commercially available calibration kit is used for color calibration, background calibration must be carried out using the background plate included in the kit.
Selection of the dyes required for calibration depends on the filters contained in the Mastercycler ep *realplex*.



- Open the dialogue **Color Calibration** in the menu under **Setup**.
- After the dialogue **Color Calibration** has been opened, the combo box is used to select the background plate included in the kit. The name of the dye is also selected here.
- The block and lid temperature are entered in the field **Incubation** in the dialogue on the right.
  The default settings are 60 °C for the block and 105 °C for the lid temperature.
- After insertion of the color calibration plate the calibration is performed by the function key **Calibrate**.
  Confirm with **OK**.
- After heating and the first reading the software will prompt to turn the plate. Turn the plate and confirm with **OK**.
- If the color calibration is successfully performed, the plate layout shows 96 green positions of the color calibration plate. The prompt **Calibration successfully finished** can be closed by confirming with **OK**.

If the color calibration failed in some positions, the calibration data can be discarded by selecting **Cancel**. In this case the former calibration data for this dye will be used. Selecting **Proceed** the color calibration data will be excepted anyhow.

This procedure is repeated for every dye.

After calibration, a sensitivity code is displayed in the panel **Calibration**. This code consists of a numerical combination, with every digit specifying the sensitivity level of the photomultiplier used to measure the respective dye in the relevant filter. The sensitivity levels for the dyes of the calibration kit are automatically preset by the software.

3.8.2.2 Calibration of customer-specific dyes

For each dye a concentration has to be prepared according to the recommended concentrations shown in the table below. For each concentration a volume of 50 µl has to be pipetted in each well of a 96-well plate. After sealing the plate a color calibration has to be performed for each dye concentration (see chapter 3.8.2.1).

Note: Use the same consumables during background and color calibration. A background calibration has to be done first.

Main filter (nm), which is used during calibration of customer-specific dye	Recommended dye concentration during color calibration in nM
520	200
550	300 – 500
580	1000 – 2000
605	2000

Note: The emission maximum of the dye must be within the main filter.

The sensitivity code should preferably not contain 1 in one of the positions. The code for the main filter should be between 2 and 8. If the sensitivity code contains 9 in the main filter, it is highly recommended to repeat the color calibration with a higher dye concentration.

3.9 Database Tool

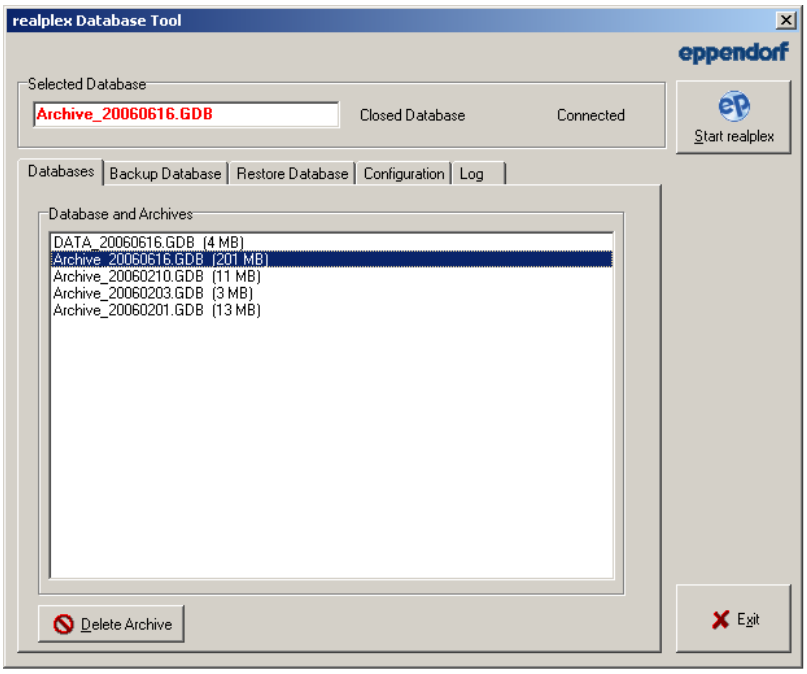
All data, analyses, settings and user structures are automatically stored in a database. It is recommended to create backups of the database at regular intervals.

The software **Database Tool** can be used for the management of databases.

Note: Database and instrument are correlated by the serial number of the *realplex* module. If a database is connected to an improper instrument, the instrument has to be calibrated first.

Double-clicking on the icon **Database Tool** displays the dialogue **realplex Database Administration**.

The upper panel **Selected Database** shows the current database and its status.



The tab sheet **Databases** shows a list of all databases and archives which are available. In parentheses, the size of the database is shown. There are two different types of databases:

– The actually used database in which all data are currently saved. The name of the database is composed of the term **DATA** and the date when this database was started. In the panel **Selected Database** this database is marked in green and as open.

– The archived databases have a read-only status and include previous data. They are named with **Archive** and the date of creation. In the panel **Selected Database** this database is marked in red and as closed. Archives can be deleted by the function key **Delete Archive**.

If one of the listed databases is selected it will be shown in the panel Selected Databases and will be connected to the *realplex* software.

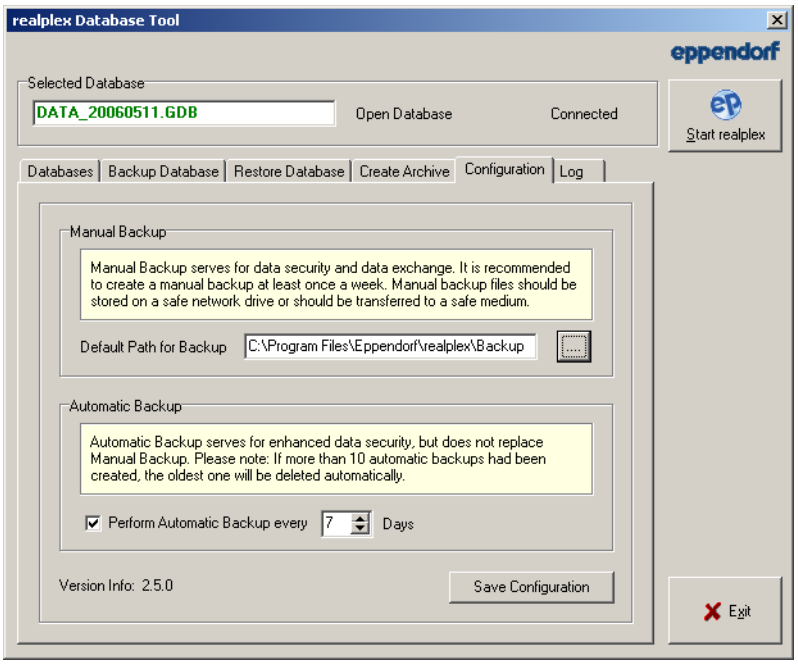
If the function key **Start realplex** is selected the *realplex* software will be opened directly.

3 Operation

3.9.1 Automatically saving of current database

After opening the *realplex* software a backup is saved automatically on a regular basis to the directory C:\Program Files\Eppendorf\realplex\Security. The settings for the automatic backup can be accessed in the tab sheet **Configuration**.

Note: Only users with administrator rights have access to the configurations (see chapter 3.9.4).

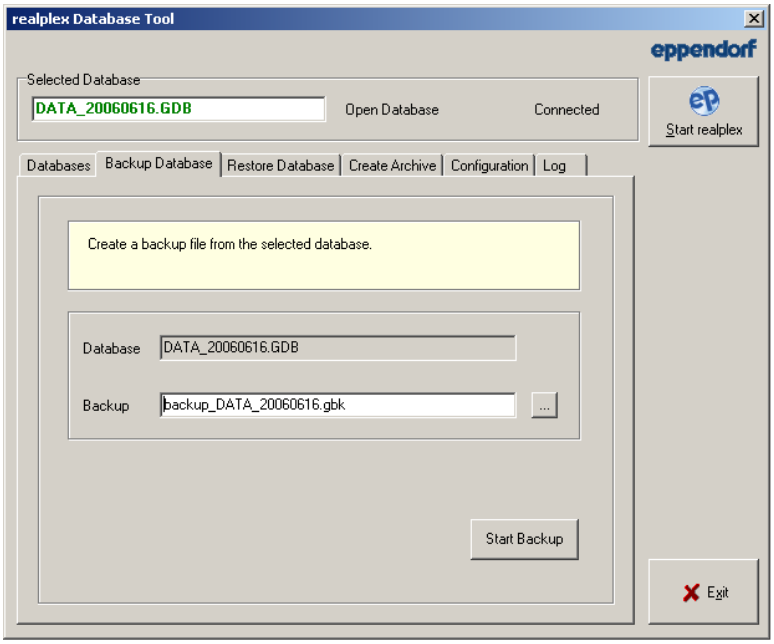


Note: If more than 10 automatic backups have been created, the oldest one will be deleted automatically. Therefore, creation of customer specific backups on a regular basis is recommended. These manual backups should be stored on an external disc or server.

3.9.2 Saving of current database

Saved copies can be made of the current database using the tab sheet **Backup Database**. This backup contains all data and settings including assays, templates, calibration data and user structure.

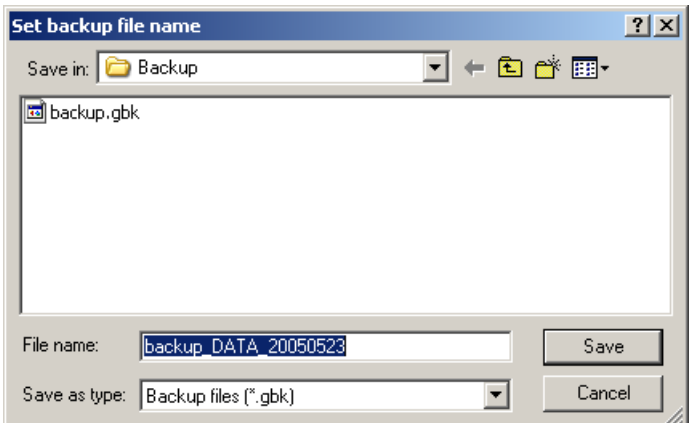
Note: It is strongly recommended to save the current database before installing a new *realplex* software version!



The panel shows the database of which a backup is to be made and which is selected in the sheet **Databases**.
In general, it is possible to create backups of databases and archives.

The name of the backup is created automatically by the software. Depending on the source the name is composed of backup_DATA or backup_ARCHIVE and the date of creation.

If the icon **Browse** is selected, the dialogue **Set backup file name** is displayed.



The current database is given a name and loaded to the panel **Backup** in GBK format using the function key **Save**.

Note: For faultless recognition of the database by the *realplex* software it is necessary to precede each name with backup_DATA or backup_ARCHIVE depending on the source!

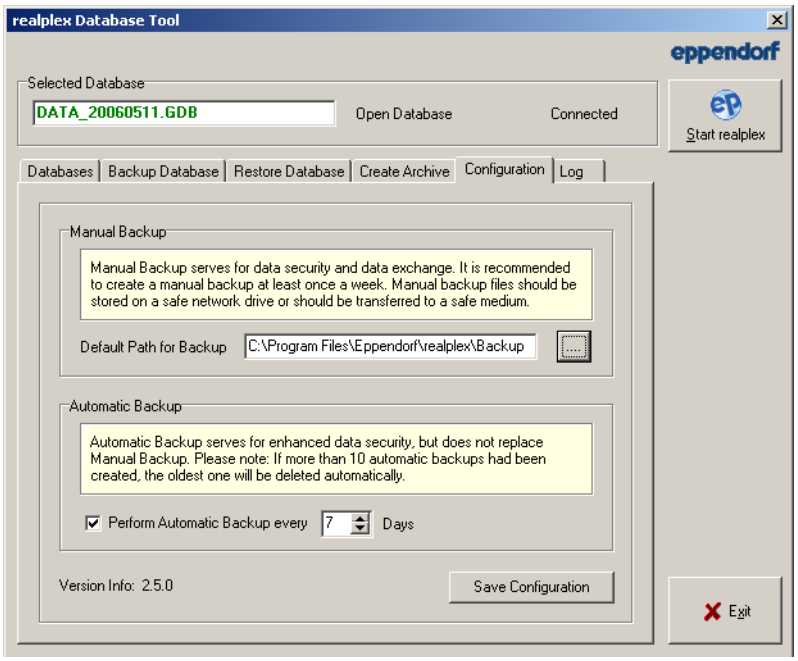
3 Operation

Start Backup

If the function key **Start Backup** is selected, a backup is created in the directory C:\Program Files\Eppendorf\realplex\Backup.
The process is documented in the tab sheet **Log**.

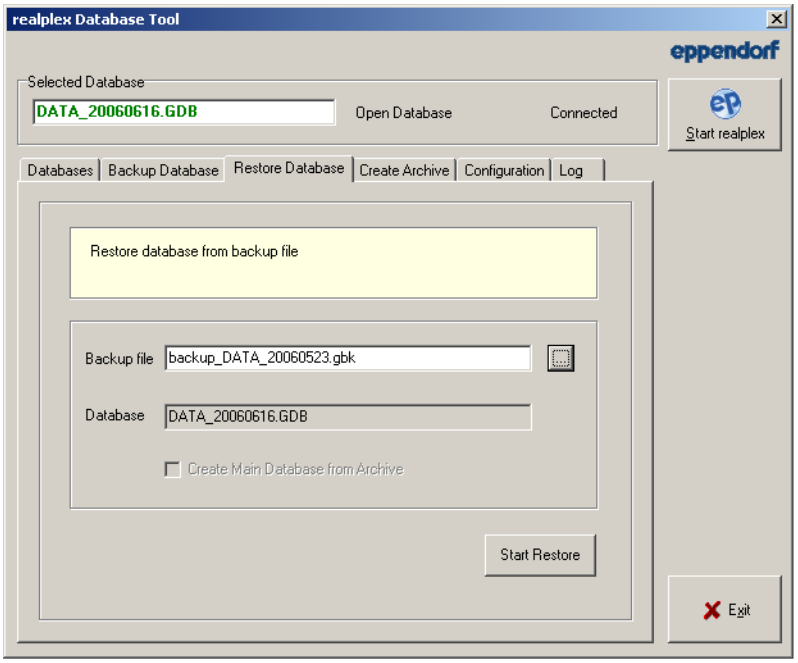
The backup is saved automatically to the directory C:\Program Files\Eppendorf\realplex\Backup. The path can be changed in the tab sheet **Configuration**. If the computer is part of a network it is recommended to select a directory on a server.

Note: Only users with administrator rights have access to the configurations (see chapter 3.9.4).



3.9.3 Importing a database

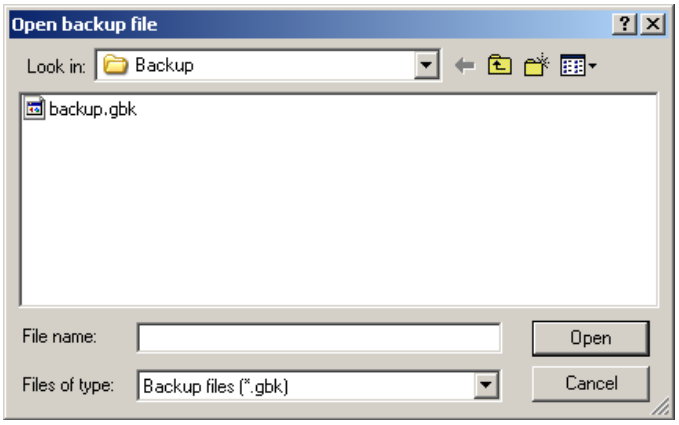
To use a backup of an older database as the active database in the *realplex* software, the current database must be replaced by a backup. To do so, first select the backup in the tab sheet **Restore Database**.



3 Operation

...

If the icon **Browse** is selected, the dialogue **Open backup file** is displayed.



A database in GBK format, is loaded to the tab sheet **Restore Database** using the function key **Open**.

Start Restore

If this function key **Start Restore** is selected, the database is loaded from the directory C:\Program Files\realplex\Backup.
The process is documented in the tab sheet **Log**.

Attention: All data including assays, settings, calibration data and user structure are overwritten by this database! It is recommended to create a backup of the currently used database before!
For using a restored database as actually used main database make sure that all calibration data are still valid.

Note: It is also possible to make a restore of the database with a backup of an archive. After this backup has been loaded to the tab sheet **Restore Database** the check box **Create Main Database from Archive** can be activated and the restore can be performed. It is recommended to create a backup of the currently used database before!

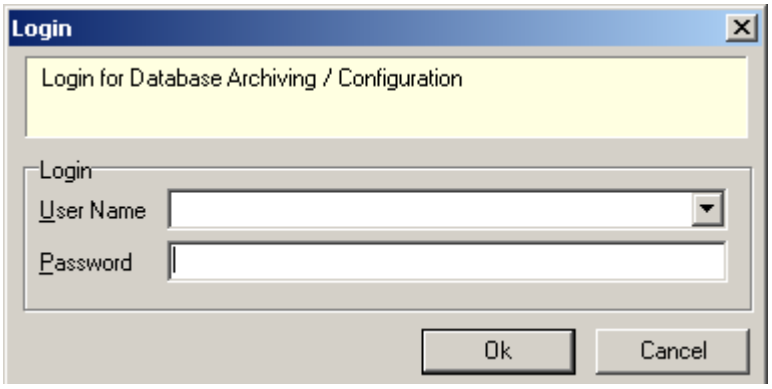
All users with Administrator rights have access rights to database management. After selection of the user name in the combo box, the password is then entered and confirmed with **OK** (see section 3.9.4). The password is the same as for the *realplex* software.

3 Operation

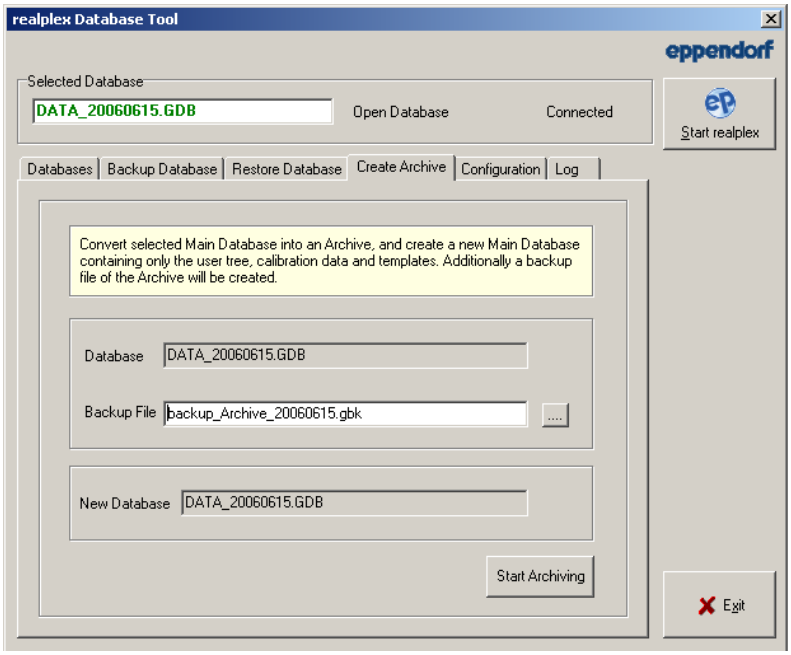
3.9.4 Archiving a database

If the database exceeds approximately 250 MB the user will be asked to archive the database after opening the *realplex* software. After archiving the user can continue with a new database including all settings concerning templates, calibration data and user structure.

All users with Administrator rights have access rights for this part of the database management. After selection of the user name in the combo box, the password is then entered and confirmed with **OK**. The password is the same as for the *realplex* software.



After login the tab sheet **Create Archive** is accessible.



The panel shows the database of which an archive is to be made and which is selected in the sheet **Databases**.

The name of the archive is created automatically by the software and it is composed of backup_ARCHIVE and the date of creation.

The backup of the archive is saved automatically to the directory C:\Program Files\Eppendorf\realplex\Backup. The path can be changed in the tab sheet **Configuration**.

In the panel **New Database** the name of the new active database is displayed. The name is created automatically by the software and has the date of creation.

Start Archiving If the function key **Start Archiving** is selected, an archive is created in the directory C:\Program Files\Eppendorf\realplex\Backup.
The process is documented in the tab sheet **Log**.

4 Programming (Assay Setup)

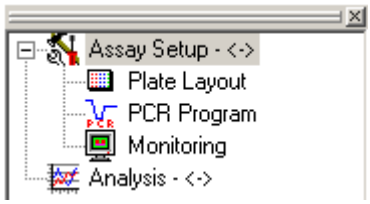
The software of the Mastercycler ep *realplex* basically consists of two modules **Assay Setup** and **Analysis**. The module **Assay Setup** is divided into the sub-items **Plate Layout**, **PCR Program** and **Monitoring**. The first two sub-items are used to edit assays, while under **Monitoring** an ongoing assay can be monitored in real-time. The module **Analysis** is described in detail in section 6.

Note: Parallel operation of both modules with two different assays is possible. While one assay is running, the analysis of a completed assay can be performed (section 6).

4.1 Creating a new assay

A new assay can be created under the menu item **File** with **New Assay**. This is first displayed without a name in the window on the left of the screen in the navigation tree with the subordinate items **Plate Layout**, **PCR Program** and **Monitoring**.

They are displayed in the work area on the right if individual sub-items are selected.
Alternatively, the different modules can also be loaded to the work area using the functions of the menu item **Commands**.



A blank plate layout is available in the work area as the default setting after opening of the software.

4.2 Creation of a plate layout

To create a plate layout **Plate Layout** is selected in the navigation tree on the left. On the right the 96 positions of a PCR plate can be seen. To edit this, the dyes to be detected have to be specified first.
To aid the user, instructions on further procedure are shown in the field **Hints** on the bottom right next to the work area.

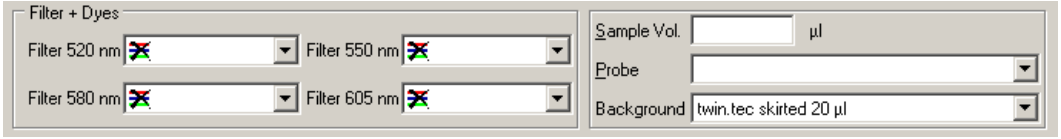
To create plate layouts, the following functions are available in the toolbar:

1 Unknown
2 Standard
3 Positive Control
4 Negative Control
5 Create Links
6 Clear Plate
7 Delete Samples
8 Group as Replicates
9 Ungroup Replicates (inactive)
10 Create Subassay
11 Delete Subassay (inactive)
12 Edit mode for Plate Layout (inactive)
13 Color for Samples

Note: Alternatively, these functions can also be accessed via the menu item **Plate Layout** or the right mouse button.

4.2.1 Determination of dye

Here the dye is selected in the panel **Filter + Dyes** with the relevant filter using the combo box. If the required dye is not included in the selection, it must be first added to the list **Dyes** under the menu item **Setup** (see 3.6). Calibration of the dye must be performed (see 3.8.2).



If the *realplex* software is connected to a Mastercycler ep *realplex*^{2} filter 580 nm and filter 605 nm are not shown in the panel.

Note: The Mastercycler ep *realplex* does not need an internal reference dye to give accurate results.

4 Programming (Assay Setup)

4.2.2 Input of sample volume

In the top right panel of the work area the volume of the samples can be entered under **Sample Vol.** Although this input is not necessary, it is recommended for full documentation of the assay.

4.2.3 Determination of probe type

In the panel on the top right in the Plate Layout the probe type can be selected using a combo box. If the required probe type is not included in the selection, it must first be added in the list **Probes** under **Setup** (see 3.7).

Note: Definition of the probe type is not absolutely necessary as it has no influence on the measuring point in the PCR program. This input is however recommended for full documentation of the assay.

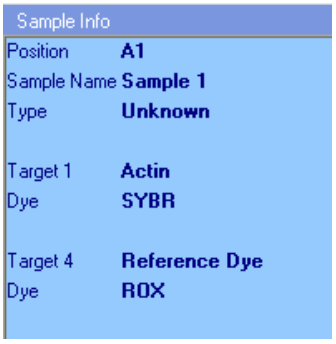
4.2.4 Determination of background

In the panel on the top right in Plate Layout the relevant plate type for the background is selected using a combo box. This has to be the plate type in which the assay is also to be performed. If the required plate type is not included in the selection, background calibration first has to be performed with this consumable (see 3.8.1).

4.2.5 Definition of sample type

The relevant dialogues are opened for editing by highlighting one or more positions in Plate Layout and subsequent selection of an icon in the toolbar for the different sample types. All functions used to edit the plate layout are also accessible via the right mouse button or the menu item **Plate Layout**.

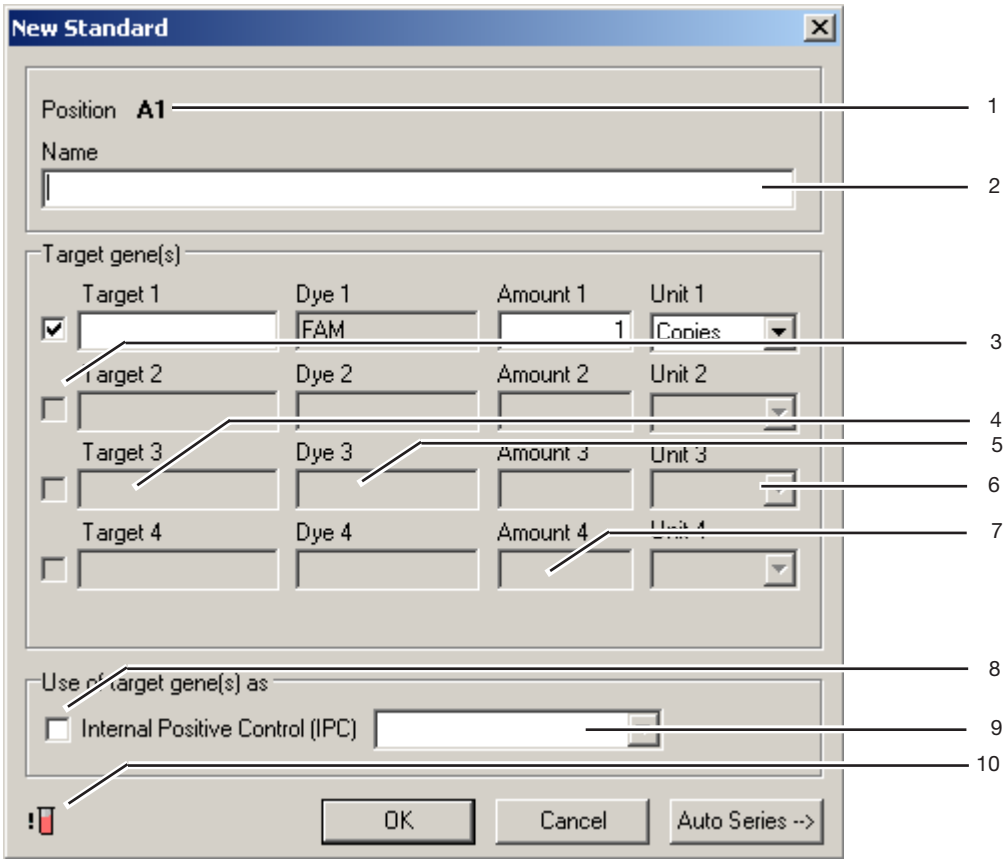
After the inputs have been made, the samples appear in the left-hand window with data relating to the type, name of the sample and position in the table **Sample**. Data for a sample selected in Plate Layout are listed in the right-hand window **Sample Info**.



4 Programming (Assay Setup)

4.2.5.1 Standards

If the icon **Standard** is selected, the relevant dialogue is opened.



1 Position of sample in the plate layout
2 Name of sample
3 Check box for selection of target gene
4 Name of target gene
5 Dye used to detect target gene
6 Unit for standard concentration
7 Concentration of standard
8 Check box for selection of an internal positive control (IPC)
9 Combo box for selection of dye for IPC
10 Symbol for sample type

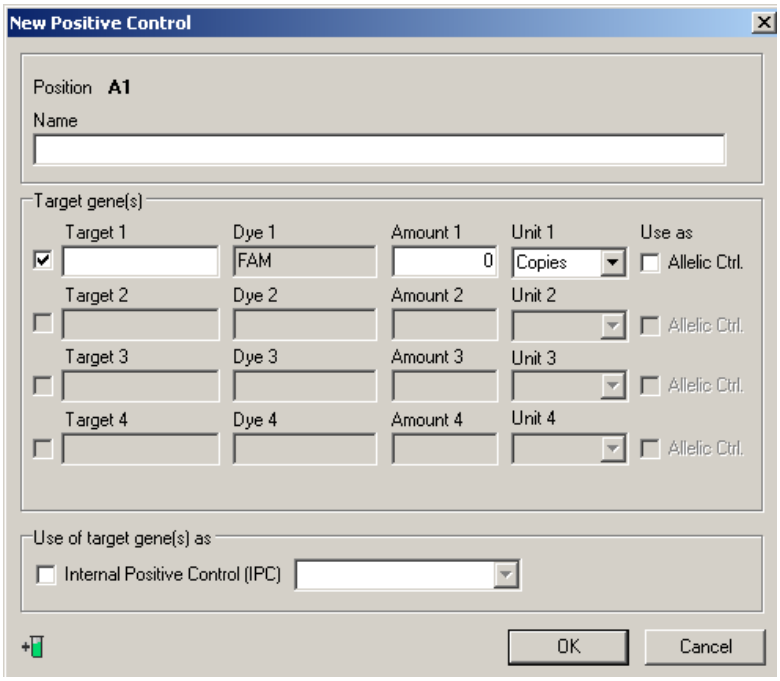
Here data are entered for the name of the standard and the target gene to be examined. It is also necessary to specify the amount or concentration of the standard in the relevant fields. Exponential values are entered for example as 1E 8. The unit **copies** and amount = 1 are preset as the default setting. Standard series can be created with the function key **Auto Series** (see 4.2.6).

Note: The number of target genes which can be edited in this dialogue depends on the number of dyes selected in Plate Layout.

4 Programming (Assay Setup)

4.2.5.2 Positive Control

If the icon **Positive Control** is selected, the relevant dialogue is opened.



Besides data for the name of the control and the target gene(s), it is possible here to define the positive controls as alleles for the analysis module **Gene identification**. To do so, it is necessary to edit a positive control as allele (see 4.2.12). Each allele is automatically assigned a color in this dialogue for subsequent evaluation under **Analysis**.

It is also possible to enter values in the field **Amount**. However this is not absolutely necessary for subsequent analysis.

4.2.5.3 Negative control

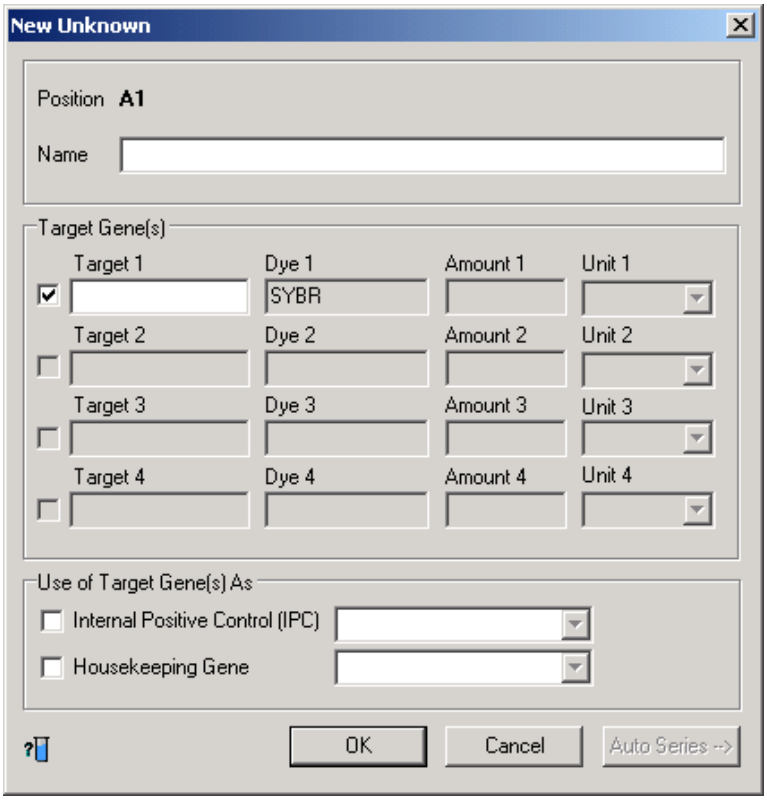
If the icon **Negative Control** is selected, the relevant dialogue is opened.

Only data for the name of the negative control and the target gene(s) are entered here.

4 Programming (Assay Setup)

4.2.5.4 Unknown sample

If the icon **Unknown** is selected, the relevant dialogue is opened.



Besides data for the name of the unknown sample and target gene(s), replicates can also be edited here using the function key **Auto Series** (see 4.2.6).

At the bottom this dialogue includes the panels **Use of target gene(s) as** and **Relative quantification / gene expression**:

Use of target gene(s) as: It is also possible to set target genes either as an internal positive control or as a housekeeping gene by selecting the relevant function and then specifying the respective dye using a combo box. The target gene for which an internal positive control or the housekeeping gene has been determined for analysis is then highlighted in yellow. You can only use one dye for every assay / subassay.

4 Programming (Assay Setup)

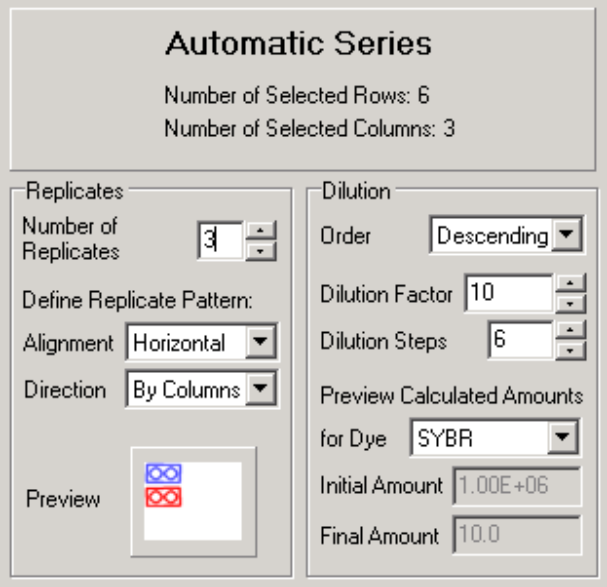
4.2.6 Automatic creation of replicates and standard series

With the sample types **Standard** and **Unknown** it is possible to create replicates automatically. When editing standards it is also possible to create standard series automatically. To do so, select the area (at least two positions) in the plate layout in which standard series and/or replicates are to be created.

Auto Series -->

To simplify the creation of standard series and replicates using the dialogue **New Standard**, it has been extended to include the option **Auto Series**.

Note: This function is only activated when more than one position is selected in the plate layout. The positions have to be vacant and contiguous.



The extended dialogue indicates how many positions in the plate layout have been selected and is divided into the areas **Replicates** and **Dilution**.

Replicates: Here it is possible to enter the number of replicates in the spin edit. In addition, this item can be used to define the pattern forming the basis for arrangement of the replicates on the plate.
The related replicates can be arranged next to each other either in horizontal or vertical alignment.
The direction in which different replicates follow each other can be in rows or columns.
The pattern is displayed in the window **Preview** for the purpose of clarification.

Dilution: The spin edits are used to specify the dilution factor and the number of dilution steps. With the combo box **Order** it can also be specified whether the dilution series is to be ascending or descending.
In the field **Preview calculated amounts** a dye can be selected via a combo box and the final concentration displayed according to the initial concentration defined for the target gene.

Note: This area is inactive in the extended dialogue **Unknowns**.

After confirmation of the dialogue with **OK** the same number of groups are created in the selected area as the number of parameters. When creating replicates with unknown samples the entire area selected is filled.

Sample names and all other associated data are allocated automatically. With standard series the concentration data for the individual standards are also displayed automatically in the plate layout.

4 Programming (Assay Setup)

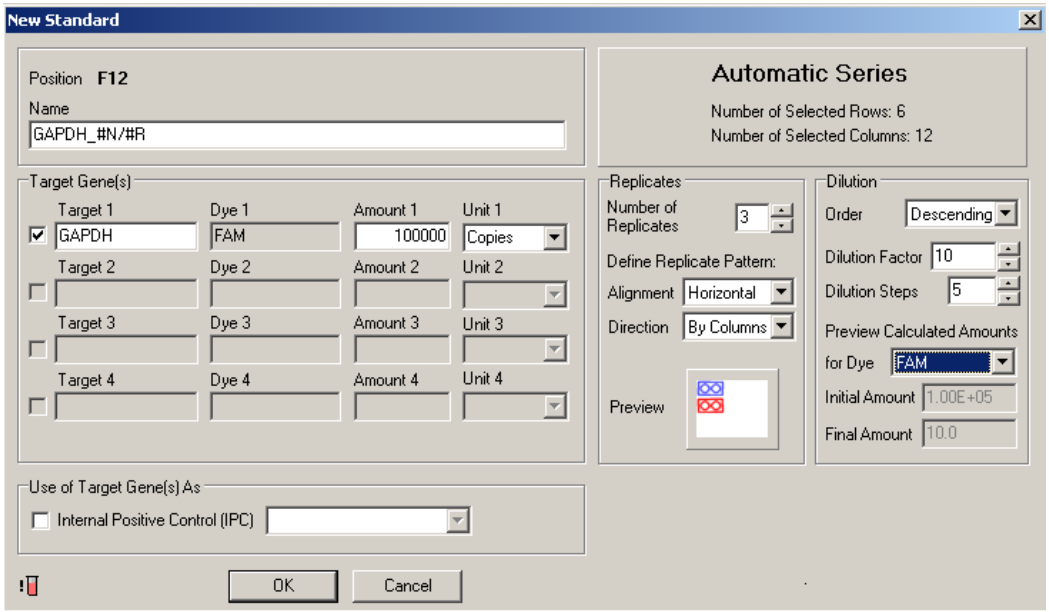
4.2.7 Sample types for quantification

To quantify the initial amount of nucleic acid in a sample a standard curve is created from samples with a known initial amount of nucleic acid. Standards are entered into the plate layout followed by unknown samples and negative controls.

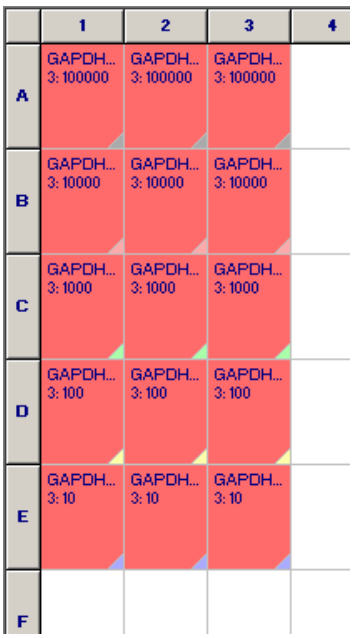
Example of programming a standard series:

The aim is to create a standard series for 10 – 100000 copies of a target gene in triplicates, with the triplicates being arranged next to each other in rows. The highest number of copies is to be at positions A1 – C1, and the dilution series is to be in descending order in steps of 10 in horizontal alignment.

- Select area A1 – E3 in the plate layout.
- Open the dialogue **Standard** and expand the dialogue by selecting the function key **Auto Series**.
- Input parameters.
- Confirm input with **OK**.



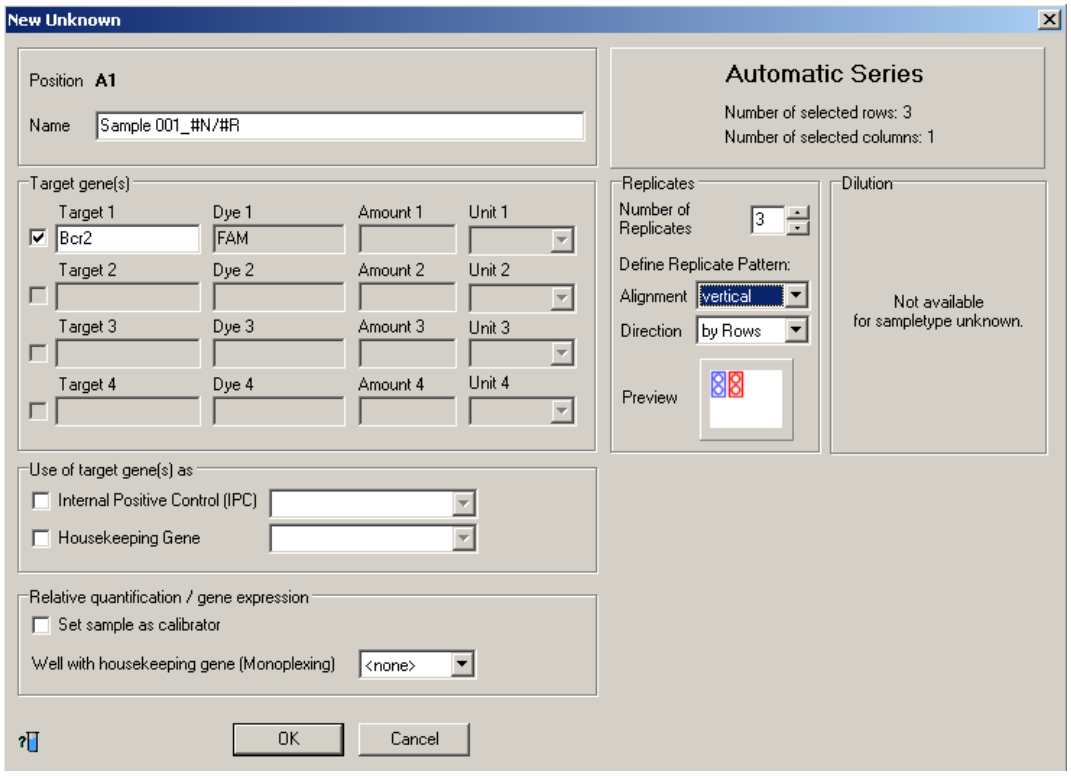
Result in plate layout:



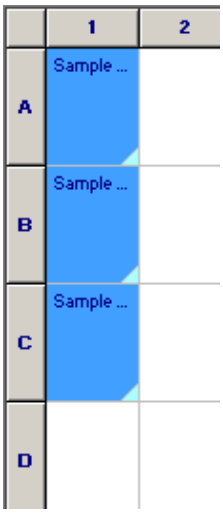
4 Programming (Assay Setup)

Example of programming replicates with unknown samples:
The aim is to define the target gene in triplicates for an unknown sample, with the triplicates being arranged next to each other at positions A1 – C1.

- Select area A1 – C1 in the plate layout.
- Open dialogue **Unknown** including additional item **Auto Series**.
- Input parameters.
- Confirm with **OK**.



Result in plate layout:



4 Programming (Assay Setup)

4.2.8 Sample types for relative quantification

Relative quantification is used for comparison of the expression levels of one or more target genes between two samples (e.g. from a cell culture subjected to different treatments or agents). The expression level(s) of the target gene(s) are normalized to the expression level of a non-regulated housekeeping gene (reference gene, endogenous control) in both samples. The normalized expression levels of one sample are then related to those of the other sample (designated as **Calibrator**) by calculating the relative expression levels according to the $\Delta\Delta C_T$ method (see chapter 6.3).

For relative quantification with the $\Delta\Delta C_T$ method no external standards are used. Therefore all samples are created as sample type **Unknown**. For each target gene a complete set of samples has to be set up according to the scheme shown below. Otherwise the Relative Quantification analysis module will not be available.

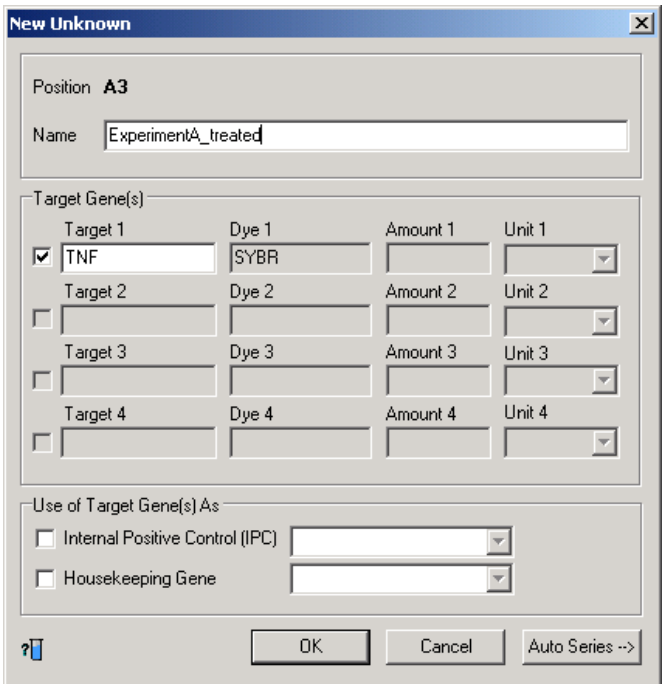
PCR Reaction type	Description	Setup
Monoplex	The target gene and the housekeeping gene are detected in separate reactions. All reactions must contain the same dye (e.g. SYBR Green or sequence specific probes for the target gene and housekeeping gene all containing FAM as reporter dye).	1 target gene reaction or replicate group linked to 1 separate housekeeping gene reaction or replicate group and 1 target gene reaction or replicate group linked to 1 separate housekeeping gene reaction or replicate group both defined as **Calibrator.**
Multiplex	The target gene(s) and its corresponding housekeeping gene are detected in the same reaction. Sequence specific probes containing different dyes for target gene(s) and housekeeping gene have to be used (e.g. in duplex reactions FAM for the target gene and JOE for the housekeeping gene).	1 reaction or replicate group for target gene and housekeeping gene and 1 reaction or replicate group for target gene and housekeeping gene defined as **Calibrator**.

Note: For each calibrator to be used, a separate subassay containing a full setup of target gene(s), housekeeping gene and calibrator has to be defined (see chapter 4.2.16).

4.2.8.1 Set up of monoplex relative quantification assays

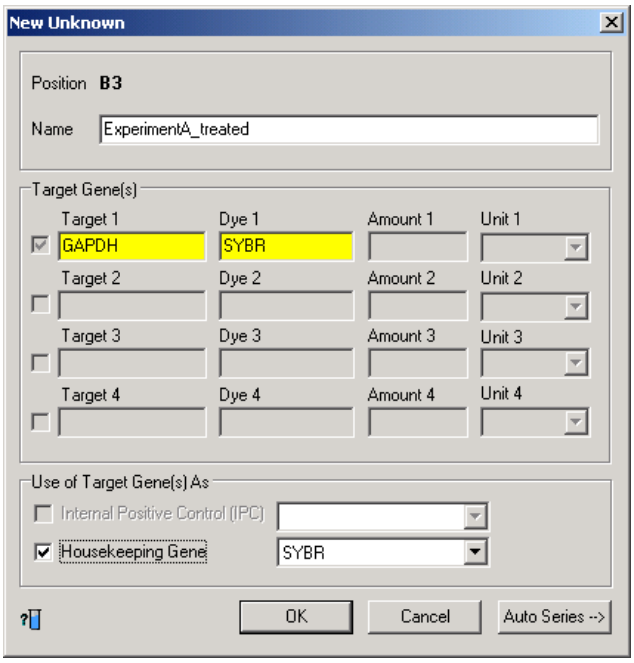
In **Assay Setup / Plate Layout** select the dye, background and probe type. Enter the reaction volume.

Select the position(s) to use for target gene reactions in the plate layout and open the **Unknown** dialog. Enter the sample and target gene names. Make sure that the checkbox for the corresponding dye is selected. Confirm with **OK**. If applicable, group the samples as replicates.



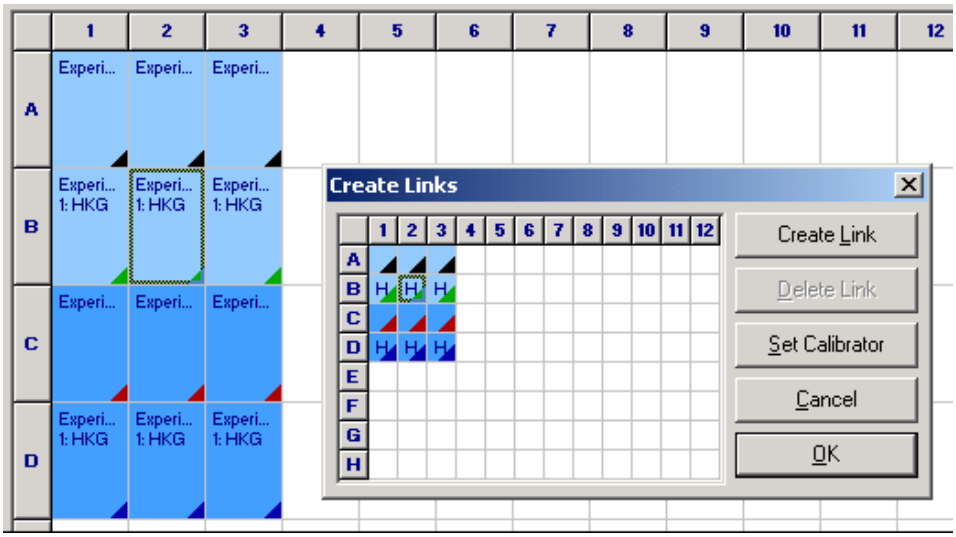
4 Programming (Assay Setup)

Select the position(s) to use for housekeeping gene reactions in the plate layout and open the **Unknown** dialog. Enter the sample and housekeeping gene names. Make sure that the checkbox for the corresponding dye is selected. Activate the checkbox **Housekeeping Gene**. Confirm with **OK** and group the samples as replicates.



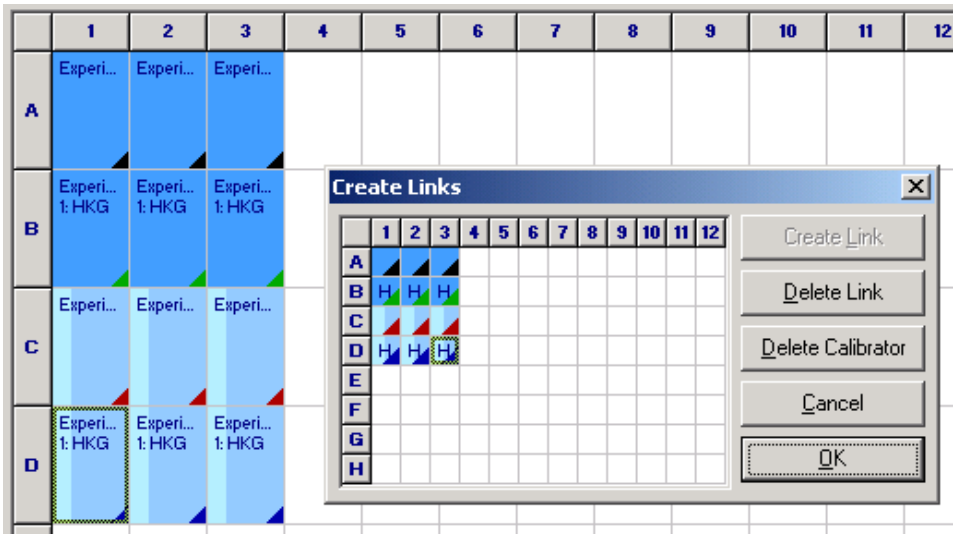
Repeat steps 2 and 3 for the calibrator samples.

Open the **Create Links** dialog by clicking the button  . In the mini plate layout select the positions containing the target gene reactions and while holding the [⇑ Shift] key select the position containing the corresponding housekeeping gene reactions. Click the **Create Link** button. Select the positions containing the target gene and the positions containing the housekeeping gene of the calibrator sample. Click the **Create Links** button.



Note: If a position is selected in the Create Links dialog, all positions currently linked to that position are highlighted automatically.

Select the calibrator group in the mini plate layout and click the **Set Calibrator** button. Confirm and close the dialog by pressing **OK**.



Calibrator positions will be marked with a light blue bar.

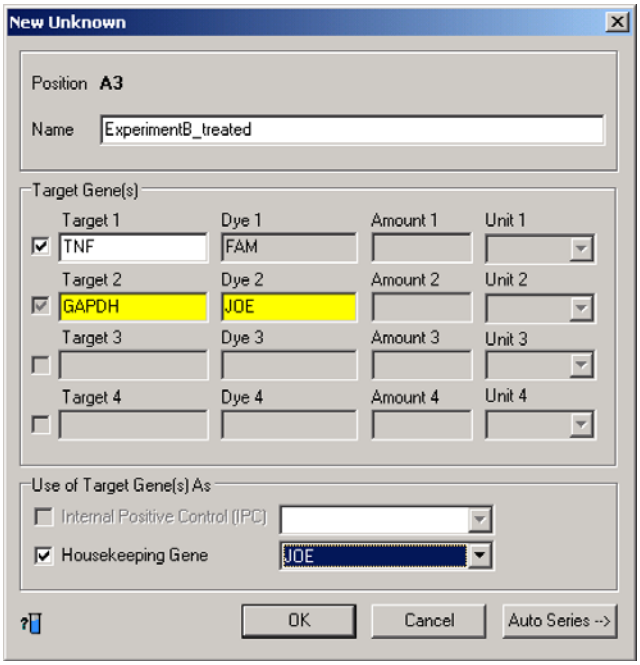
Note: If more than one replicate group for the target gene and / or housekeeping gene is present in the calibrator set, a confirmation dialog concerning the Relative Quantification calculation will be shown. In this case, the arithmetic average C_T values of all target genes and / or housekeeping genes in the calibrator will be used in the calculation of the relative expression levels. To accept and proceed click the **OK** button.

Arrange all corresponding samples in a subassay before adding additional samples to the plate layout.

4.2.8.2 Set up of multiplex relative quantification assays

In **Assay Setup / Plate Layout** select the dyes, background and probe type. Enter the reaction volume.

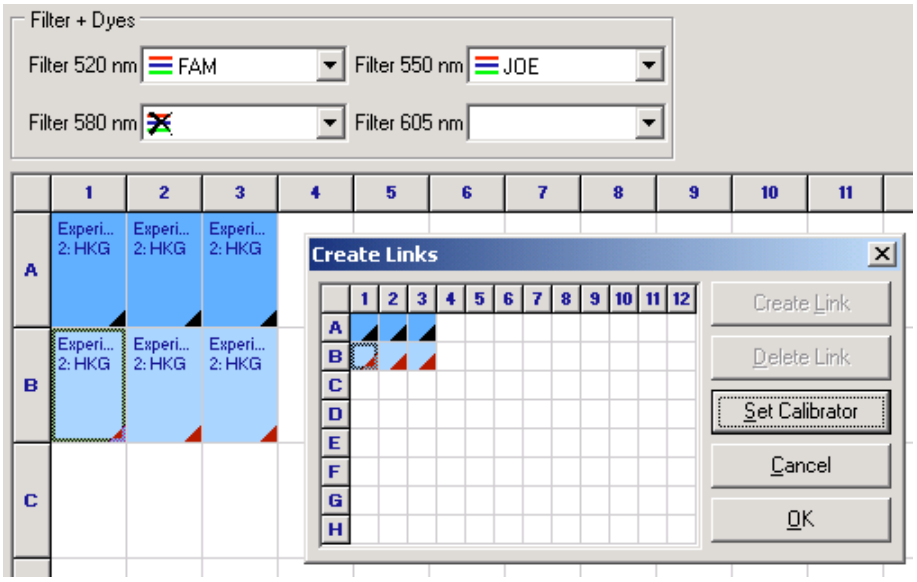
Select the position(s) to use for the combined target gene(s) and housekeeping gene reactions in the plate layout and open the **Unknown** dialog. Enter the sample name and the name(s) of the target gene(s). Make sure that the checkbox for the corresponding dyes is selected. Activate the checkbox **Housekeeping Gene** and select the dye to use for the detection of the housekeeping gene in the combo box. Confirm with **OK**. If applicable, group the samples as replicates.



Repeat step 2 for the calibrator samples.

4 Programming (Assay Setup)

Open the **Create Links** dialog by clicking the button  . In the mini plate layout select the positions containing the calibrator sample. Click the **Set Calibrator** button. Confirm and close the dialog by pressing **OK**.



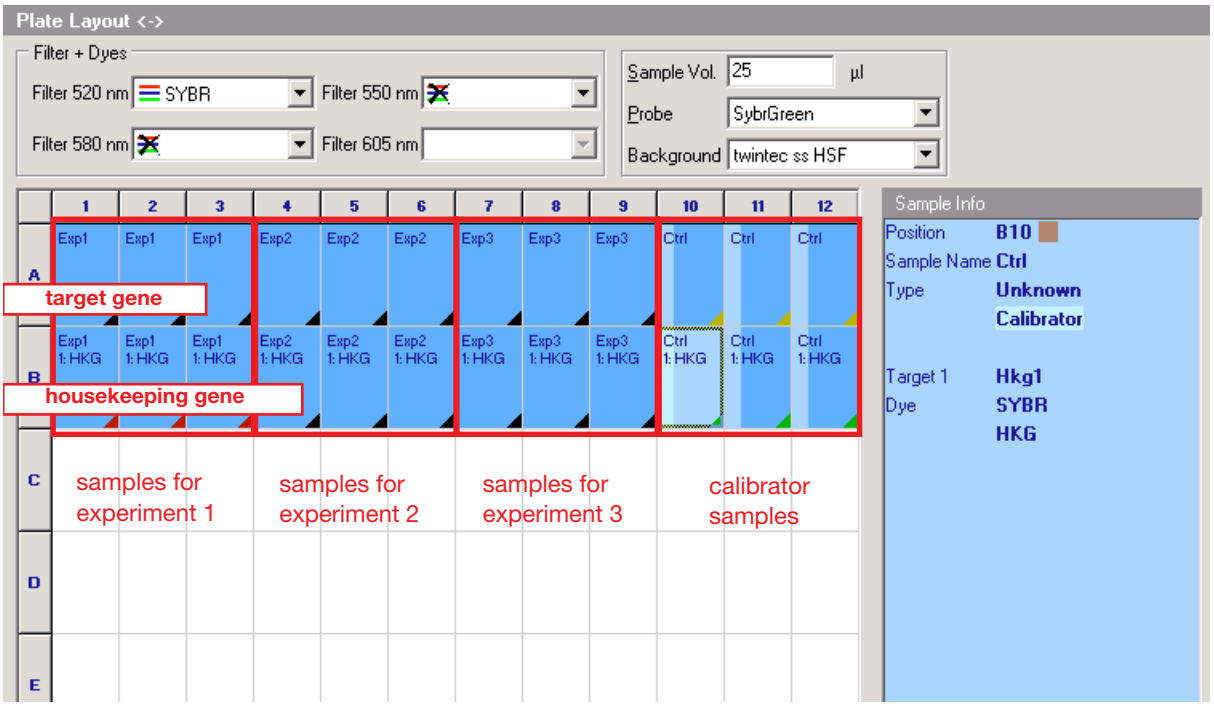
Calibrator positions will be marked with a light blue bar.

Note: If more than one replicate group for the target gene and / or housekeeping gene is present in the calibrator set, a confirmation dialog concerning the Relative Quantification calculation will be shown. In this case, the arithmetic average C_T values of all target genes and / or housekeeping genes in the calibrator will be used in the calculation of the relative expression levels. To accept and proceed click the **OK** button.

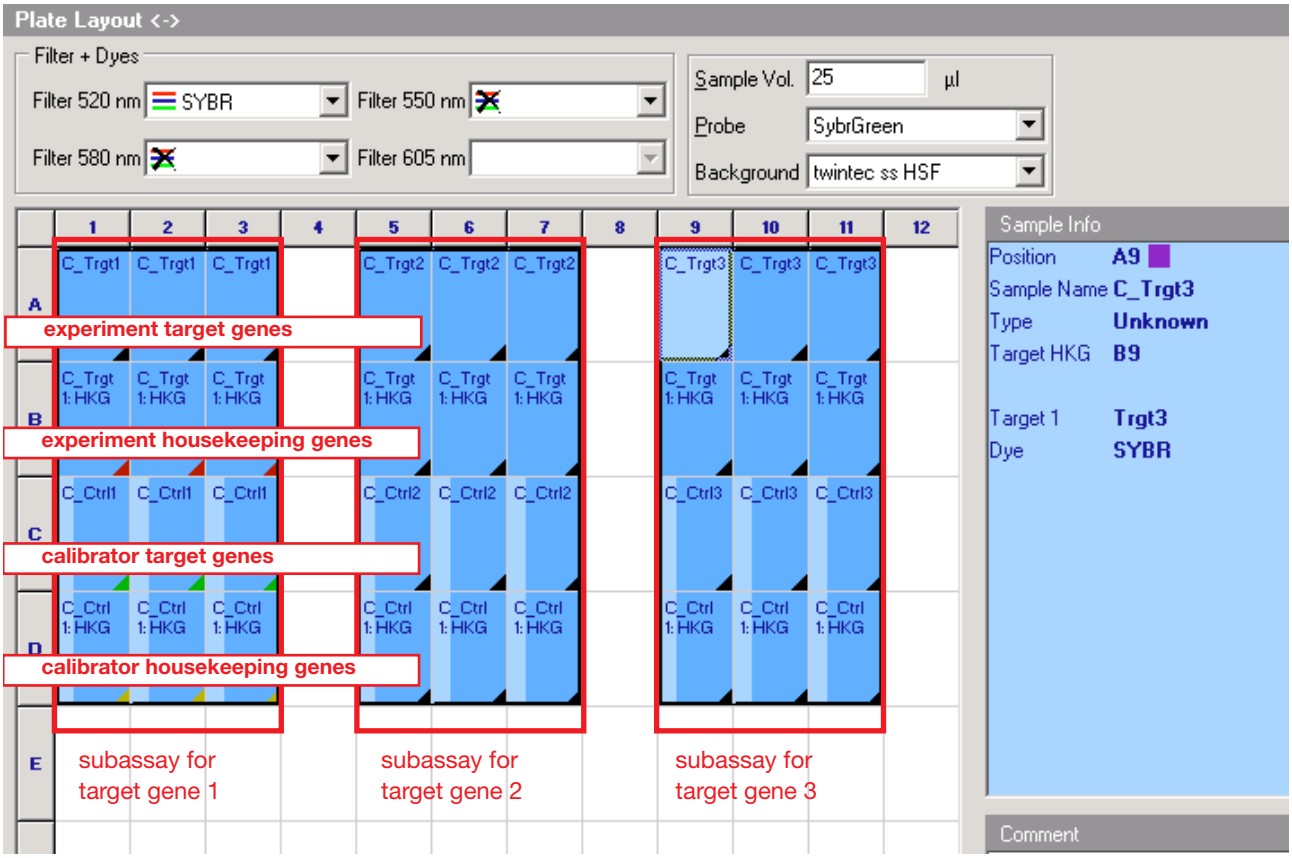
Arrange all corresponding samples in a subassay before adding additional samples to the plate layout.

4.2.8.3 Examples

Example 1: Expression of 1 target gene is compared between samples from 3 different experiments and a control sample. The control sample is used as calibrator. Triplicate monoplex reactions employing SYBR Green are used.

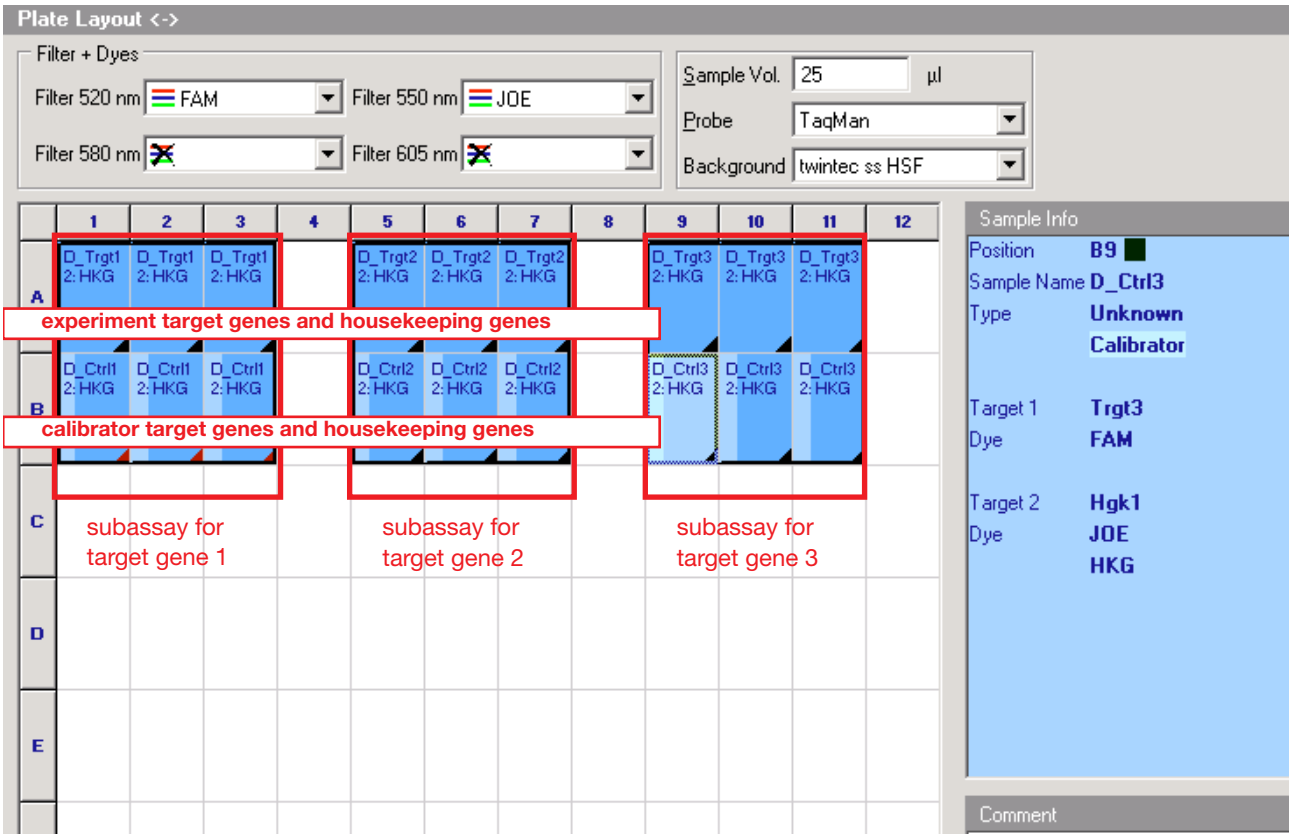


Example 2a: Expression of 3 target genes is compared between an experiment sample and a control sample. The control sample is used as calibrator. Triplicate monoplex reactions employing SYBR Green are used.



4 Programming (Assay Setup)

Example 2b: Expression of 3 target genes is compared between an experiment sample and a control sample is used as calibrator. Triplicate duplex reactions employing sequence specific probes labeled with FAM for the target genes and JOE for the housekeeping gene.



4.2.9 Sample types for melting point analysis

No special aspects have to be taken into account for melting curve analysis.

4.2.10 Sample types for endpoint determination

To perform endpoint determination standards, negative controls and unknown samples need to be ascertained. See section 4.2.5 for further details.

4.2.11 Sample types for a +/- assay

To carry out a +/- assay positive and negative controls have to be defined in addition to the unknown samples.

4 Programming (Assay Setup)

4.2.12 Sample types for gene identification

To identify genes and alleles in unknown samples, a positive control has to be defined for every gene or allele. This is shown in the following example.

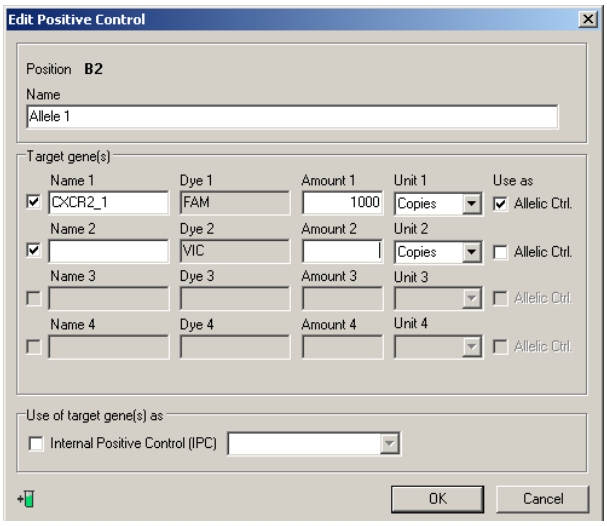
Allele 1

- Select area B2 – B4 in the plate layout.
- Open dialog **Positive control**.
- Select both alleles in the panel **Target gene(s)**.
- The concentration of the positive control can be specified if known.
- Selection of the genes as Allelic Control using the relevant check box.
- Confirm with **OK**.
- Grouping of the samples at positions B2 – B4.

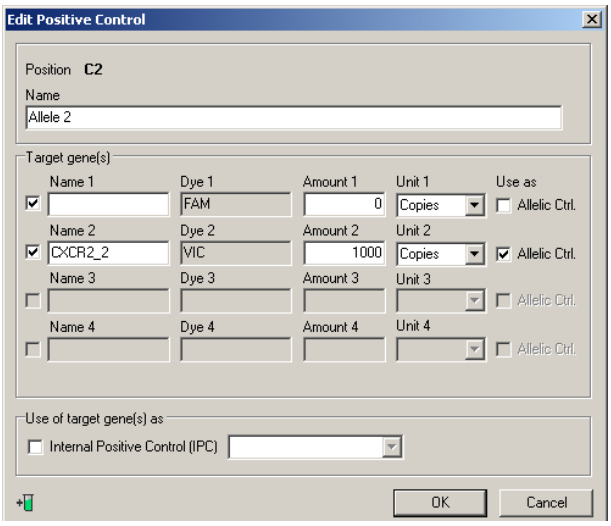
Allele 2

- Select area C2 – C4 in the plate layout.
- Open dialog **Positive control**.
- Select both genes in the panel **Target gene(s)**.
- The concentration of the positive control can be specified if known.
- Selection of the genes as Allelic Control using the relevant check box.
- Confirm with **OK**.
- Grouping of the samples at positions C2 – C4.

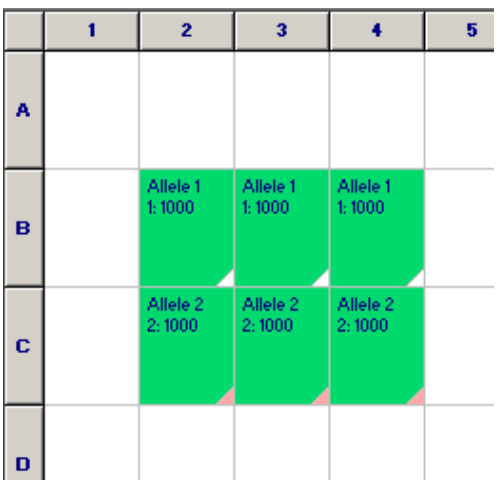
Allele 1



Allele 2



After the samples have been edited, the plate layout looks as follows:



4 Programming (Assay Setup)

4.2.13 Copying and cutting of samples

The commands **Copy**, **Cut** and **Paste** required for this procedure can be accessed either via the right mouse button or the menu item **Plate Layout**.
To copy samples of the plate layout, these are first selected and then copied to the clipboard using the **Copy** command. Then a position in the plate layout is selected and the sample type inserted using the **Paste** command.
The **Cut** command can be used to cut samples and then insert them at another location in the plate layout using the **Paste** command.

4.2.14 Create Links

Create Links is used for special settings in a relative quantification assay:
- To define samples as calibrators.
- To link samples (target and housekeeping gene).

For details see section 4.2.8.

4.2.15 Deleting samples

To delete one or more samples they are first selected and then deleted by selecting the icon **Delete Samples**.

If the icon **Clear Plate** is selected, all samples of a plate layout are deleted.

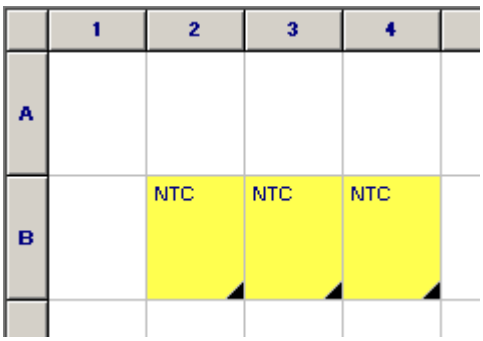
Deleting samples in assays, which are already started or completed is possible (see section 4.2.18). The software automatically saves all fluorescence data concerning a certain position in the plate layout. Only entries like name, amount etc. are deleted.

4.2.16 Grouping of several samples

In addition to the option described in section 4.2.6, it is possible to create replicates by grouping together samples already present in the plate layout. The prerequisite for grouping is that the sample type and all other data correspond. The sample names in a group do not have to be identical.

If several samples are highlighted in the plate layout and the icon **Group** then selected, these samples are grouped together to permit the automatic statistical analysis of replicates.

The grouped samples are marked in the plate layout by highlighting in color on the bottom right. The color can be changed by reselecting the icon **Group as replicates**.



Grouping can be undone using the icon **Ungroup replicates**.

4 Programming (Assay Setup)

4.2.17 Determination of subassays

To perform various assays on a PCR plate, the individual assays are marked as subassays to permit separate analysis by the software at a later date. This means it is possible to carry out multiple assays in parallel, whereby each assay may involve different samples with different target genes and dyes. An example is shown in the plate layout, where two different target genes are to be determined in two different standard series and different unknown samples.

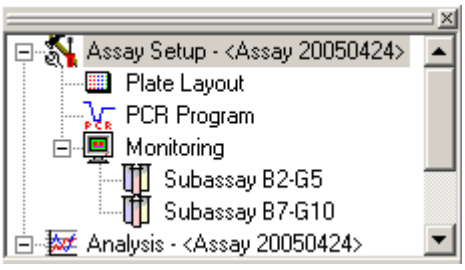
The prerequisite for this is that both assays include the same PCR program and that acquisition of the fluorescence data is carried out in the same temperature step.

If all samples of a subassays are highlighted in the plate layout and the icon **Create Subassay** selected, these samples are marked by a frame.

Note: Alternatively blank positions in the plate layout may initially also be selected and defined as a subassay. This may be followed by determination of the individual sample types of the assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		TNF_1E... 1: 1E9	TNF_1E... 1: 1E9	TNF_1E... 1: 1E9	Mr Jones		HIV_1E11/1 2: 1E11	HIV_1E11... 2: 1E11	HIV_1E11... 2: 1E11	Sample 1		
C		TNF_1E... 1: 1E8	TNF_1E... 1: 1E8	TNF_1E... 1: 1E8	Mr Jones		HIV_1E1... 2: 1E10	HIV_1E1... 2: 1E10	HIV_1E1... 2: 1E10	Sample 1		
D		TNF_1E... 1: 1E7	TNF_1E... 1: 1E7	TNF_1E... 1: 1E7	Mr Jones		HIV_1E9/1 2: 1E9	HIV_1E9/2 2: 1E9	HIV_1E9/3 2: 1E9	Sample 1		
E		TNF_1E... 1: 1E6	TNF_1E... 1: 1E6	TNF_1E... 1: 1E6	Mrs Li		HIV_1E8/1 2: 1E8	HIV_1E8/2 2: 1E8	HIV_1E8/3 2: 1E8	Sample 2		
F		TNF_10... 1: 100000	TNF_10... 1: 100000	TNF_10... 1: 100000	Mrs Li		HIV_1E7/1 2: 1E7	HIV_1E7/2 2: 1E7	HIV_1E7/3 2: 1E7	Sample 2		
G		NTC	NTC	NTC	Mrs Li		NTC	NTC	NTC	Sample 2		
H												

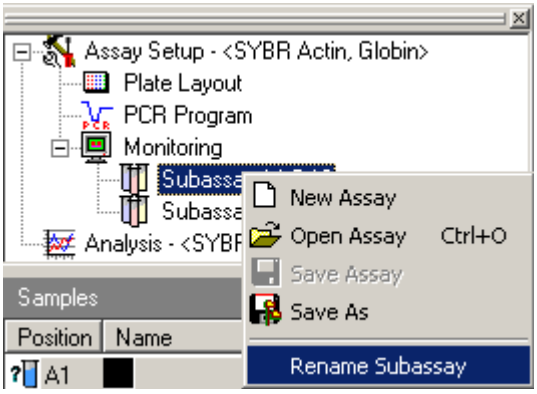
The display of the samples of a subassay during performance or analysis (see section 6.1.1.2) is performed separately and data is saved under separate subitems in the navigation tree.



4 Programming (Assay Setup)

The individual subassays and their position in the plate layout are shown under the node **Monitoring** and **Analysis**. If individual subassays are selected, the related data are loaded to the work field.

The name of a subassay is generated automatically and includes the well positions of the subassay (e.g. Subassay B2-G5). The subassay can be renamed by right mouse clicking the name field in the navigation tree. 32 characters are allowed in length. For specification of the characters which are permitted see section 3.5.3.



Grouping can be undone using the icon **Delete Subassay**. To do so the samples first have to be selected.

Note: The prerequisite for the performance of two different assays in one PCR plate is that both assays are performed with the same PCR program and that acquisition of the fluorescence data is carried out in the same temperature step.

4.2.18 Editing of a plate layout of a completed assay

After the PCR program of an assay has been started or the assay is completed, the plate layout is locked. To make subsequent changes, the plate layout has to be set to **Edit Mode**.

The blocked plate layout of a completed assay can be reactivated by selecting the icon **Edit Mode** for plate layout. Once editing has been performed, the plate layout is deactivated again by reselecting the icon.

All subsequent changes in the plate layout are documented in the UserLog.

EditMode allows changing and deletion of initially defined samples using the tools described in chapters 4.2.5. to 4.2.17. Deleted samples will be marked with a gray background color indicating that fluorescence data for these positions had been or is being collected during the run. Deletion of samples in Edit Mode excludes this data from analysis but the data itself is not deleted from the assay. The data can be reaccessed by redefining samples at these positions.

Note: Note that no data is acquired for positions where no samples were set up before starting the assay.
By using Edit Mode already present analysis settings are reset to default.

4.2.19 Color for Samples

If the icon **color for samples** is selected, the color of the sample and the corresponding curves can be changed.

Different basic colors are available. User-specific colors can be defined via the color field. For allocating the same color to different samples, select the corresponding positions before the dialogue **color** is opened.

4 Programming (Assay Setup)

4.3 Creation of a PCR program

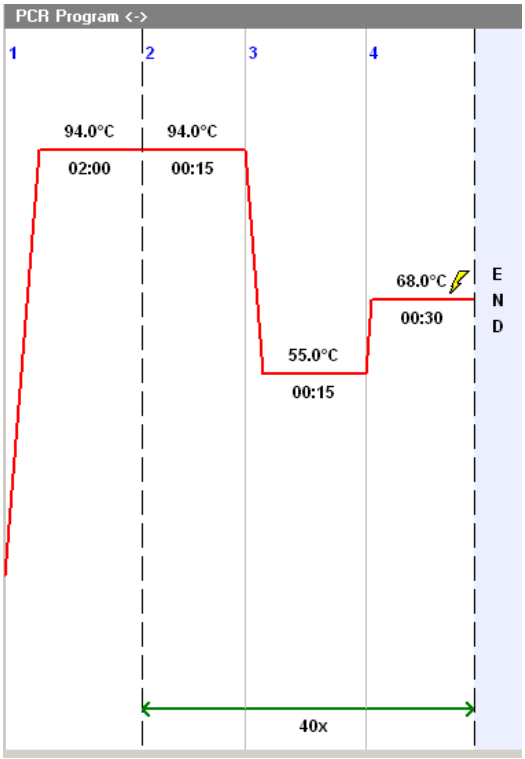
The following functions are available in the toolbar to edit the PCR program:

1 Insert Step
2 Edit Step
3 Delete Step
4 Clear PCR Program
5 Show Gradient
6 Set Measuring Point

Note: Alternatively these functions can also be accessed via the menu item **PCR Program** or the right mouse button.

4.3.1 Create a PCR program with a program template

If the sub-item **PCR Program** is selected in the navigation tree, a program template, which consists of a 3 Step Cycle, will be displayed in the work field for a new assay.



To edit the program, individual steps are selected and the icon **Edit Step** then used to call up the dialogue in which the data for temperature, time and number of cycles can be amended.
Alternatively the dialogue **Edit Step** can be opened by double-clicking on a step.
In addition, it is possible to use this dialogue to make all other settings for increments, gradient function and heating and cooling rates (see section 4.3.3.1).

Alternatively the data for temperature, time and number of cycles can be amended directly in the PCR program by selecting and then adding the change.

Note: The properties of the heated lid and the thermoblock can be amended in **Program Header** (see section 4.3.7).

4.3.2 Create a PCR program without a program template

To create a program without a template, the icon **Clear PCR program** can be used to delete the entire program and a new program created by inserting program steps.

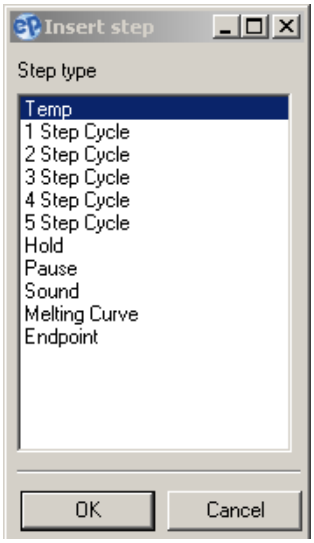
4 Programming (Assay Setup)

4.3.3 Insertion and editing of program steps

The cursor is used to select the area of the PCR program in which the new program step is to be inserted. Steps are always inserted to the left of the highlighting.

If the icon **Insert Step** is selected, the relevant dialogue is displayed and can be used to insert various program steps.

If the program step is selected, it will be inserted in the PCR program to the left of the highlighting.



After the program step has been inserted, the data for temperature, time and number of cycles can be directly amended in the PCR program. To do so, they are selected and the change then made.

If other adjustment options are to be amended for program steps, the icon **Edit Step** can be used to call up the dialogue in which all other settings and changes can be made.

4.3.3.1 Temp

A temperature step can be inserted at any point in the program. The main parameters of the temperature step, temperature and holding time, are shown in the work area of the PCR program and can be directly edited there.

TEMP	nominal temperature in degrees Celsius
Permissible values:	4.0 °C to 99.0 °C
Input steps:	0.1 °C
Time:	holding time in minutes and seconds
Permissible values:	00:01 to 99:59 mm:ss
Input steps:	1 s

Additional functions of the temperature step can also be accessed via this window.

Temp incr. – Temperature increment:
Increase / decrease in the temperature of the programmed temperature step to the next cycle by this amount.

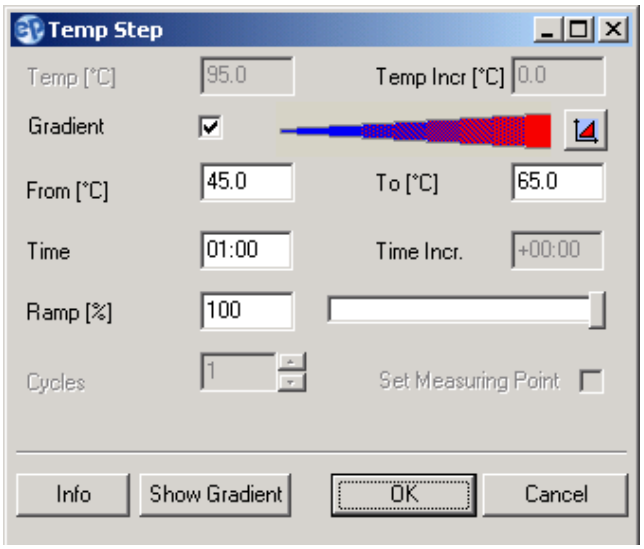
Default setting:	0.0 °C
Permissible values:	0.0 to 10.0 °C
Input steps:	0.1 °C
Temperature increase:	+
Temperature decrease:	–

4 Programming (Assay Setup)

The increment is marked by a star in the step of the PCR program: 55 °C* / 00:30

Gradient:
When using the gradient function, every column of the thermoblock is set to a different temperature. To program a temperature gradient activate the **Gradient** check box and add the minimum and the maximum temperature into the **From** and **To** fields, respectively. Activating **Gradient** disables the **Temp Incr** and **Time Incr** fields.

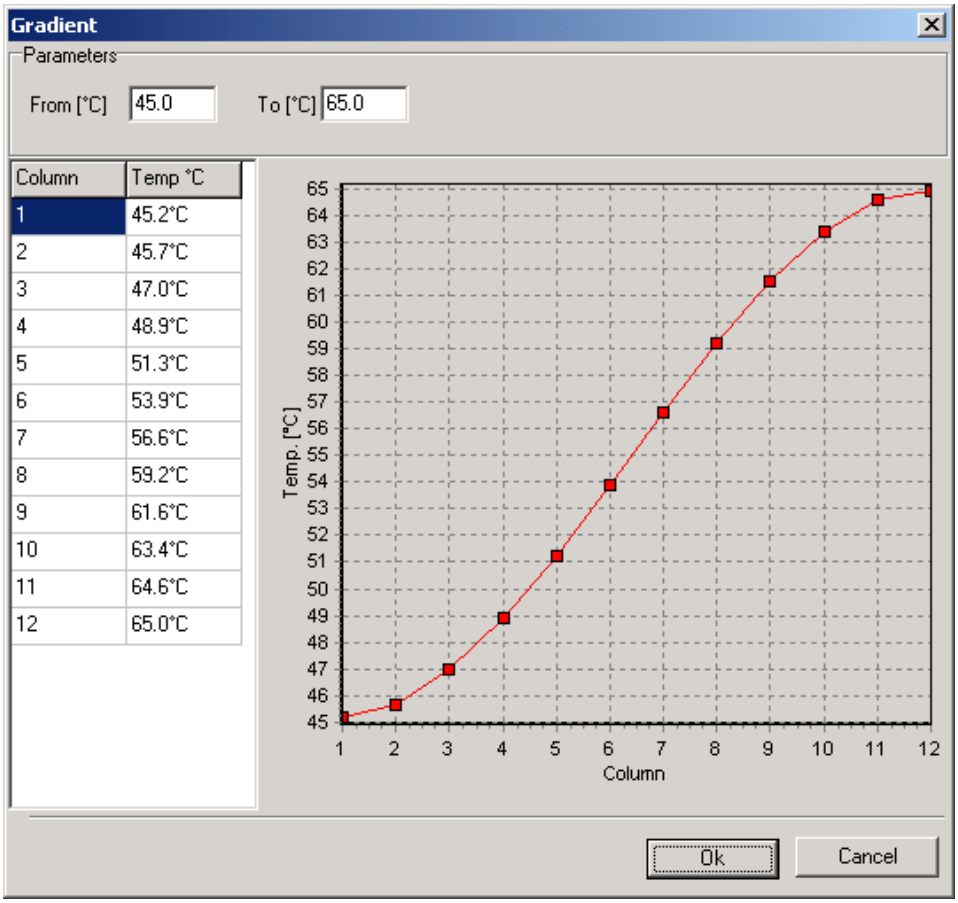
Default setting:	deactivated
from temp:	temperature of left block side
Permissible values:	30.0 °C to 99.0 °C
Input steps:	0.1 °C
to temp:	temperature of right block side
Permissible values:	30.0 °C to 99.0 °C
Input steps:	0.1 °C



If the icon **Show Gradient** is selected, a dialogue is displayed, showing the temperatures of the individual columns.

A thermoblock can be selected in the panel Parameters. In addition, the temperatures of the gradient can be entered here.

4 Programming (Assay Setup)



The gradient is marked in the step of the PCR program by additional underlining. 55 °C / 00:30

The maximum spread of the gradient depends on the thermo module used (see Technical data). If the gradient function is switched off, there is homogeneous temperature distribution over the entire block.

Time incr. – Time increment:
Increase / decrease in the holding time of the programmed temperature step to the next cycle by this amount.

Default setting:	00:00
Permissible values:	00:00 to 01:00
Input steps:	1 s
Time increase:	+
Time decrease:	–

The increment is marked by a star in the step of the PCR program. **55 °C** / **00:15***

Ramp – Heating and cooling rate:
Speed at which the set temperature of the programmed step is achieved.

Default setting:	100 %
Permissible values:	1 to 75 % and 100 %
Input steps:	1 %

For procedures involving slow heating or cooling, different values can be entered for the ramp or the bar in the graph moved using the cursor. The current value will then be displayed in the input field. The heating and cooling rates depend upon the thermo module used (see Technical data).

4 Programming (Assay Setup)

4.3.3.2 Cycle (1 – 5 Step Cycle)

The Cycle command contains the number of temperature steps (1 – 5) in one cycle and the number of repetitions used for execution of the temperature commands.

Default setting:	40
Permissible values:	1 to 99
Input steps:	1

4.3.3.3 Hold

The program holds the temperature at the entered value until the PCR program is ended. To proceed press Start.

Default setting:	4.0 °C
Permissible values:	4.0 °C to 99.0 °C
Input steps:	0.1 °C

4.3.3.4 Pause

The program is stopped, and the temperature remains at the value of the last temperature command. The program continues after the key **Start program** is pressed.

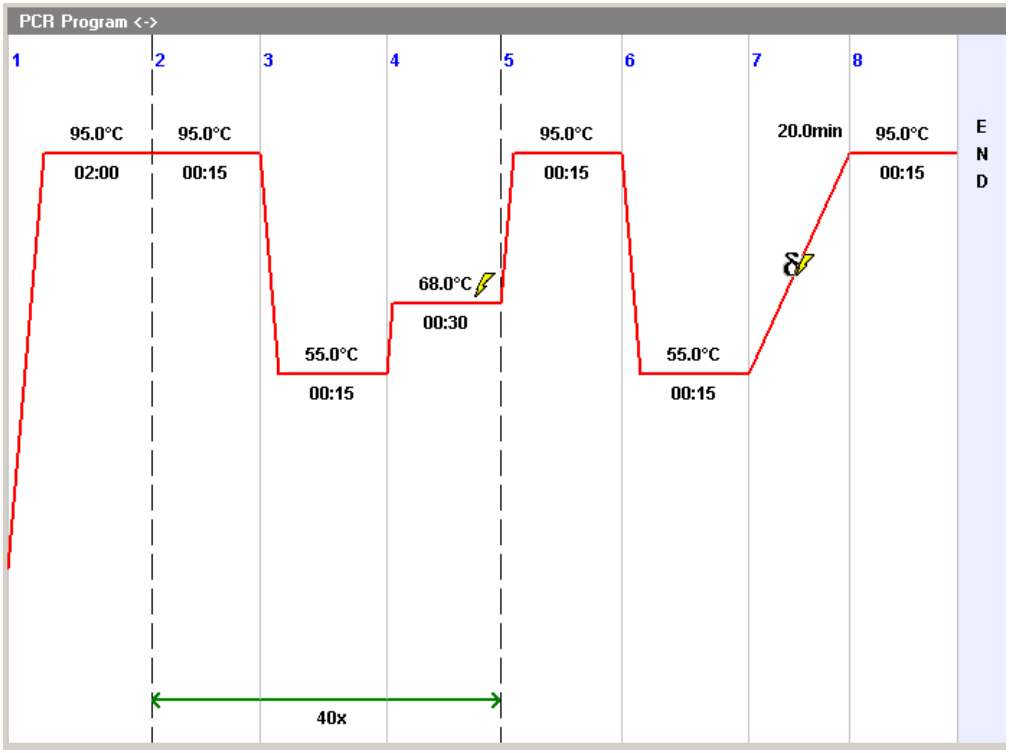
4.3.3.5 Sound

It is possible to emit an acoustic signal, e.g. to announce a programmed pause or the end of the program.

Default setting:	9
Permissible values:	1 to 9 (number of signals)
Input steps:	1

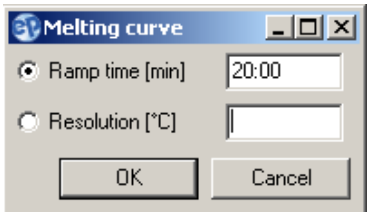
4.3.3.6 Melting Curve

Selection of the item **Melting Curve** in the dialogue **Insert Step** automatically inserts four program steps.
Besides the actual step of the melting curve, this includes an initial denaturation step as well as a temperature step each for the initial and final temperature.



To amend the parameters for determination of the melting point, the step of the melting curve is selected, followed by the icon **Edit step**.

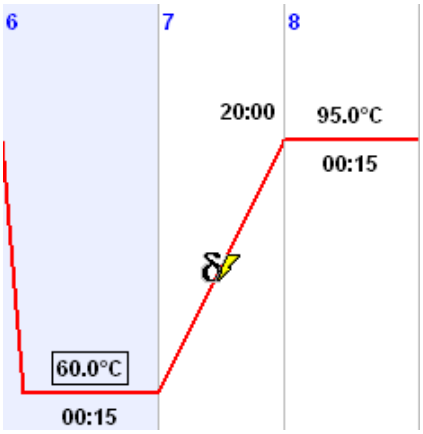
4 Programming (Assay Setup)



Ramp time: Input of the time in which the melting curve is to be created.
Permissible values: 5 – 30 min
Input steps: 1 s
Fluorescence data are measured continously.

Resolution: Alternatively the measuring rate for melting curve can be entered.
Permissible values: 0.1 – 2.0 °C
Input steps: 0.1 °C
Fluorescence data are measured after heating of x °C is finished.

In the PCR program the melting curve is marked by a symbol.



4.3.3.7 Endpoint

After the dialogue **Insert Step** has been opened choose the entry **Endpoint**. The data for temperature and duration can be easily edited by clicking at the values in the graph.

TEMP	nominal temperature in degrees Celsius
Permissible values:	4.0 to 99.0 °C
Input steps:	0.1 °C
Time:	holding time in minutes and seconds
Permissible values:	01:00 to 99:59 mm:ss
Input steps:	1 s

4.3.4 Copying of program steps

The commands **Copy** and **Paste** required for this procedure can be accessed either via the right mouse button or the menu item **PCR program**. To copy a program step, this is first selected and then copied to the clipboard using the **Copy** command. Then a position in the PCR program is selected and the program step inserted there using the **Paste** command.
Here the program step is always inserted on the left next to the highlighting.

4.3.5 Deletion of program steps

Individual temperature steps are deleted by first being selected and then deleted by selecting the icon **Delete Step**.

4 Programming (Assay Setup)

4.3.6 Determination of measuring point

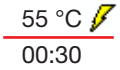
In general, data acquisition can be carried out during PCR program, endpoint assay or melting point analysis. Each item can be programmed once in an assay.

The measuring point is determined in a temperature step by highlighting a step and selecting **Set measuring point** in the **Temp Step** dialogue.

Attention:
The measuring point is defined by the probe that is used. It is not set up by the software automatically!

Note: Data acquisition can only be carried out in one temperature step within the PCR cycle. The PCR program can be composed of repeated PCR cycles. Data acquisition can be carried out in each PCR cycle, but the software based evaluation is only possible in the first PCR cycle.

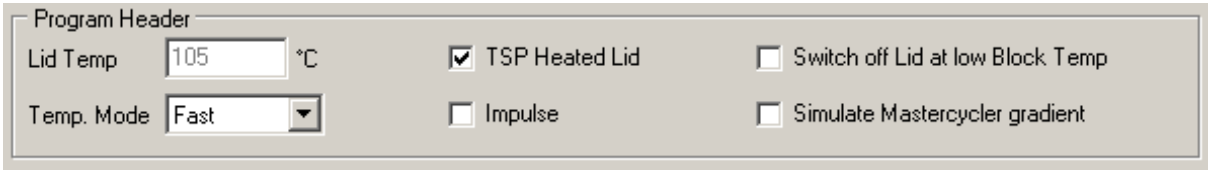
Attention: When setting measuring points it should be ensured that the duration of the temperature steps is sufficient. With one dye per filter rotor 8 seconds are required for data acquisition while the measuring time is extended to 16 seconds for two dyes per filter rotor.

The measuring point is marked in the step of the PCR program by a relevant symbol. 

To remove the measuring point the relevant temperature step is first selected and then the check box **Set measuring point** deactivated in the dialogue **Edit step** or the icon **Set measuring point** selected in the toolbar.

4.3.7 Header settings

The header settings form the program head in which the controller strategy and the behavior of the heated lid are each set on a program-related basis.



Lid Temp
The heated lid temperature of 105 °C is preset and cannot be changed.

Temp.Mode
This command is used to specify the type of regulation for the block temperature mode.

Standard — Default setting / block temperature mode for standard applications. This mode should be used for sample volumes in the range from 20 µl to 50 µl. Additionally, this setting will be recommended if the mode **fast** results in poor amplification results.

Fast — The temperature at the thermoblock is measured and the nominal temperature then set. This mode should be used for Fast-PCR and low sample volumes (< 20 µl) as well as for templates with low GC-content. The program run time is decreased in comparison to the mode **standard**.

TSP Heated Lid
The PCR program is only started after the lid temperature has been achieved. If this function is not selected, the PCR begins directly after the program start. For endpoint assays deactivation of the combo box is not possible.

Impulse
For devices with silver block (thermo module Mastercycler ep gradient S) it is possible to accelerate the first heating process using the Impulse function. If another block is selected or if the function **Simulate Mastercycler gradient** is selected, this function is not active.

Switch off lid at low block temp
Depending on the input the heated lid is switched off at low block temperatures. If block temperatures are held below 15 °C for more than 5 minutes, the heated lid is switched off automatically.
If this function is not selected, the lid heating is only switched off at the end of a program.

Note: If the last command in a program is a Hold step, the heated lid remains switched on until the key is operated. The prerequisite for this is that the temperature of the Hold step is <15 °C and the function **Switch off lid at low block temp** is deactivated.

Simulate Mastercycler gradient
PCR programs adopted from other Cyclers may have to be re-optimized as the temperature characteristics vary and may influence the PCR results.

Simulate Mastercycler gradient permits simulation of the temperature characteristics of the Mastercycler gradient to guarantee the simple transfer of established programs. This setting allows the temperature control behavior of a Mastercycler gradient to be simulated and thus to establish protocols from the Mastercycler gradient in general without further optimization on the Mastercycler ep *realplex*.
To do so, the PCR program of the Mastercyclers gradient is entered in Assay Setup under the PCR program and the check box **Simulate Mastercycler gradient** then activated in the program header.
After being saved the program can be started on the Mastercycler ep *realplex* without re-optimization of the parameters.

5 Operation (Monitoring)

5.1 Sample loading

The Mastercycler ep *realplex* can be equipped with up to 96 PCR tubes or one 96-well PCR plate. To ensure uniform pressure of the heated lid when using tubes it is recommended positioning a tube in each corner of the thermoblock. Other tubes should be distributed evenly in the block.
Please remember that with real time PCR analyses both the tubes and the sealing systems selected must be suitable for fluorescence detection (see Ordering information).

For optimum temperature transition it should be ensured that the tubes are properly positioned in the thermoblock and do not wobble. Tubes that are not sufficiently heat resistant (approx. to 120 °C) should not be used. This generally also applies to covers used to seal PCR plates.

Note: Different tube types should not be used together as they will then not be sealed properly by the heated lid. In addition, no suitable calibration data have been stored.
When using individual 0.2 ml PCR tubes, tubes should also be positioned in each corner of the thermoblock.

Attention: PCR plates should only be labeled at the side as labeling may also be fluorescent!

5.2 Block and Lid temperature control

For incubation experiments or to accelerate the start of a method, both the thermo module and the heated lid of the *realplex* module can be subjected to temperature control. To do so, the required block or lid temperature is selected under the cycler node in the dialogue **realplex System configuration** (see section 3.7).

Note: At the start of a program the settings of the respective program have priority. During an ongoing program separate block temperature control of the thermoblock cannot be switched on.

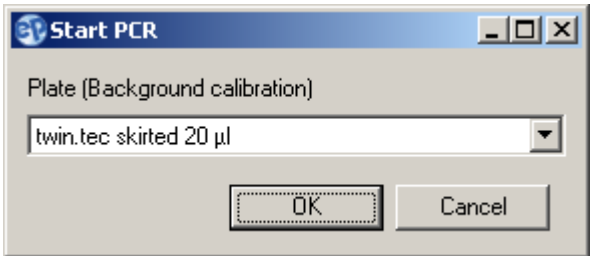
5.3 Start assay

After the created assay has been saved and the sample tubes inserted, the heated lid is slid forwards. The sealing clamp is then moved down and the program started.

Note: Make sure that the instrument has been warmed up for at least 15 minutes before starting an assay.

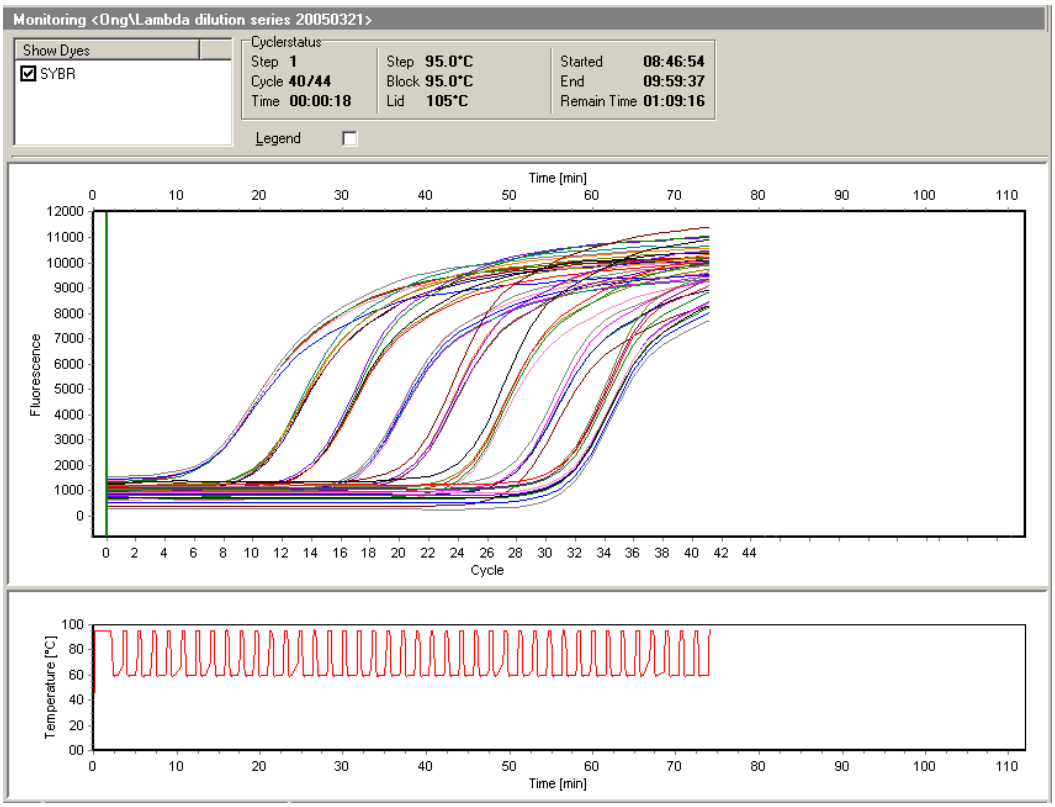
If the icon **Start current cycler program** is selected, the assay is started.
Alternatively this function is also available under the menu item **Command**.

After the assay has started, the dialogue **Start PCR** is displayed and can be used to select the correct background plate with a combo box.



5 Operation (Monitoring)

If a background plate has already been specified in the plate layout, this is automatically shown in the combo box. It is possible to select a background plate other than the one already specified in the plate layout. If this is done, it is documented in the report (see section 6.1.10).



The data acquisition can be observed in the mode **Monitoring**.

The fluorescence intensity is shown here as a function of the number of cycles and time. Underneath the temperature profile is shown in the course of time.
If the check box **Legend** is selected, a legend is inserted on the right of the fluorescence profile.

With the acquisition of measured data for multiplex assays it is also possible to choose between individual dyes in the panel **Show Dyes** by selecting the relevant check box.

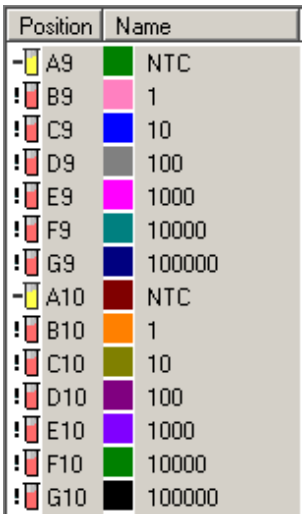
5.3.1 Quick start of an assay

To quick start an assay, select the dyes and the background to be used and enter the sample volume. Then select all positions in the plate layout and define them as **Unknowns**. All fields of the dialogue may be left blank. Enter the PCR program and start the run. While the assay is running or at any later time before analysis, the actual plate layout can be entered and unused samples deleted using **Edit Mode** (see 4.2.18).

5 Operation (Monitoring)

5.4 Selection of individual samples

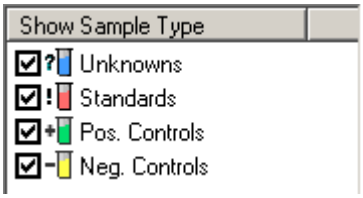
All samples are listed in the navigation tree under **Samples**.
This table shows the names of the individual samples and their positions in the plate layout. In addition, the samples are marked with the symbols of the sample types. To facilitate the assignment of individual fluorescence curves to the samples, the color of the fluorescence curves is also shown in the table.



If one or more samples are selected, the related fluorescence curves are shown in the work area. To select several samples in one row, first the upper sample is highlighted and then the lowest sample by simultaneously pressing the <Shift> key. If several samples which are not positioned next to each other are to be selected, the <Ctrl> key has to be pressed during selection.

5.5 Selection of individual sample types

To show or hide individual sample types they are activated or deactivated in the window **Show Sample Type**.



5.6 Cycler Status

Directly after the start all information for the ongoing PCR program is shown under **Monitoring** in the panel **Cycler Status**. The progress of an ongoing program can be monitored in the status display.

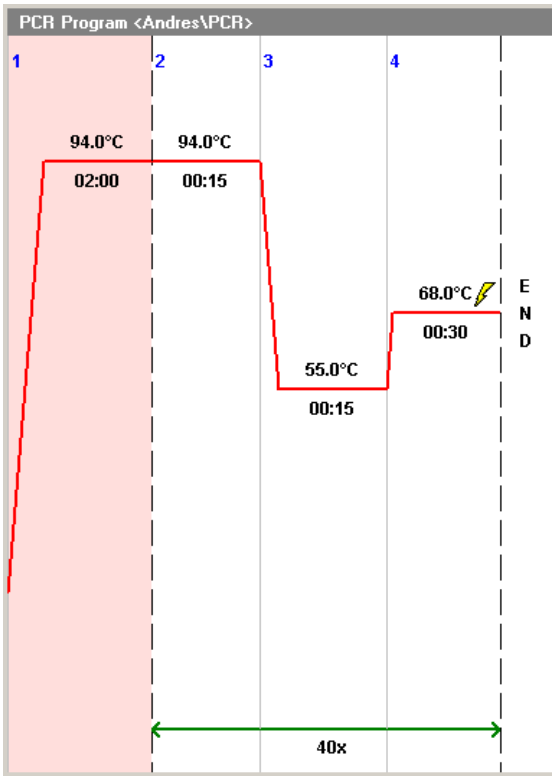
The current cycle is shown in the left-hand block of the panel, in addition to the current temperature step and the time still remaining for this temperature step.

The middle block of the panel **Cycler Status** shows the nominal temperature of the current program step and the current temperatures of the block and heated lid.

The right-hand block provides information about the start and end time of the ongoing PCR program and the time period for which this program will last.

5 Operation (Monitoring)

By changing from **Monitoring** to **PCR Program** it can also be seen in the PCR program which is the current temperature step. This is shown in red.



5.7 Interruption of an ongoing assay

The assay can be interrupted, e.g. to add a reagent. The current temperature of the block and the lid remain unchanged.

The icon **Pause current cycler program** can be used to interrupt the ongoing assay.

Cycler Status: Pause The status display then shows **Pause**.

The program can be continued at any time (see section 5.8) or aborted (see section 5.9).

5.8 Continue assay

If the assay is to be continued, the heated lid has to be closed first.

A program that has been stopped can be continued at any time with the icon **Start current cycler program**.

Cycler Status: Running After the assay has been continued, the status display then shows **Running**.

5.9 Abort assay

An assay is interrupted when the icon **Pause current cycler program** is selected.

Afterwards the assay is aborted by selecting the icon **Abort current cycler program**.

Cycler Status: Idle The information **Idle** is shown in the status display.

5 Operation (Monitoring)

5.10 Use of Mastercycler ep *realplex* for standard PCR

The Mastercycler ep *realplex* can also be used solely as a thermo cycler. To do so, a new assay is opened (see section 3.10.2) and only a PCR program created in the Assay-Setup (see section 3.5.1). Make sure that no measuring point is set in the case of a standard PCR. After creation of the PCR program it is saved and started.

Note: The plate layout remains blank, and no dye should be selected as otherwise there will be an error message on start.

5.11 Use of Mastercycler ep *realplex* as Fluorimeter

The Mastercycler ep *realplex* can also be used for fluorescence measurement independently of a PCR. In this case solely endpoint determination is carried out (see section 4.2.10).

Here it must be remembered that background calibration is also necessary for such determination. In addition, color calibration must be performed for every dye to be detected.

Note: On calibration the temperature of the block and heated lid must correspond to the conditions of subsequent fluorescence measurement.

5.12 Shut down

To shut down the Mastercycler ep *realplex* the ongoing assay is first ended and saved.
The software is then closed, computer shut down and the device switched off.

Saving of assays is only neccessary if the data were evaluated, the raw data will be saved automatically.

Note: The *realplex* module should be kept in a closed position when the Mastercycler ep *realplex* is in off state to prevent contamination of the block.

6 Analysis

An assay is analyzed by first being opened with the command **Open Assay** and then selecting the node **Analysis** in the navigation tree. If the assay finished shortly before and no other assay was opened afterwards, the data will be automatically accessible under **Analysis**.

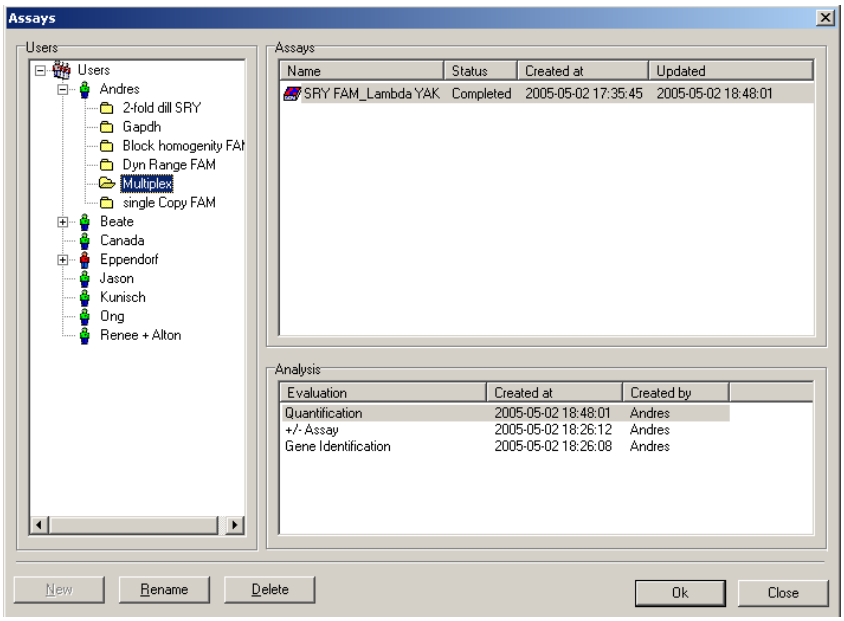
Note: While one assay is running, it is possible to analyze an assay that has already been completed under **Analysis** (see section 6.1.1.1).

Information about the plate layout and PCR program can be viewed under the relevant node of the navigation tree when the relevant assay also appears under **Assay Setup**. If another assay is running at the same time, access to these data is not possible.

6.1 General

6.1.1 Open Assay

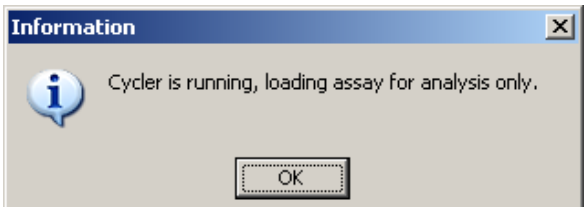
Open an assay using **Open Assay**.



After the assay is opened, the raw data are displayed automatically under **Analysis** in the right-hand work field. These data are the fluorescence values acquired during detection which were set against the factors from the background and color calibration. Display of the raw data differs depending on the analysis module and is described in the individual sections for evaluation of the different analysis modules.

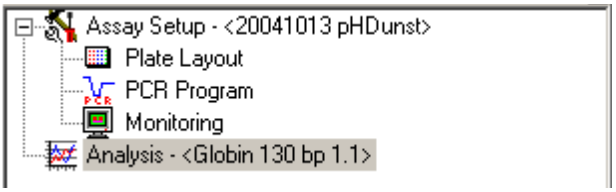
6.1.1.1 Open assay during an ongoing assay

During data acquisition of an ongoing assay it is possible to analyze an assay that has already been completed. To do so it is selected in the dialog **Assays**. Another dialog appears, stating that the assay is for evaluation only as another assay is currently running.



After confirmation with **OK** the assay is displayed under **Analysis**.

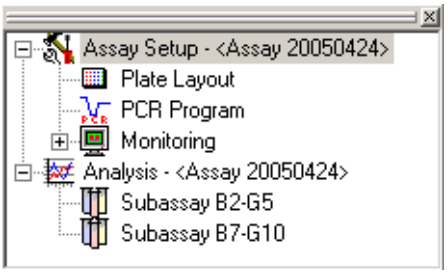
6 Analysis



Note: During data acquisition of an ongoing assay it is not possible to view information on the plate layout or PCR program of the assay already completed. Sample types and their positions can be seen in the Mini Plate Layout under Analysis.

6.1.1.2 Open subassay

If subassays have been defined in the plate layout, they are shown under **Analysis** as individual nodes. The designation **Subassay B2-G5** indicates the position in the plate layout (for renaming a subassay see section 4.2.18). If a subassay is selected, only the data associated with the subassay are loaded to the work field where they can be analyzed.



All analyses of the sub-assays are saved under the assay.

6.1.2 Selection of individual samples

All samples are listed in the Navigator under **Samples**.

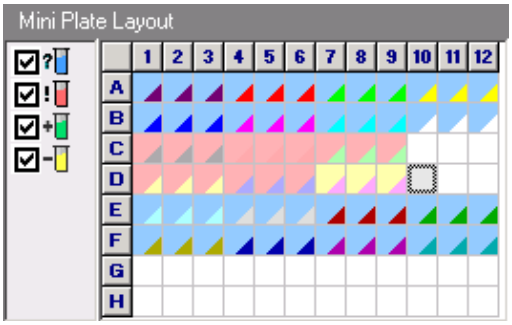
Position	Name
A9	NTC
B9	1
C9	10
D9	100
E9	1000
F9	10000
G9	100000
A10	NTC
B10	1
C10	10
D10	100
E10	1000
F10	10000
G10	100000

This table shows the names of the individual samples and their positions in the plate layout. In addition, the samples are marked with the symbols for the sample types. The color is also shown in the table to simplify the assignment of individual fluorescence curves to the samples. In the individual analysis modules this table is extended into a table of results.

If one or more samples are selected, the associated fluorescence curves are shown in the work area. To select several samples in one row, first highlight the top sample and then the bottom sample simultaneously pressing the <Shift> key. If several samples which are not positioned next to each other are to be selected, the <Ctrl> key has to be pressed during selection.

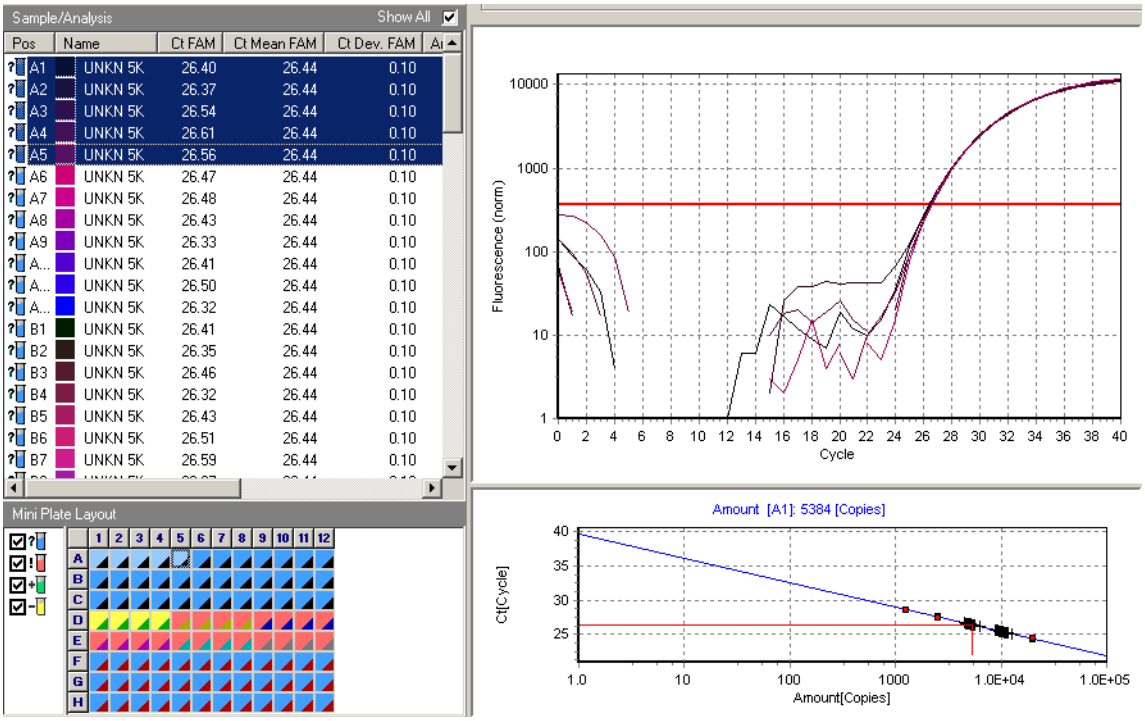
The sequence of the samples can be changed by double-clicking on the heading of individual columns in the sample table.

Alternatively, samples can be selected using the Mini Plate Layout:



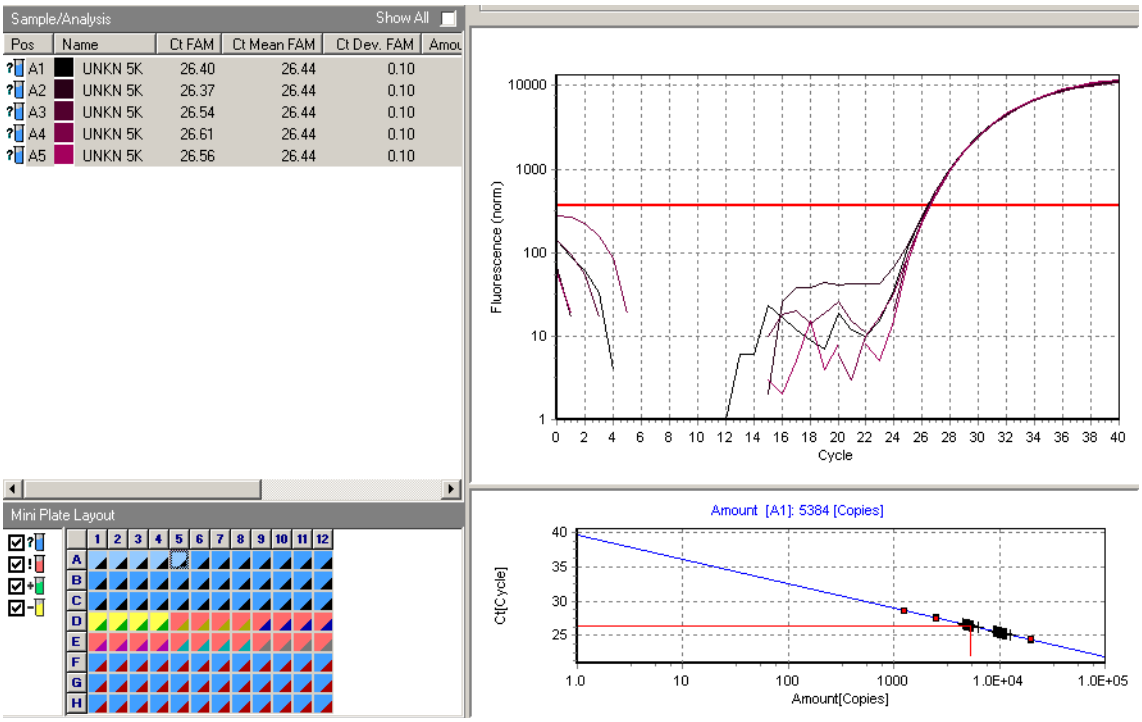
If the Mini Plate Layout is not visible it can be activated using the **Show / Hide Mini Plate Layout** button.

In the default view mode the check box **Show All** in the right upper corner of the results table is activated. Whereas in the work area only the selected samples are presented as curves or columns, all samples which have been defined in the plate layout are listed in the sample table.



6 Analysis

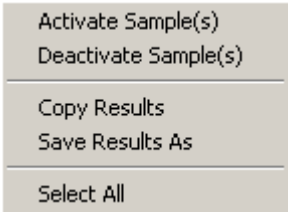
By deactivating the check box **Show All** another mode of sample presentation is chosen. In this case the results table shows only the samples selected in the **MiniPlate Layout**. If an assay contains a lot of samples, the sample table will be more clearly arranged by this way.



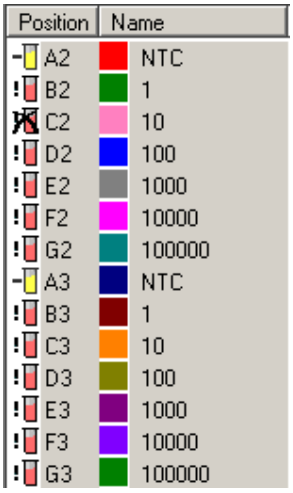
Note: A Report shows the currently selected view mode.

6.1.3 Deactivation of samples

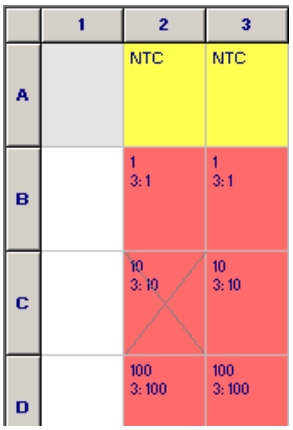
To exclude samples from analysis they can be deactivated. To do so one or more samples are highlighted in the sample table under **Analysis** and the function **Deactivate Sample(s)** selected using the right mouse button (alternatively: menu item "Commends").



The deactivated samples are marked in the sample table by a cross through the sample type symbol.



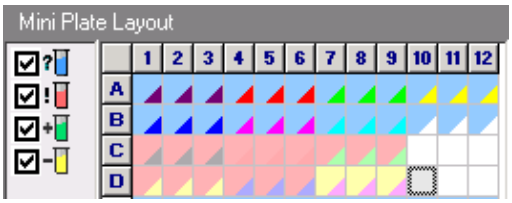
In addition, the deactivated sample is marked with a cross in the plate layout.



The analysis is then updated automatically. In order to reactivate the deactivated samples, the sample in the sample table is highlighted again and the function **Activate Sample(s)** selected using the right mouse button.

6.1.4 Selection of individual sample types

To show or hide the curves of individual sample types in the work field, they are activated or deactivated in the window **Mini Plate Layout** using the appropriate check box.

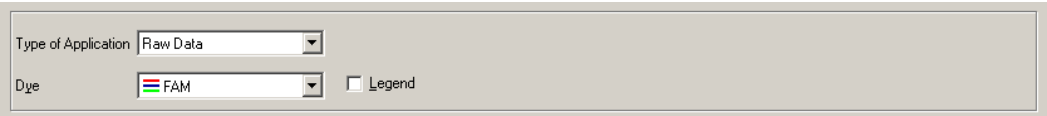


This selection does not apply to the sample table, nor does it affect the analysis – unlike the function **Deactivate Sample(s)** or **Activate Sample(s)**.

6 Analysis

6.1.5 Selection of analysis module

In the top panel different analysis modules can be selected using the combo box **Type of Application**. These data are then displayed in the work field according to the module. In addition, a legend can be inserted by selecting **Legend** on the right.



Note: If the required analysis module is not available in the selection, the settings necessary for this analysis have not been made in the plate layout.
Subsequent alteration of the plate layout is possible in the mode **Edit mode for plate layout** (see section 4.2.19).
If analysis modules are not available, this may also be due to the fact that settings necessary for this analysis are not included in the PCR program. In this case subsequent alteration is not possible.

With multiplex assays, the combo box **Dye** can also be used to select individual dyes. It is possible to display the data selectively for one dye or all of the dyes at the same time via **all dyes**.
Special settings for analysis (e.g. setting of threshold) are not possible in the view **all dyes**.

6.1.6 Zooming charts

The following icons are available to magnify and shrink the data in the work field:


The zoom tools are deactivated using **Unselect Zoom Functions**.


Parts of the charts can be magnified using **Zoom In**.

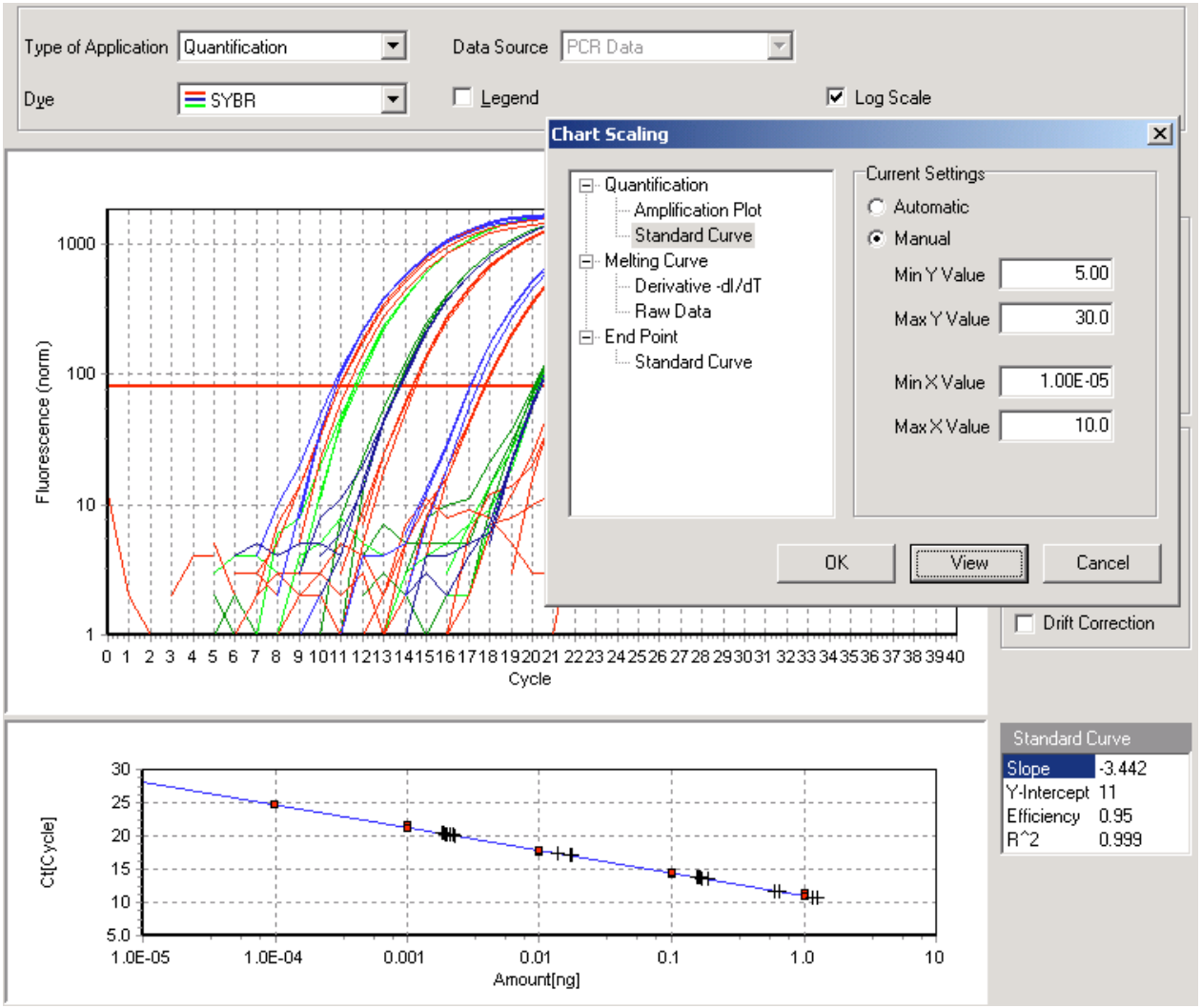

Charts can be shrunk by selecting **Zoom Out**.


If the icon **Unzoom** is selected, altered charts will be displayed according to the default.

To magnify or shrink charts, first select the relevant icon and then click on the chart with the tool then available.
This process is repeated until the chart attains the required size. To deactivate the tool select the icon **Unselect Zoom Functions**.

6.1.7 Modifying the scaling of charts

By default, all charts generated in the **Analysis** section are auto scaled. To set the axis scalings manually, right click inside the chart area and select **Scaling** from the context menu.



In the **Chart Scaling** dialog all charts from the modules that have been included in the analysis are listed in the tree. Select the radio button **Manual** for the chart to be rescaled and enter the minimum and maximum values for the X- and Y-axis. By clicking the **View** button, the modified chart can be previewed.

Note: The View button is inactive if the selected chart belongs to an analysis module other than the currently active.

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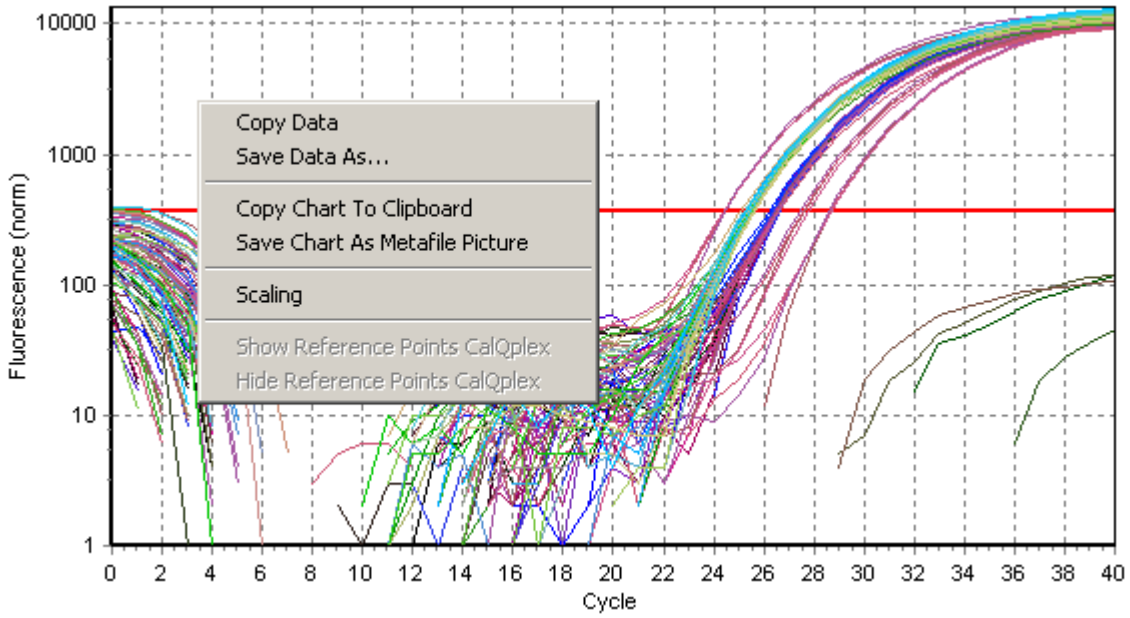
6.1.8 Restore default settings

Restore Defaults If this function is selected, the defaults will be restored for all parameters altered.

6.1.9 Copy and save data

For further analysis in other software programs, copy and save of fluorescence data is possible. Fluorescence data (raw data, PCR data, and melting curve data) are exported as indicated in the software (taking into account e.g. curve smoothing and base line correction).



To copy and save fluorescence data of samples the corresponding entry in the popup menu of the chart has to be selected. The software exports the data which are presented in the chart, i.e. only the samples selected in the sample table. Data of deactivated samples are not included. The data are displayed line-by-line (A1 –> A12).

The command **Copy Data** can be used to first copy the fluorescence data to the clipboard and then insert it in other documents.

If the command **Save Data As…** is selected, the file selection dialogue will be shown. The following data formats can be chosen: Text files (*.txt), comma separated (*.csv), and Microsoft Excel 97 (*.xls).

Note: Copying data of melting curves with a variety of data points per well could result in difficulties if pasted to Microsoft Excel or other spreadsheet applications due to a limitation in maximum column numbers.

6.1.10 Copy and save charts

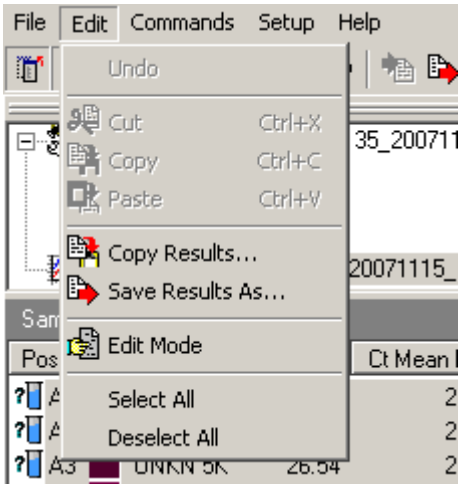
To save and copy individual charts first select with the cursor and then press the right mouse button. A popup menu will appear in the chart (see figure section 6.1.9).

If the command **Save Chart As Metafile Picture** is selected, the chart can be saved in EMF format.
The chart can be opened in Windows Explorer by double-clicking on it. In the program <Windows Picture and Fax Viewer> the chart can then be saved in other image formats or transferred to the image editing program <Windows Paint>.

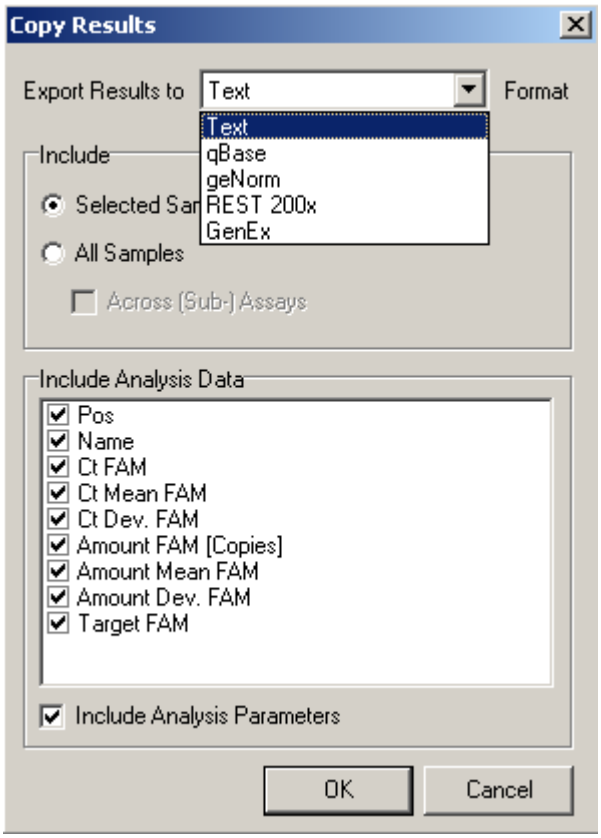
The command **Copy Chart To Clipboard** can be used to first copy the chart to the clipboard and then insert it in other documents.

6.1.11 Copy and save results as TXT, CSV, and XLS data format

Relevant commands in the menu item **Edit** can be used to copy and save results from the table **Samples/Analysis** in the navigation tree. Alternatively, the table Sample/Analysis can be selected with the cursor and a popup menu then opened with the right-hand mouse button.



The selection dialogue enables user-specific settings like data format as well as sample and analysis selection.



Note: Deactivated samples will not be exported by the software.

The following settings can be selected:

- Data format: The appropriate data format can be chosen by selecting the corresponding entry in the combo box.
- Sample selection: The selection can be carried out by activating the radio buttons for **Selected Samples** or **All Samples**. By choosing **Selected Samples**, only the samples selected in the results table are exported. **All Samples** refers to all samples present in the plate layout. The check box **Across (Sub-) Assays** will only be of interest, if Subassays are in use.

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- Selection of Analysis data (only selectable for Text format): Depending on what is selected all given columns of the results table or only a limited number of columns will be exported. For deselection of columns, the corresponding check box has to be deactivated. **Include Analysis Parameters** gives information concerning the analysis settings, e.g. threshold and baseline settings.

After the command **Copy Results** is selected the required settings have to be made in the selection dialogue. After confirming the dialogue by **OK**, the corresponding data is saved to the clipboard and can be inserted in another document. **Save Results As…** can be used to save the data from the table Sample/ Analysis as TXT, CSV, and XLS data format. For this purpose choose the format TXT first and close the dialog by **OK**. File directory, file name, and file type have to be chosen in the file selection dialogue **Save data as…**.

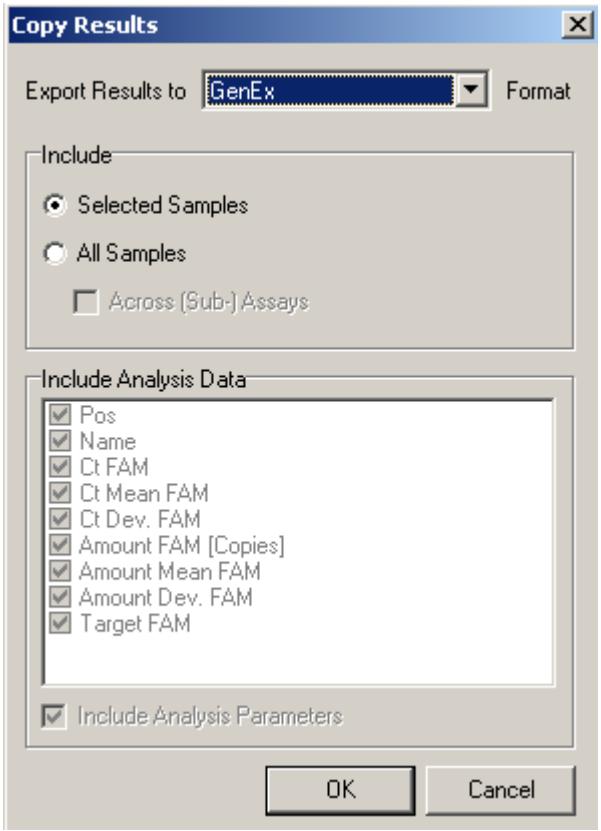
6.1.12 Copy and save results to special software tools for relative quantification

There are several specific software tools for calculation of gene expression and related relative quantification assays. The *realplex* application enables export of data to the most commonly used of these software tools (qBase, geNorm, REST and GenEx).

Note: For further information concerning the software tools please see corresponding literature and internet websites. In this manual, only the procedure of exporting data to these tools is described.

qBase	http://medgen.ugent.be/qBase/
geNorm	http://medgen.ugent.be/genorm/
REST	Relative Expression Software Tool http://www.gene-quantification.com
GenEx	http://www.multid.se

Access to **Copy Results** and **Save Results As…** is given as described in section 6.1.11. In the selection dialogue the software tool of interest can be selected in the combo box.



The entries qBase, geNorm, REST 200x and GenEx will only be present, if the relevant assay contains PCR data. Melting curve and endpoint data can not be used. Because results and sample information have to be arranged in a format specific to each of these software tools, the analysis data selection is deactivated.

The following options are available:

- Software tool
- **All Samples or Selected Samples**
- In the case of All Samples the check box **Across (Sub-) Assays** has to be activated if necessary.

File names of exported data are created based on the assay name and the corresponding ending of the file type. The software checks the exported data sets automatically with regard to completeness and consistency in sample and target name. If necessary, replicate sample numbers are removed automatically. If the requirements for the corresponding software tool are not fulfilled, one of the following error messages will be displayed.

- "The plate layout contains replicate groups with inconsistent sample names and targets. To resolve this, make sure that all samples within a replicate group have the same sample names and targets."
- "The plate layout contains samples with undefined sample names and/or target. To resolve this, enter sample names and target names for all samples."
- "The plate layout contains samples with duplicate combinations of sample name and target, which are not grouped as replicates. To resolve this, either group these samples as replicates or rename the samples."

In this case inconsistencies have to be corrected first. Afterwards **Copy / Save Results** can be repeated.

6.1.12.1 qBase

Export of real time PCR results to qBase is offered for qBase Version 1.3.5 and higher. Only samples of type Unknown, Standard, and Negative Control are exported in this format. In the All Dyes view, the format qBase can not be selected. If results of multiplexed assays are to be transferred to qBase, export the data of each dye to an extra file and import them in qBase to a single experiment data set. Output of data occurs line-by-line, sorted by well position.

Note: When importing assay data in qBase select "qBase" in the import dialog, since the "Eppendorf" import is not yet implemented in the current version of qBase.

Available file formats for qBase are: XLS (recommended), TXT, and CSV.

6.1.12.2 geNorm

Export of real time PCR results to geNorm is offered for geNorm Version 3.4 and higher. Only samples of type Unknown are exported in this format. Output of data based on C_T values is computed as described in the geNorm documentation and is arranged in a matrix of sample names and target names.

Available file formats for geNorm are: XLS (recommended), TXT, and CSV.

6.1.12.3 REST 200x

Export of real time PCR results to REST is offered for REST 200x Version 1.9.9 and higher. Only samples of type Unknown and Standard are exported in this format. For REST, the following information is exported from the Plate Layout: target names, housekeeping genes, calibrators. REST automatically calculates amplification efficiency for a target gene if a corresponding standard curve is present.

For REST 200x the only file format available is REST.

6.1.12.4 GenEx

Export of real time PCR results to GenEx is offered for GenEx Version 4.2. and higher. Only samples of type Unknown are exported in this format. Amounts of Mean Amounts (for replicate groups) are exported if a corresponding standard curve is present for each target to be exported. Otherwise C_T values or Mean C_T values are exported. Data are arranged as a matrix of sample names and target names.

Available file formats for GenEx are: XLS (recommended), TXT, and CSV.

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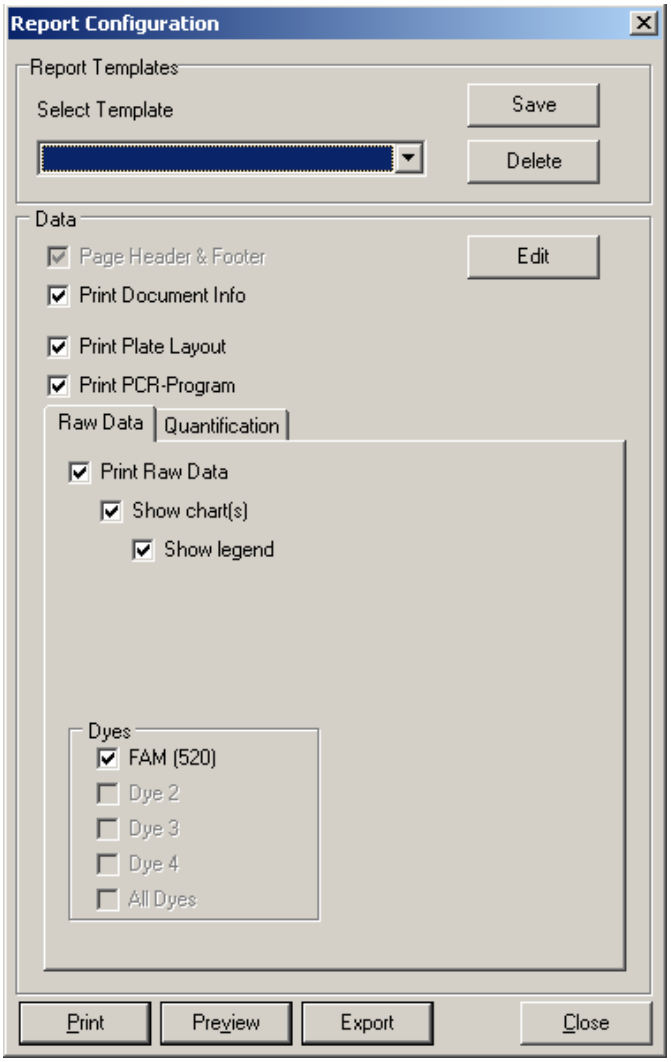
6.1.13 Compile reports

Reports can be used to make extensive compilations of experimental parameters and results which go beyond the standard screen display. They are output as a preview on screen, a printout to a printer or as a printable file in PDF format. After the function **Report** is selected under the menu item **File**, the dialog **Report Configuration** appears. This function is only active when in the module **Analysis**.

Alternatively, the dialog **Report Configuration** can also be opened using the icon **Print Report**.



By checking individual check boxes in the panel **Data** the user can select the data to be listed in the report. This includes general document information and the data from the assay setup. This is followed by tab sheets for raw data and the analyses, which have been saved for the assay or currently created. By selecting the appropriate check box the charts of the fluorescence data and the analyses can be inserted in the report as well as the associated legends. In addition, the user can select the dyes for which data are to appear in the report. To exclude complete analyses and all raw data from the report the top check box is deactivated in the relevant tab sheet. In the default setting all data of an assay are included in the report.

Note: It is only possible to select the analyses already performed by the user with the data record concerned under **Analysis** or previously saved by him. The function keys **Preview**, **Print** and **Export** are only accessible if at least one printer driver is installed on the computer!

With the function **Page Header & Footer**, items such as company names and logos can be inserted in the header line of the report.

Edit Alternatively, the dialog **Edit Page Header & Footer** can also be opened with the function key **Edit**.

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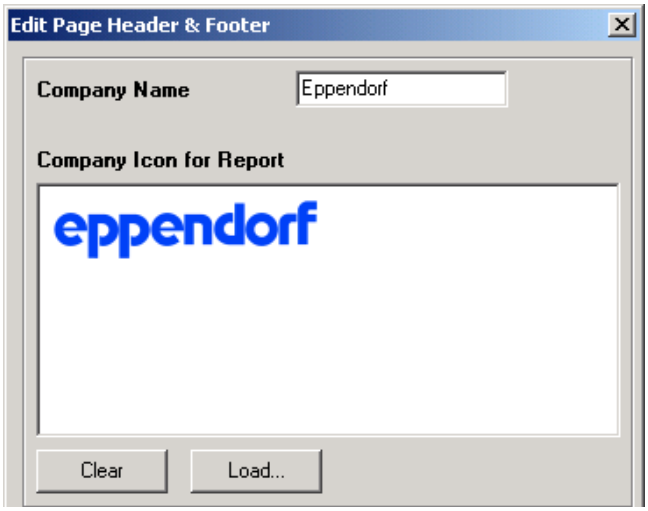


The name of the company or institute can be entered in the field **Company Name**.

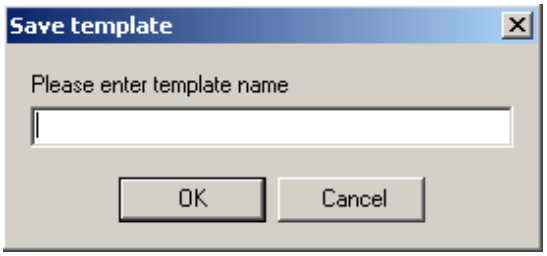
Clear Logos already present in the field **Company Icon for Report** can be deleted.

Load... If this function is selected, logos are loaded to the field **Company Icon for Report**.
Note: Only icons in BMP format can be inserted (size: max. 250 x 50 Pixel).

6.1.13.1 Work with report templates

User-specific report templates can be saved in the panel **Report Template** to simplify the preparation of reports.

Save After amending the report this function key is used to open the dialog **Save template**.
The name of the report is then entered and the input confirmed with **OK**.



To use a report template to prepare a report, a template is selected after opening the dialog **Report Configuration** using the combo box **Select Template** in the panel **Report Templates**.

Delete Report Templates can be deleted with this function key.

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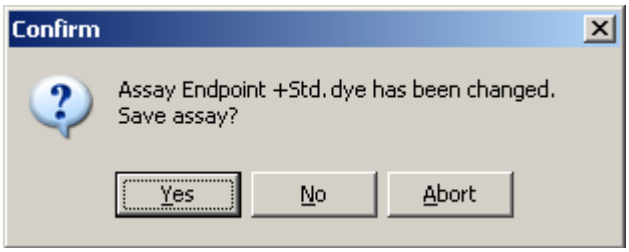
6.1.14 Save analyses

If the acquired data of an assay are analyzed, the user is automatically prompted to save the settings on opening another assay or closing the assay.



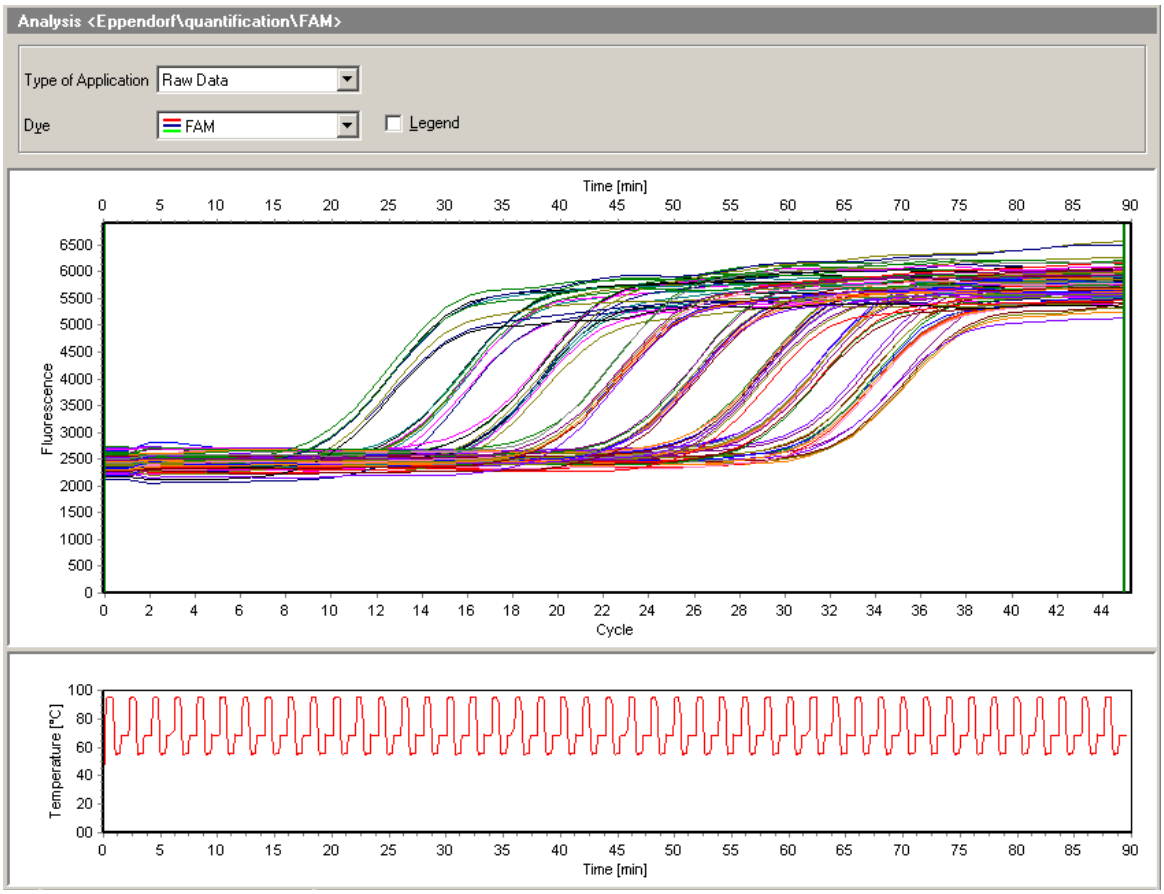
This also applies when changes have been made to an existing analysis.

6.1.15 Call up analyses

When opening an assay for which analyses have already been saved, the raw data first appear in the work field in the mode **Analysis**. If the relevant analysis is selected in the combo box **Type of Application**, the analysis appears with all saved settings.

6.2 Quantification

After completing an assay or opening a completed assay the raw data are loaded to the work area under **Analysis**. Here the fluorescence intensity is displayed as a function of the number of cycles and time. Underneath the temperature profile is shown in the course of time.



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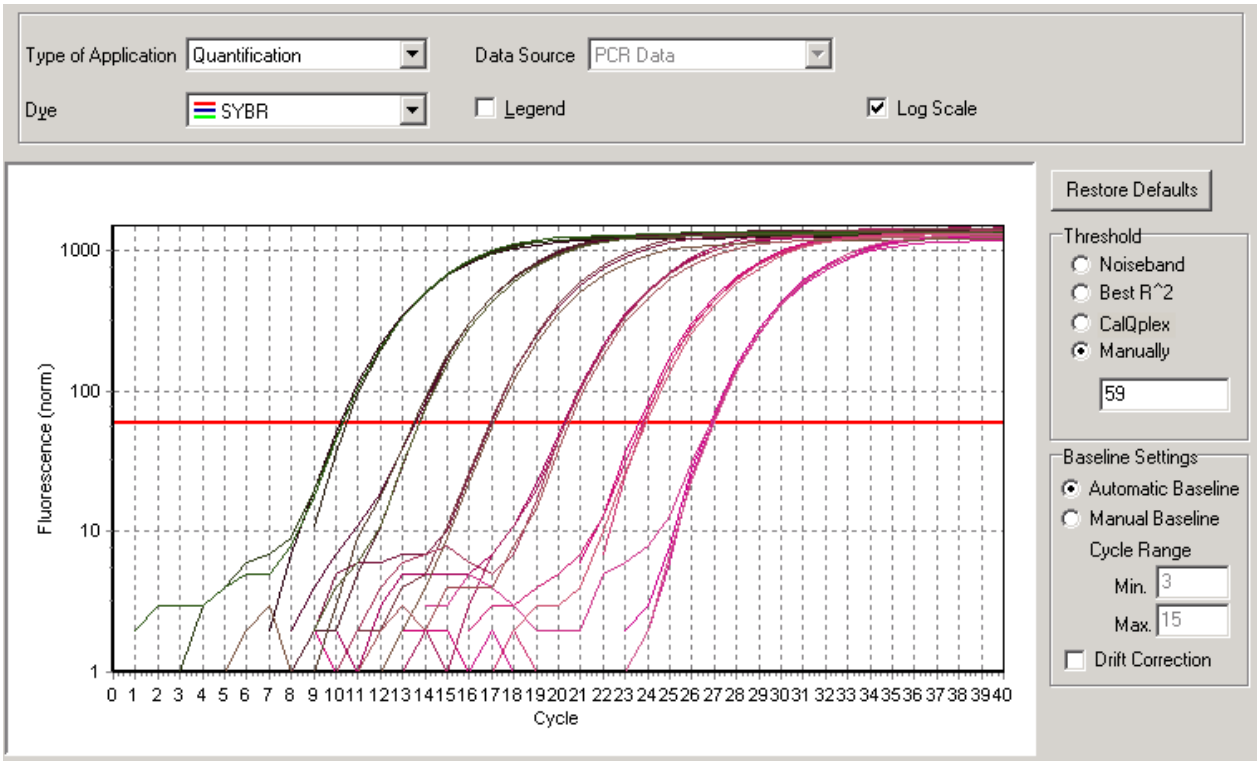
If **Quantification** is selected in the combo box **Type of Application**, the fluorescence curves are automatically shown in logarithmic display. If the data are to be presented in logarithmic display, the check box **log scale** must be activated.



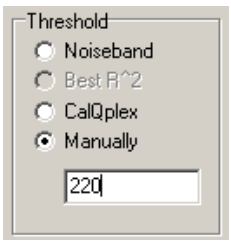
The header now also shows the field **Data Source**, indicating which data source was used as the basis for the analysis displayed. Basically there are three different data sources: endpoint determination, PCR data and melting curve analysis. The data sources which are available for analysis are determined by the PCR program in Assay Setup.

Note: Different data are only available for selection under **Data Source** with the analysis modules Gene Identification and +/- Assay.

6.2.1 Determination of threshold

The threshold is used to specify C_T values of samples. Here the C_T value is the cycle in which the fluorescence signal intersects with the threshold. The threshold is determined using the field on the right-hand side, with the data points of the exponential phase of the amplification curves being used to calculate the C_T values.

Three different methods for specifying the threshold are available in the panel **Threshold**:



The radio button **Noiseband** can be used for automatic determination of the threshold by the software. Here the threshold is specified so that it is significantly (10 times the standard deviation) above the noise of the baseline. **Noiseband** is preselected as the default setting by the software.

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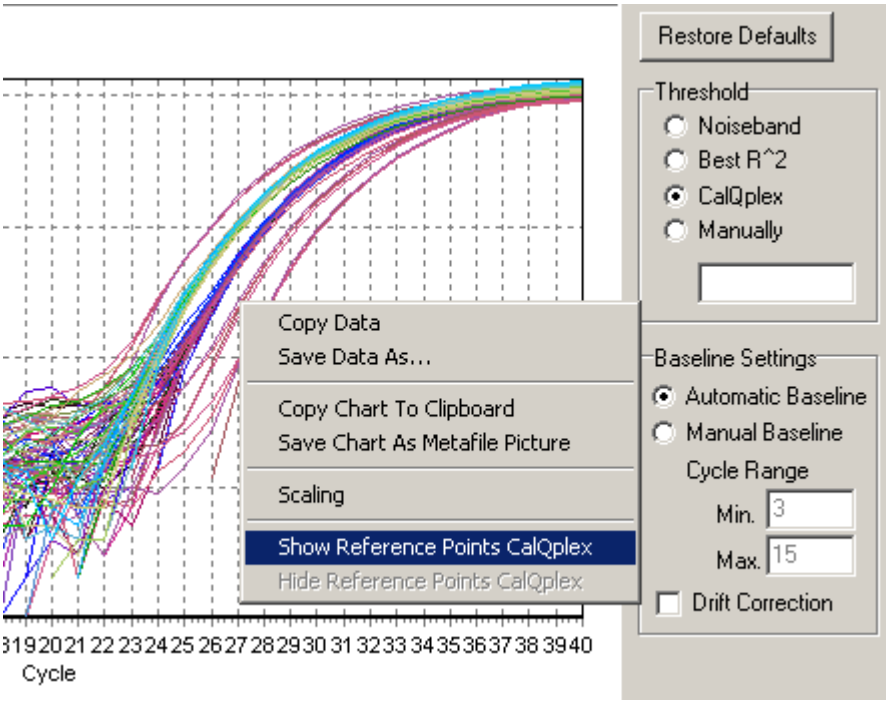
The function **CalQplex** can be used for automatically analysing single curves (Eppendorf algorithm). Fluorescence data are analysed based on a continuous growth model in combination with a baseline correction. The parameters of a perfect PCR curve are aligned to the experimental fluorescence curve of a given sample. The **CalQplex** method results in a reference cycle number for each fluorescence curve. This method does not rely on a threshold level. The reference cycle numbers are referred to as **Reference Points**. They correspond to the C_T values of the conventional methods and will be therefore displayed in the column C_T values. In default view mode the Reference Points are not shown in the amplification plots. To change this, the item **Show Reference Points CalQplex** has to be chosen in the right mouse popup menu. Reference Points are marked as crosses.



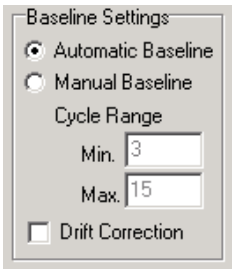
With the function **Best R^2** the software locates the threshold in the area in which all curves of the standards have a maximum value for correlation. If no standards have been defined, this function is inactive. At least three standard concentrations are necessary for this function to be active.

If the radio button **Manually** is selected, the location of the threshold is specified manually by inputting a fluorescence value. Alternatively, the threshold can also be directly moved in the chart using the left mouse button.

6.2.2 Determination of baseline

The panel **Baseline Settings** is used to specify the parameters for calculation of the baseline.

Automatic calculation of the baseline is selected as the default setting. In this case the optimum baseline is calculated for every sample individually.



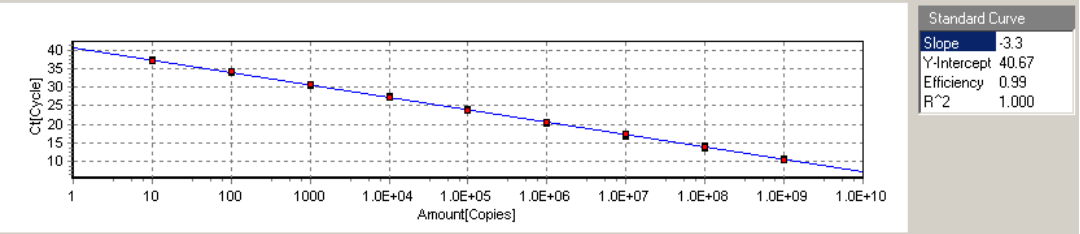
To adjust the baseline the radio button **Manual Baseline** is selected, now making the previously inactive **Cycle Range** accessible. In the relevant fields the user can enter the minimum and maximum cycle between which the baseline is to be calculated for all curves. Here the area between cycle 3 and 15 is given as the default setting.

In addition, the drift in the baseline can be corrected in this panel by activating the check box **Drift Correction**. This correction will affect all curves equally.

Note: All settings made in this panel affect the curve shapes and the C_T values.

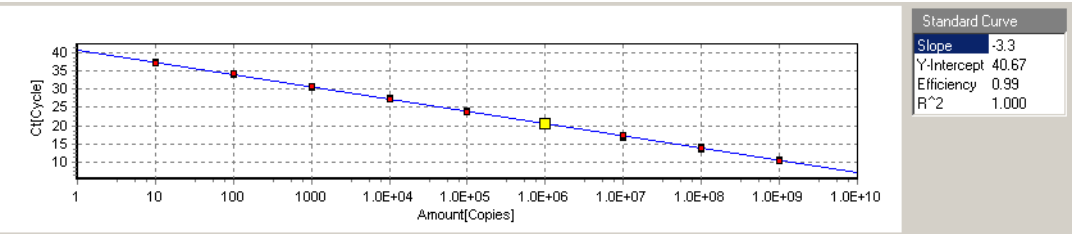
6.2.3 Standard curve

The software automatically uses the C_T values of the standards to calculate a standard curve which shows the C_T values as a function of the amount of nucleic acid used. The unit of the standard concentrations was determined when editing the standards in the plate layout.

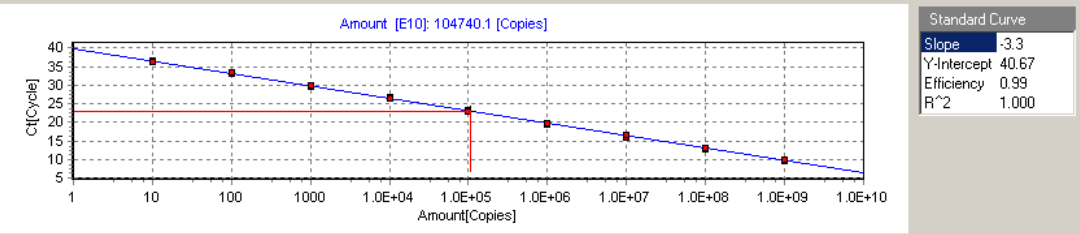


Slope	Increase in standard curve
Y-Intercept	Point at which the standard curve intersects with the Y-axis (based on amount = 1 [unit])
Efficiency	Efficiency of the PCR $E = 10^{[-1/slope]} - 1$ The value for E should be approx. 1
R^2	Correlation coefficient of standard curve

The measured values of the standards are marked with red squares (see above) and of the unknown samples with black crosses. If individual standards are selected in the sample table, the associated measured value is indicated by a yellow square in the standard curve.



If individual unknown samples are selected, the calculated C_T value and associated concentration of the unknown sample are indicated by red lines in the standard curve.



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6.2.4 Analysis

The standard curve allows the user to conclude the initial amount of nucleic acid of the unknown samples from a specific C_T value. The results are calculated automatically by the software and can be found in the table **Sample/Analysis**.

Sample/Analysis

Pos	Name	Ct FAM ...	Ct Mean ...	Ct Dev. ...	Amount ...	Amount Mean ...	Amount Dev. ...	Target ...
! A1	Std 1	11.69	11.62	0.07	1.00E+08			Lambda
! A2	Std 1	11.59	11.62	0.07	1.00E+08			Lambda
! A3	Std 1	11.57	11.62	0.07	1.00E+08			Lambda
! A4	Std 1	11.64	11.62	0.07	1.00E+08			Lambda
! A5	Std 1	11.60	11.62	0.07	1.00E+08			Lambda
! A6	Std 1	11.58	11.62	0.07	1.00E+08			Lambda
! A7	Std 1	11.74	11.62	0.07	1.00E+08			Lambda
! A8	Std 1	11.56	11.62	0.07	1.00E+08			Lambda
? A9	Sample A	11.72	11.79	0.08	8.21E+07	7.88E+07	4.15E+06	Lambda
? A...	Sample A	11.90	11.79	0.08	7.34E+07	7.88E+07	4.15E+06	Lambda
? A...	Sample A	11.81	11.79	0.08	7.78E+07	7.88E+07	4.15E+06	Lambda
? A...	Sample A	11.72	11.79	0.08	8.19E+07	7.88E+07	4.15E+06	Lambda
! B1	Std 2	14.99	15.07	0.12	1.00E+07			Lambda
! B2	Std 2	15.17	15.07	0.12	1.00E+07			Lambda
! B3	Std 2	15.11	15.07	0.12	1.00E+07			Lambda
! B4	Std 2	15.29	15.07	0.12	1.00E+07			Lambda
! B5	Std 2	15.13	15.07	0.12	1.00E+07			Lambda

Pos — Specifies the position of the sample in the plate layout. For simple assignment the samples are also marked with the symbols for the respective sample type.

Name — Name of sample

Ct — C_T value
If an internal reference dye is used in a concentration that gives relative fluorescence signals < 100, the entry **Ref.dye too low** will appear. In this case, no curve is shown in the amplification curve.

IPC — Internal positive control (only present if selected in plate layout)

Amount — Initial amount of nucleic acid, specified by the user with these standards. With unknown samples and negative controls this is calculated by the software based on the standard curves.

Mean — Mean value for C_T value and amount, respectively, when determining unknown samples in replicates.
Note: Only included when replicates defined in plate layout.

Deviation — Standard deviation for C_T value and amount, respectively, when determining unknown samples in replicates.
Note: Only included when replicates defined in plate layout.

Target Gene — Target gene (name determined in plate layout when editing sample).

Note: The sequence of the samples can be changed by double-clicking on the heading of individual columns in the sample table.

6.2.5 Multiplex analysis

If different genes are quantified in parallel in a multiplex analysis, a separate standard curve must be created for every gene. To do so the relevant dye is selected in the top panel using the combo box **Dye**.

If **All Dyes** is selected in the combo box **Dye**, the curve shapes for all dyes are displayed. In this mode it is not possible to display the standard curves at the same time.

6.3 Relative quantification

The Relative Quantification analysis module is used to compare expression levels of a target gene among differently treated samples. The expression levels are calculated using the $\Delta\Delta C_T$ method (Livak and Schmittgen. 2001, *Methods* 25(4): 402–408). A prerequisite to yield valid relative expression values is that the efficiency is comparable *and* close to 1 in the PCR systems of both target gene and housekeeping gene.

The expression levels *R* are calculated as follows:

$$R = 2^{-\Delta\Delta CT} = 2^{-(\Delta CT \text{ sample} - \Delta CT \text{ calibrator})}$$

$$= 2^{-[(CT \text{ sample} - CT \text{ housekeeping gene}) - (CT \text{ calibrator} - CT \text{ housekeeping gene})]}$$

The assignment of target and housekeeping gene pairs and calibrators is made through the **Create Links** dialog. The relative quantification analysis option is only available if a suitable set of samples has been defined in the plate layout (see chapter 4.2.9) and the PCR run is completed.

6.3.1 Performing a relative quantification analysis

1. In the navigator window move to the **Analysis** section and select **Relative Quantification** from the **Type of Application** combo box.
2. Select a **Dye** from the combo box and mark the samples to be included in the **Sample / Analysis** list.
   The amplification curves are then displayed in the graph window. Adjust the **Threshold** and **Baseline Settings** according to chapters 6.2.1 and 6.2.2.
3. For multiplex assays, repeat step 2 for all additional dyes used.

The resulting expression levels are displayed in the **Sample / Analysis** list.

6.3.2 Results table for monoplex assays

For every sample individual C_T values and mean C_T and standard deviation of replicates are displayed. The mean expression levels and the range of individual expression levels are listed in columns **Expr. Level** and **Range** for positions where a non-calibrator target gene is defined. The expression level of the calibrator target gene is 1 per definition and is not displayed. Expression level values correspond to a fold expression of the target gene compared to the calibrator sample.

Sample/Analysis

Pos	Name	Ct FAM...	Ct Mean FAM ...	Ct Dev. FAM...	Expr. Level FAM...	Range FAM...
B5	Exp_B	24.78	24.65	0.14	2.1	1.8 - 2.5
B6	Exp_B	24.74	24.65	0.14	2.1	1.8 - 2.5
B7	Exp_B	24.47	24.65	0.14	2.1	1.8 - 2.5
B8	Exp_B	24.60	24.65	0.14	2.1	1.8 - 2.5
B9	Exp_C	22.56	22.47	0.11	10	8.8 - 11
B...	Exp_C	22.33	22.47	0.11	10	8.8 - 11
B...	Exp_C	22.55	22.47	0.11	10	8.8 - 11
B...	Exp_C	22.45	22.47	0.11	10	8.8 - 11
C1	Control	25.56	25.80	0.19		
C2	Control	26.02	25.80	0.19		
C3	Control	25.79	25.80	0.19		
C4	Control	25.82	25.80	0.19		
C5	Exp_A	26.01	25.85	0.17	0.93	0.80 - 1.1
C6	Exp_A	25.76	25.85	0.17	0.93	0.80 - 1.1
C7	Exp_A	25.97	25.85	0.17	0.93	0.80 - 1.1

Pos — Specifies the position of the sample in the plate layout. For simple assignment the samples are also marked with the symbols for the respective sample type. The sample defined as the calibrator in the plate layout is marked with a C.

Name — Name of sample.

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Ct FAM	C_T value for target gene (here: FAM-marked probe). Mean value and standard deviation for C_T value are shown, when determining unknown samples in replicates.
Expr. Level...	Expression level of sample calculated according to $\Delta\Delta C_T$ method.
Range	Range of Expression level according to $\Delta\Delta C_T$ method.

6.3.3 Results table for multiplex assays

If **All Dyes** is selected from the **Dyes** combo box, individual C_T values and mean C_T and standard deviation of replicates for all dyes used are displayed. The mean expression levels and the range of individual expression levels are listed in columns **Expr. Level** and **Range** for all dyes used for detection of a non-calibrator target genes is defined. The expression level of the calibrator target gene is 1 per definition and is not displayed.

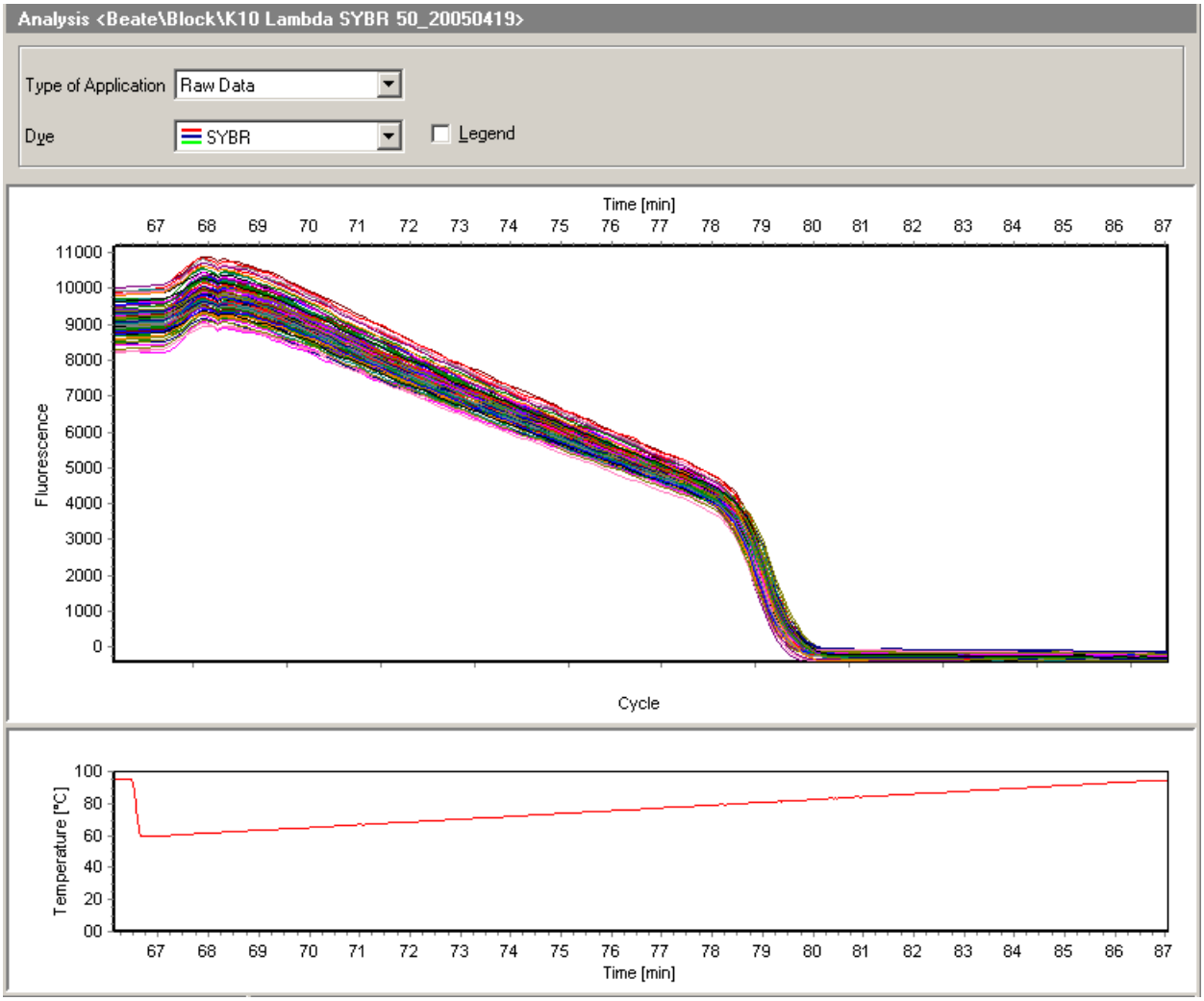
Sample/Analysis

Pos	Name	Ct FAM...	Ct Mean FAM...	Ct Dev. FAM...	Expr. ...	Range F...	Ct JOE...	Ct Mean JOE...	Ct ...
A1	Control	25.92	25.10	0.74			28.84	27.87	0.80
A2	Control	24.33	25.10	0.74			27.36	27.87	0.80
A3	Control	25.50	25.10	0.74			28.18	27.87	0.80
A4	Control	24.65	25.10	0.74			27.09	27.87	0.80
A5	Exp_A	25.00	25.53	0.40	0.92	0.53 - 1.6	27.39	28.17	0.87
A6	Exp_A	25.44	25.53	0.40	0.92	0.53 - 1.6	28.71	28.17	0.87
A7	Exp_A	25.83	25.53	0.40	0.92	0.53 - 1.6	27.46	28.17	0.87
A8	Exp_A	25.85	25.53	0.40	0.92	0.53 - 1.6	29.10	28.17	0.87
A9	Exp_B	24.57	24.78	0.17	1.8	1.1 - 2.7	28.36	28.37	0.52
A...	Exp_B	24.75	24.78	0.17	1.8	1.1 - 2.7	29.00	28.37	0.52
A...	Exp_B	24.96	24.78	0.17	1.8	1.1 - 2.7	27.72	28.37	0.52
A...	Exp_B	24.86	24.78	0.17	1.8	1.1 - 2.7	28.40	28.37	0.52
B1	Exp_D	22.92	22.85	0.14	6.5	4.2 - 10	28.48	28.32	0.77

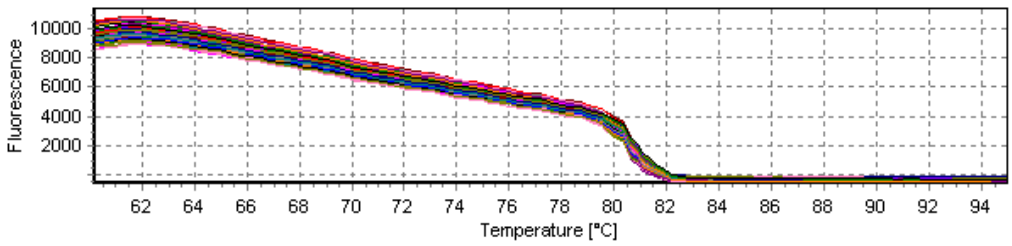
6.4 Melting curve analysis

Melting curve analysis is generally performed after a PCR by continuously measuring the fluorescence intensity of the reaction samples during a slow increase or fall in temperature. This process can be used to determine the melting temperature (Tm) of a PCR product (e.g. marked with SYBR-Green I) or when using specific probes, the melting temperature of the resulting hybrids.

After completing an assay or opening a completed assay the raw data are loaded to the work area under **Analysis**. Here the fluorescence intensity is displayed as a function of time. Underneath the temperature profile is shown in the course of time.



To analyze the melting points the analysis module **Melting Curve** first has to be selected in the combo box **Type of Application**. The fall in the fluorescence intensity is shown in the chart below as a function of temperature. At the melting point the measured signal curves show a maximum in the fall in fluorescence intensity.



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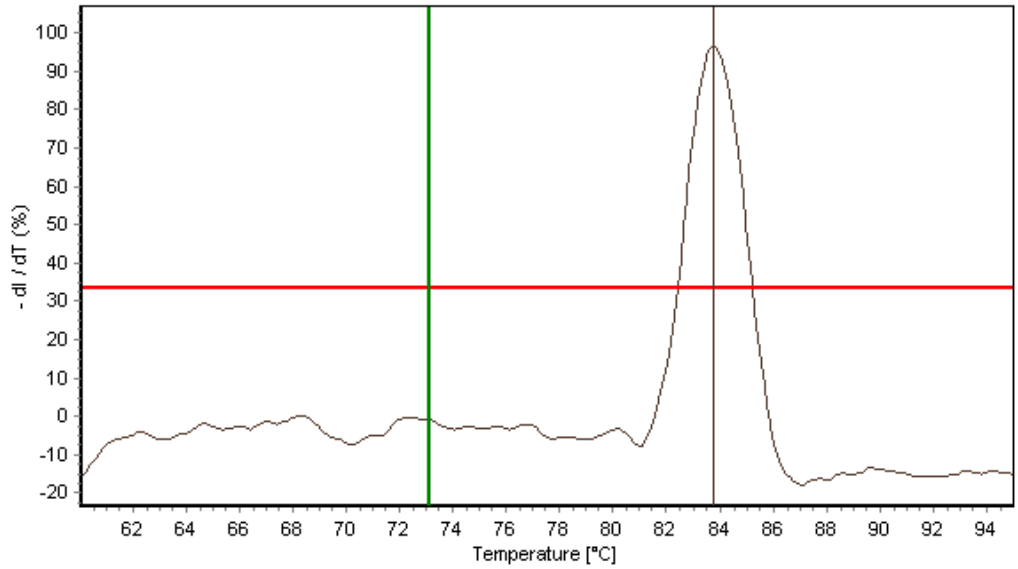
To allow the maximum intensity changes to be identified more easily the melting curves are shown as peak curves. These peak curves are the negative first derivative of the melting curves as a function of temperature (-dI/dT). The unit of the y-axis is given in %, with 100 % representing the highest peak of the assay.

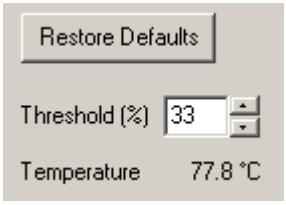


The location of the peak maximum corresponds to the melting temperature of the product. This is determined by the software and marked by vertical lines. A vertical line will only appear if a single sample is selected.

6.4.1 Determination of threshold

A threshold is determined for the melting curve analysis. Here it should be borne in mind that it is the peaks above the threshold that are analyzed, not those below. This is shown as a horizontal line in red and can be moved using the spin edit to the right of the chart. The default setting is 33 %.

Alternatively, the threshold can also be changed by manually moving the red line.



Melting points of individual samples can be checked manually using the green vertical line. To do so it is moved to the point of the peak maximum, and the melting point can then be read off on the right under the threshold input.

6.4.2 Analysis

The melting points of the samples determined by the software can be found in the table **Sample/Analysis**.

Sample/Analysis — Show All

Pos	Name	No. Tm...	Tm x (°C) SYBR	Tm y (°C) SYBR
A1	NTC	1	84.0	
A2	NTC	1	83.8	
A3	NTC	1	83.7	
A4	500	1	83.8	
A5	500	1	83.9	
A6	500	1	83.9	
A7	1000	1	83.9	
A8	1000	1	83.9	
A9	1000	1	83.9	
A...	2000	1	83.8	
A...	2000	1	83.9	
A...	2000	1	84.0	

Pos — Specifies the position of the sample in the plate layout. For simple assignment the samples are also marked with the symbols for the respective sample type.

Name — Name of sample

No. Tm — Indicates whether and how many melting points were identified.

Tm(s) (°C) — Melting point of sample determined by the software.

Tm x (°C) — Melting point no.1 of sample determined by the software.
In the case of two melting points, the lower temperature is listed here.

Tm y (°C) — Melting point no.2 of sample determined by the software.
In the case of two melting points, the higher temperature is listed here.
If only one melting point is present, there will be no entry in this column.

Mean — Mean value for Tm(s) when determining unknown samples in replicates.
Note: Only included when replicates were defined in the plate layout and only one melting point is determined per sample and dye.

Deviation — Standard deviation when determining unknown samples in replicates.
Note: Only included when replicates were defined in the plate layout and only one melting point is determined per sample and dye.

The software is capable of determining max. two melting points per sample. If a curve has more than two peaks above the threshold, no entry will be made in the sample table. In this case analysis can be carried out manually.

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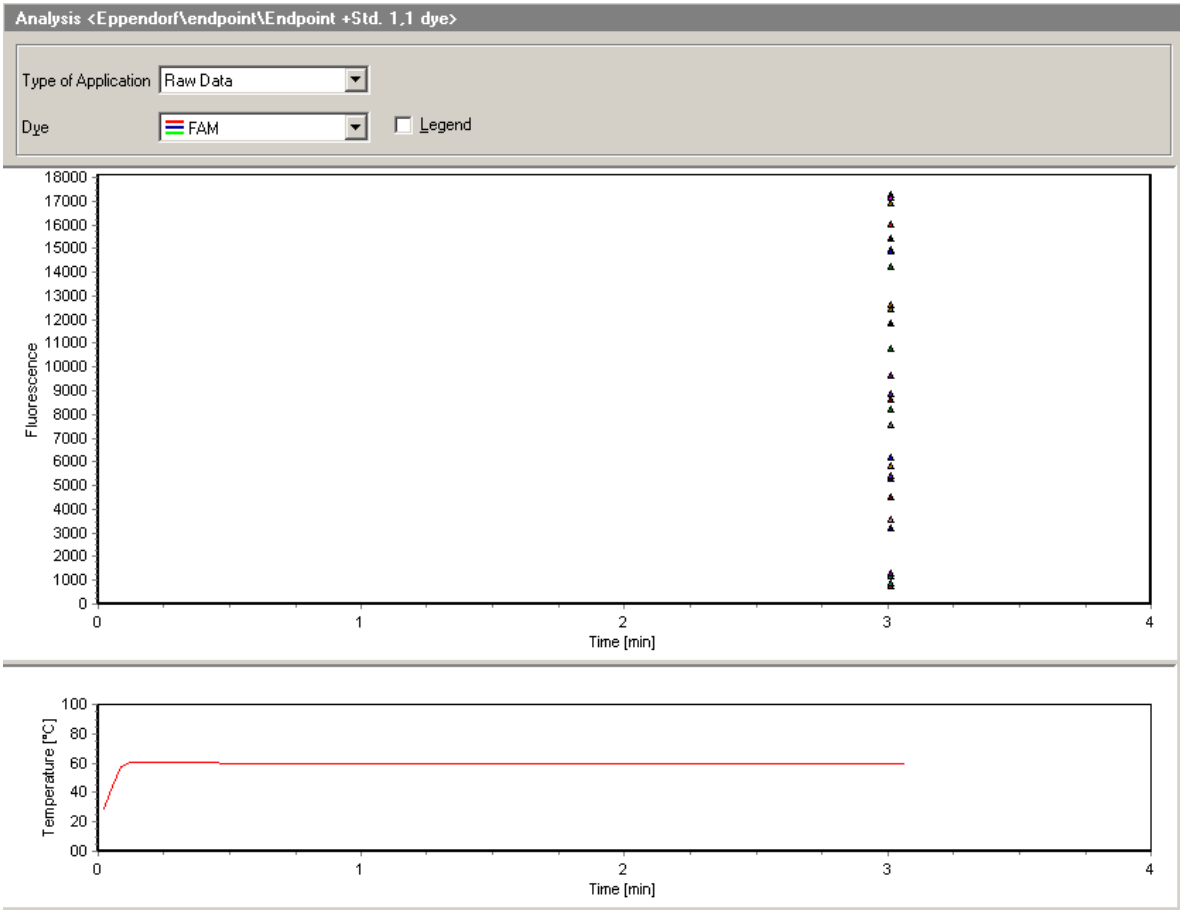
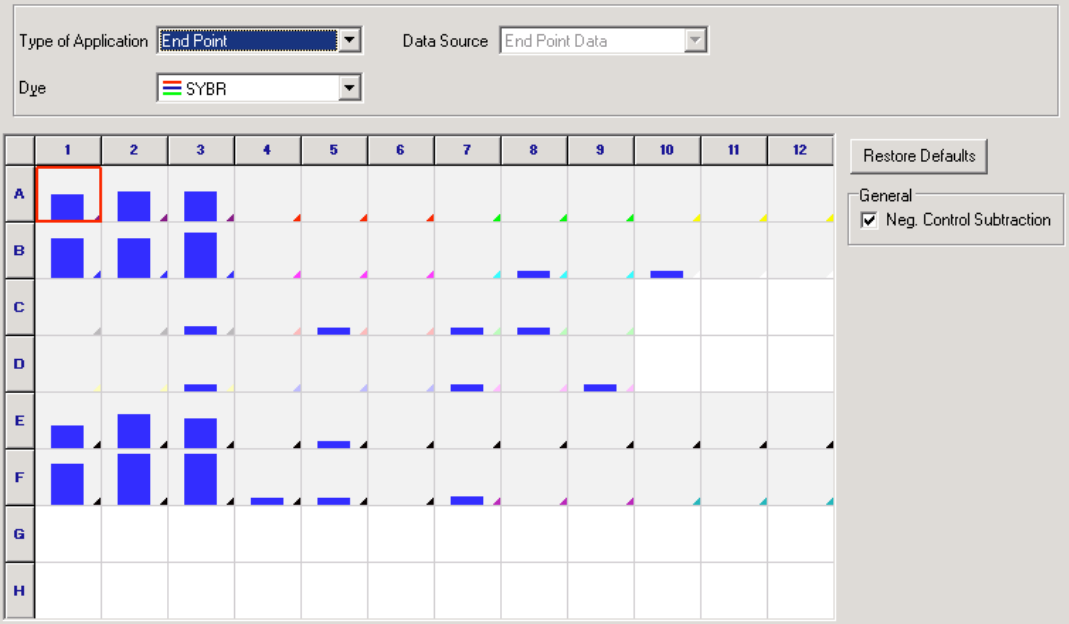
6.5 Endpoint measurement

Endpoint measurement is performed by including the step **Endpoint** in the PCR program. Here data acquisition can take place after the creation of PCR data, but also independently of data acquisition during a PCR (see section 5.12).

After completing an assay or opening a completed assay the raw data are loaded to the work area under **Analysis**. Here the fluorescence intensity is displayed as a function of the time. Underneath the temperature profile is shown in the course of time.



If **Endpoint** is selected in the combo box **Type of Application**, the fluorescence data of the endpoint measurement are shown as bars in all allocated positions of the PCR plate or block when using individual tubes. The scale range of the height of the bars is related to the maximum value ascertained on endpoint measurement. This takes up the entire height. Negative values are not shown here but can be found in the sample table. Replicates defined in the plate layout as a group are indicated by colored triangles (see section 4.2.16).

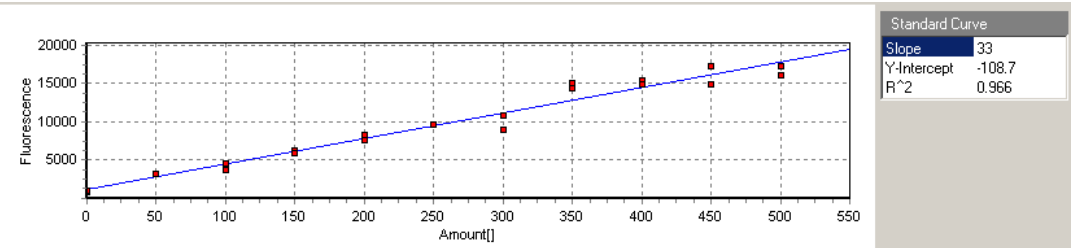


If samples have been defined as negative controls in the plate layout, the fluorescence values of the negative controls can be deducted from all other measured values by activating the check box **Neg. Control Subtraction** in the panel **General**. With more than one negative control their mean value is subtracted. In the presence of more than one group of negative controls, the mean of all of them is used.

All measured values can be found in the table **Samples/Analysis**.

6.5.1 Standard curve

If standards have been defined in the plate layout, a standard curve is calculated by the software. This shows the fluorescence intensity as a function of the amount used. The unit is determined when editing the standards in the plate layout.



Slope	Increase in standard curve
Y-Intercept	Point at which the standard curve intersects with the Y-axis
R^2	Correlation coefficient of standard curve

The measured values of the standards are marked with red squares and of the unknown samples with black crosses.

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6.5.2 Analysis

The standard curves are used to calculate the amount for every measured fluorescence value of an unknown sample or negative control. They can be found in the table **Samples/Analysis**.

Sample/Analysis

Pos	Name	Endpoint SYBR	Amount SYBR [ng/µl]	Mean SYBR	Dev. SY...
H2	Control	115	5.85E-02	-1.02E-02	9.72E-02
H3	Control	74	-7.89E-02	-1.02E-02	9.72E-02
F2	Sample B	206	0.364	0.431	9.48E-02
F3	Sample B	246	0.498	0.431	9.48E-02
B2	Sample C	2322	7.46	7.53	0.107
B3	Sample C	2367	7.61	7.53	0.107
A2	Std 1	3085	10.0		
A3	Std 1	3181	10.0		
C2	Std 2	346	1.00		
C3	Std 2	388	1.00		
D2	Std 3	260	0.500		

Pos — Specifies the position of the sample in the plate layout. For simple assignment the samples are also marked with the symbols for the respective sample type.

Name — Name of sample

Endpoint — Fluorescence intensity of sample measured during endpoint determination.

Amount — Initial amount of nucleic acid, specified by the user with these standards. With unknown samples and negative controls this is calculated by the software based on the standard curves.

Mean — Mean value when determining unknown samples in replicates.
Note: Only active when replicates were defined in plate layout.

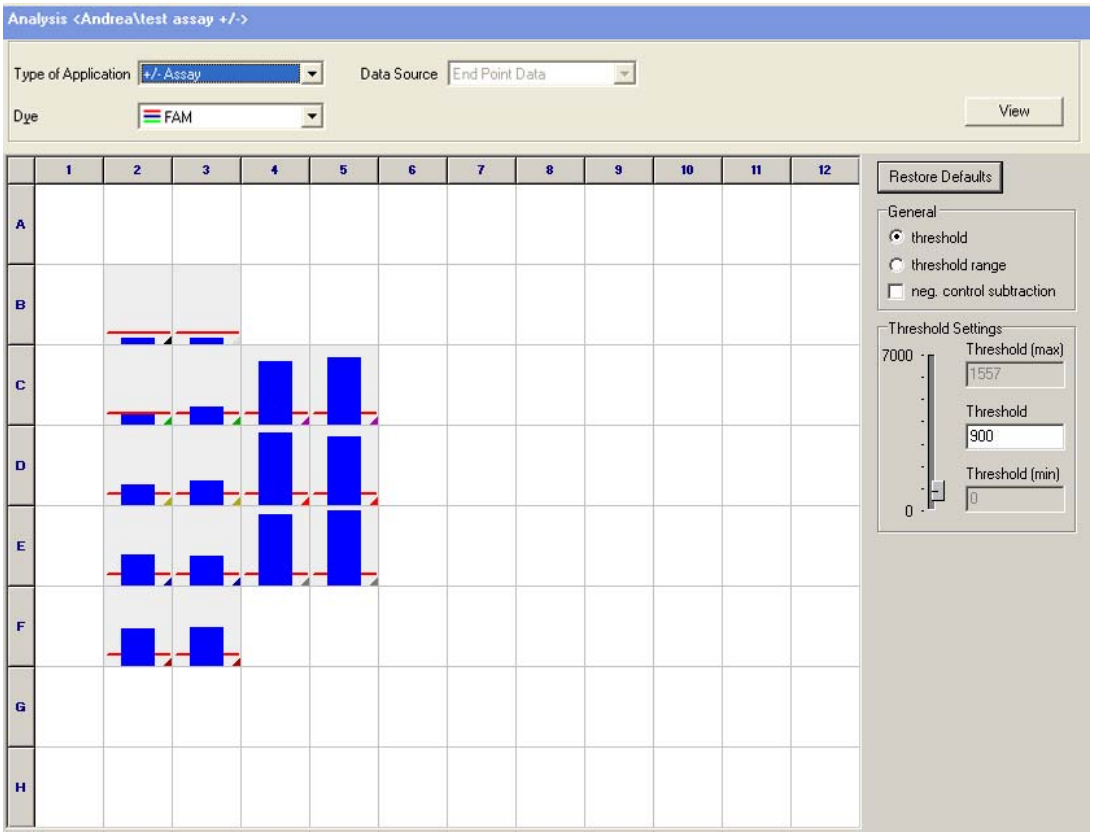
Deviation — Standard deviation when determining unknown samples in replicates.
Note: Only active when replicates were defined in plate layout.

6.6 +/- Assay

Data from endpoint measurement or a PCR can be analyzed in the analysis module **+/- Assay**. When the assay is opened, raw data for a PCR run and/or for endpoint determination will appear accordingly in the right-hand work field.

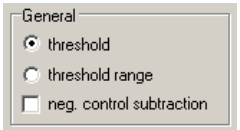
6.6.1 +/-Assay with endpoint determination as data source

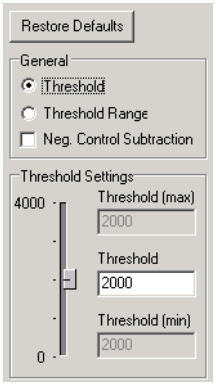
If **+/-Assay** is selected using the combo box **Type of Application** followed by the data source **Endpoint Data** with the combo box **Data Source**, the fluorescence data of this endpoint measurement are shown as bars in all allocated positions of a PCR plate. Here grouped replicates are indicated by colored triangles (see section 4.2.16).



6.6.1.1 Determination of threshold

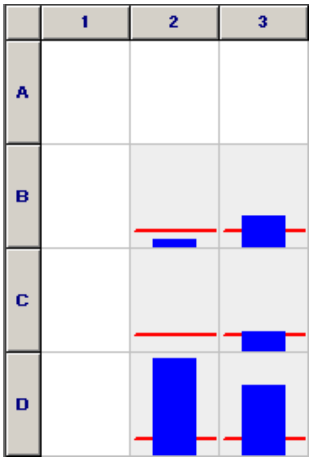
The determination of a threshold defines when a sample should be seen as positive (+) or as negative (-). Here the samples below the threshold are categorized as negative and those above it as positive. To do so the type of threshold first has to be specified in the panel **General**. The threshold is shown as a red line in all occupied positions of the PCR plate.





If the radio button **Threshold** is selected, a fixed threshold can be specified in the panel **Threshold Settings**. This can be carried out either by entering a numerical value or by moving the track bar. The greatest possible threshold corresponds to the maximum value determined on endpoint measurement.

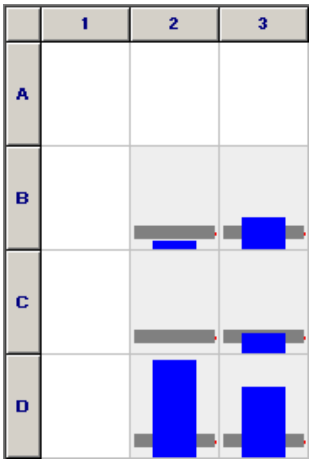
The location of the threshold is marked by a red line.



Note: Every change in the threshold has a direct influence on whether a sample is categorized as negative or positive.

If the radio button **Threshold Range** is selected, it is possible to enter a fluorescence range for the threshold in the panel **Threshold Settings**. Here too either a numerical value can be directly entered or the left track bar moved. If samples show a fluorescence within the range, they will be automatically categorized as inconclusive.

The threshold range is marked in grey.



Note: If the fluorescence range of the threshold is changed, this may lead to a change in the categorization as negative, positive and inconclusive results.

If samples have been defined as negative controls in the plate layout, the fluorescence intensity of the negative controls can be deducted from all other measured values by activating the check box **Neg. Control Subtraction**. If more than one negative control has been edited in the plate layout, the mean value is subtracted.

6.6.2 +/-Assay with PCR data as data source

If **+/-Assay** is selected using the combo box Type of Application followed by **PCR Data** under **Data Source**, the fluorescence curves will appear in the right-hand work field.

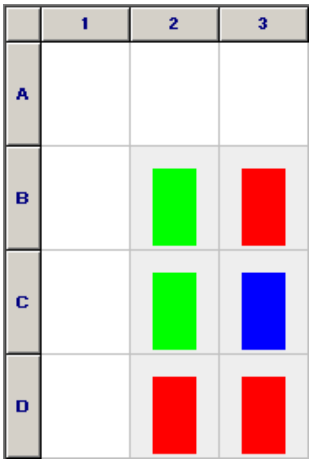
6.6.2.1 Determination of threshold

The determination of a threshold defines when a sample should be seen as positive (+) or as negative (-). Here the samples below the threshold are categorized as negative and those above it as positive. There are no inconclusive results. See section 6.2.1 for further details for the determination of thresholds.

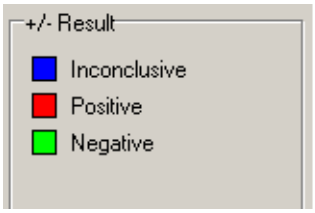
6.6.3 Analysis

View The function **View** in the top panel can be used to display the results in the work area.

The results are shown as different-colored bars of the same height in the allocated positions of the PCR plate.



The legend is shown in the panel **+/- result**. Besides positive and negative results inconclusive results are also displayed. They are indicated by lying within the **Threshold range** and being marked as **inconclusive**. If no **Threshold range** but a **Threshold** was specified, a result marked **inconclusive** is not possible.



In addition, the results are shown in the table **Samples/Analysis**. The descriptions positive, negative and **inconclusive** are shown in the column **+/- result [Dye]** beside the fluorescence data.

Sample/Analysis

Pos	Name	+/- Result	Endpoint
F7	sample 8	positive	16913
E7	sample 7	positive	11881
D7	sample 6	inconclusi...	8214
C7	sample 5	negative	5401
F6	sample 4	positive	12453
E6	sample 3	positive	12638
D6	sample 2	inconclusi...	8614
C6	sample 1	negative	5306
B6	Neg. Control	negative	1214
B7	Neg. Control	negative	1300
F4	Pos. Control	positive	16058
F5	Pos. Control	positive	17138

Evaluation If this function is selected, the measured values of endpoint measurement or of the PCR are loaded to the work area again.

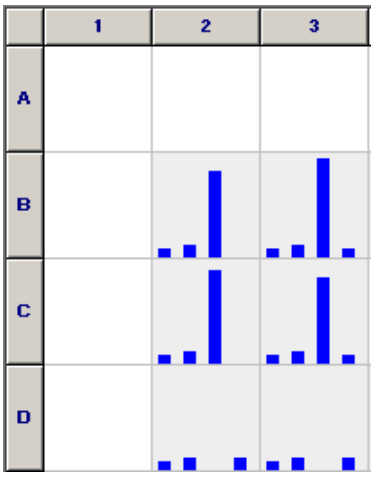
6.6.4 Multiplex analysis

If different genes are determined in parallel in a multiplex analysis, separate analysis must be carried out for every target gene. To do so the relevant dye is selected in the top panel using the combo box **Dye**.

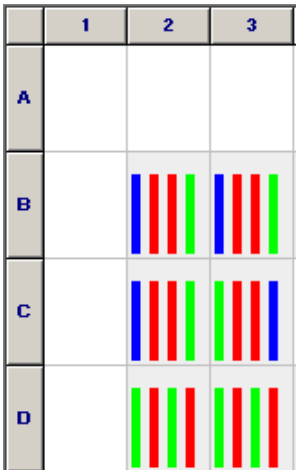
All results can be displayed if **All Dyes** is selected in the combo box **Dye**.

With endpoint determination in the mode **Evaluation** the fluorescence values of the different dyes are shown as blue bars for every sample. The assignment of the bars to the target genes analyzed can be found in the legend.

Note: If PCR Data is selected in the combo box **Data Source**, the relevant curve shapes will appear in the mode **Evaluation**.



If you switch to the mode **View** the results of analysis will be shown as different-colored bars for every sample.
The assignment of the bars to the target genes analyzed can be found in the legend.
All results are listed in the table **Samples/Analysis**.



6.7 Gene Identification

Gene identification can be based either on a PCR or endpoint determination as the data source. After the analysis module **Gene Identification** has been selected in the header as **Type of Application**, the data from the source forming the basis for gene identification will appear in the work area. If more than one data source is available, they can be selected using the combo box **Data Source**. For defining allelic controls under plate layout see 4.2.13.

6.7.1 Determination of threshold

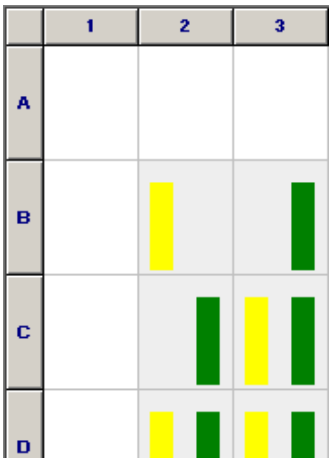
Before actual analysis of the gene identification can be performed, the relevant dye must be selected for every allele in the top panel using the combo box **Dye**. Subsequent determination of the threshold then depends on whether a PCR or endpoint determination is used as the data source. The process can be found in the relevant sections for quantification (6.2.1) or +/- Assay (6.6.6.1).

6.7.2 Analysis

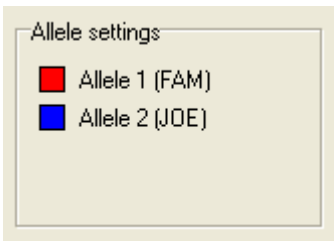
Once the threshold has been determined separately for every allele, all dyes are selected in the top panel using the combo box **Dye**.

View The function **View** in the top panel can be used to display the results in the work area.

The results are shown as different-colored bars in the allocated positions of the PCR plate. If one allele is detected, it is shown as a single bar. If both alleles are detected in a sample, two different colored bars are seen.



The panel **Allele settings** shows the legend indicating the color-coded assignment of the alleles automatically set by the software. The dye used to detect the relevant allele is also indicated.



The results are listed in the table **Sample/Analysis**.

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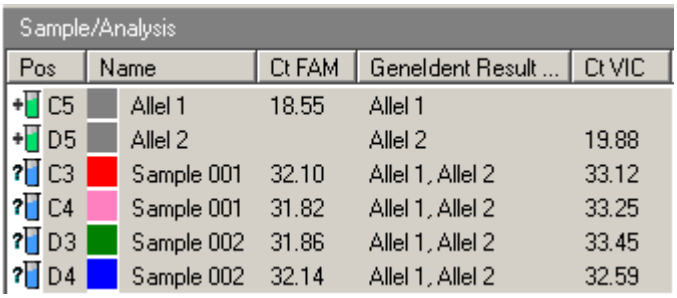


Sample/Analysis

Pos	Name	Ct FAM	GeneIdent Result ...	Ct VIC
C5	Allel 1	18.55	Allel 1	
D5	Allel 2		Allel 2	19.88
C3	Sample 001	32.10	Allel 1, Allel 2	33.12
C4	Sample 001	31.82	Allel 1, Allel 2	33.25
D3	Sample 002	31.86	Allel 1, Allel 2	33.45
D4	Sample 002	32.14	Allel 1, Allel 2	32.59

Pos — Specifies the position of the sample in the plate layout. For simple assignment the samples are also marked with the symbols for the respective sample type.

Name — Name of sample

Ct FAM — C_T value for allelle 1 (here: FAM-marked probe).

GeneIdent Result... — The alleles detected for the sample are given under **GeneIdent Result**.

Ct VIC — C_T value for allelle 2 (here: VIC-marked probe).

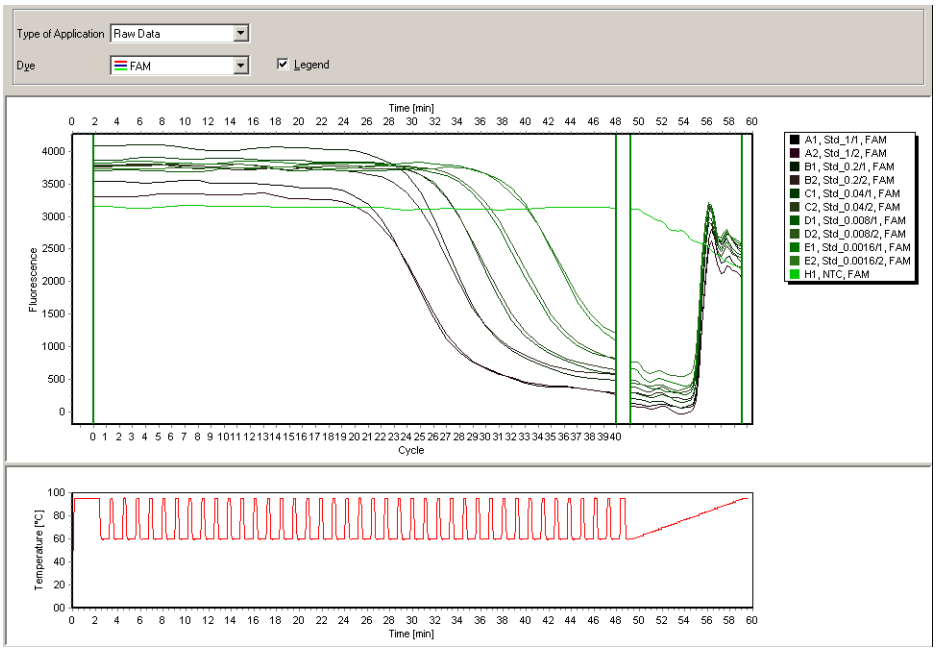
Results for the unknown samples are only shown in the column **GeneIdent Result** if a C_T value has been determined for the positive controls for the alleles or the values are above the threshold, in the case of endpoint determination.

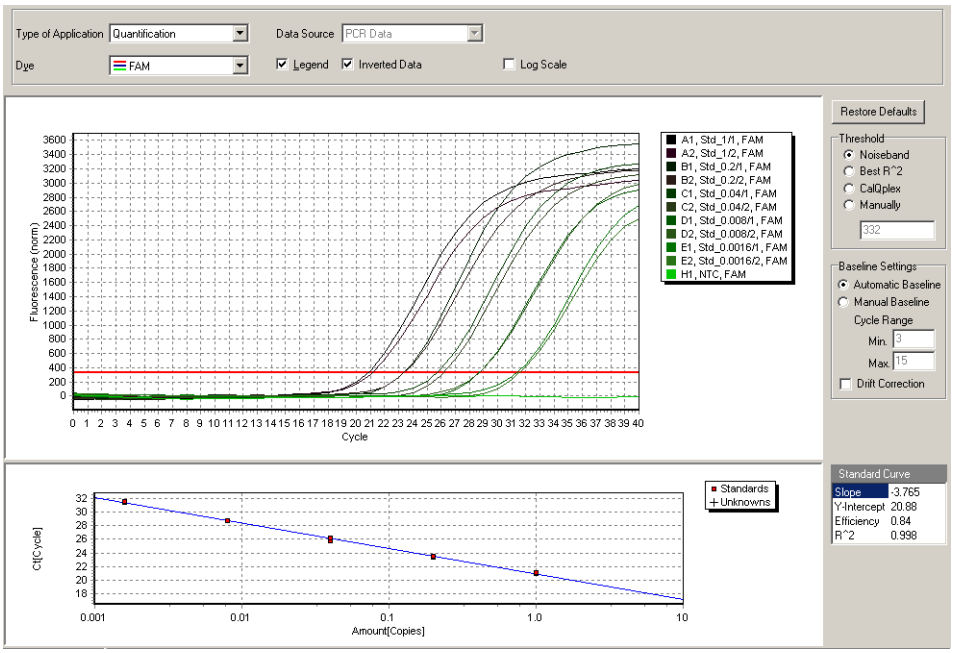
Note: Positive controls for alleles are always given as positive in the column **GeneIdent Result**, including where no C_T value was determined for them or the values are below the threshold, in the case of endpoint determination.

Evaluation — If this function is selected, the measured values of endpoint measurement or of the PCR are loaded to the work area again.

6.8 Inverted data

There are real-time PCR chemistries which result in decreasing fluorescence signals the more PCR product is generated. Curves with decreasing fluorescence levels are shown in the Raw Data View. For analyzing these data in the different analysis modules of the realplex software, activate the check box **Inverted Data**. Subsequently, the curves are inverted and threshold and baseline adjustment can be done. Analysis can be performed as described in chapter 6.2 – 6.7.





Note: Analysis of endpoint data will not be possible for assays showing decreased fluorescence levels. Activation of the check box **Inverted Data** comes along with an entry in the document information of the report (see chapter 6.1.13).

7 Maintenance

The device may only be opened by a qualified technician. The warranty for the device will become null and void if there is unauthorized intervention in the device!

7.1 General instructions on cleaning of device

When maintenance and cleaning are carried out, the device must be switched off and the plug removed beforehand.

The thermoblock, the inner side of the heated lid and micro test tubes / PCR plates can reach temperatures of over 50 °C very quickly. There is a risk of burns!

The heated lid should be kept closed until temperatures of approx. 30 °C or less are reached.

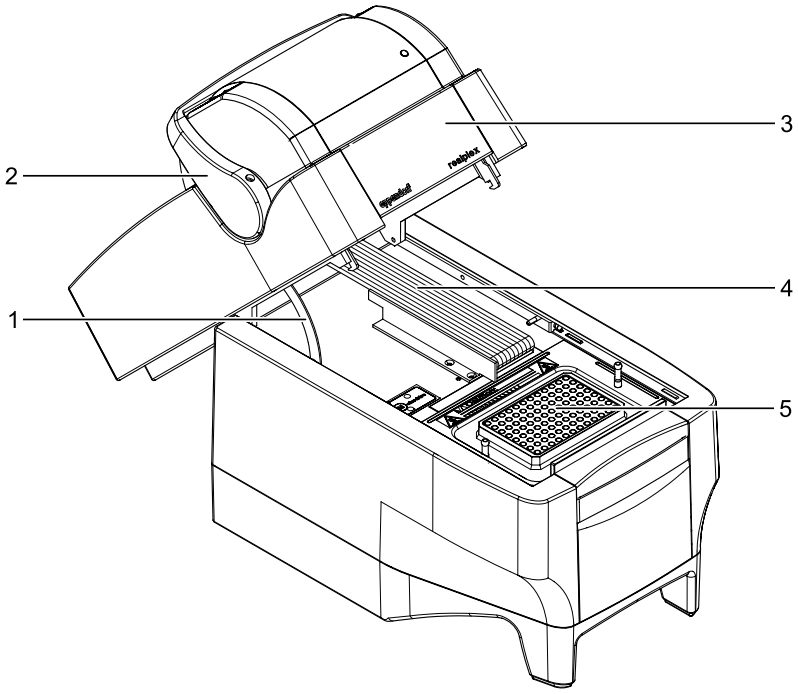


Figure 7: Cleaning position

1 Head lock arm (locked)
2 Sealing clamp
3 *realplex* module (tilted)
4 Rollflex cable
5 Thermoblock

For cleaning the device, the heated lid can be opened. To do so the *realplex* module must be moved to the rear position. The heated lid can then be put in a vertical position and then locked in position using the head lock arm (see Figure 7).

While the *realplex* module is lifted up, the sealing clamp (see Figure 7) of the lid should not be lowered. This could damage the photomultiplier!

When moving down the heated lid, it should be held with one hand and the head lock arm pulled forwards with the other hand. The heated lid is then lowered carefully.

Do not insert your fingers between the lid and the housing of the device when opening or closing the lid or when opening to the cleaning position as they may get caught.

It should be borne in mind that when cleaning the device, the sharp edges of the heated lid may cause injury e.g. rollflex cable and guide rail.

The device should be protected from organic solvents and aggressive chemicals. Make sure that no liquids enter the device.

The operator is responsible for carrying out appropriate decontamination if hazardous material is split on or into the device.

The surface of the Mastercycler ep *realplex* may be wiped down with a damp cloth or a mild lab cleaner.

Note: Cleaning of the heated lid must not be performed. For further information, please call the Eppendorf Service.

7 Maintenance

Before cleaning or decontamination methods other than those specified by the manufacturer are used, the user should check with the manufacturer that the intended method will not damage the device.

Note: Cleaning and decontamination may also be necessary as precautionary measures if the device or accessories are to be sent in for maintenance, repair or passed on. The manufacturer supplies a form which the operator can use to confirm that the device has been subjected to such treatment.

Note: When switched off the *realplex* module should always be kept closed to prevent contamination of the block.

7.2 Cleaning of thermoblock

Before cleaning of thermoblock turn off the instrument and let instrument cool for 10 – 15 minutes.

- Clean contaminated positions using a cotton swap soaked with 70 % ethanol (stock solution: Ethanol p.a. (>99.8 %) undenatured). Turn the cotton swab carefully in the well.
- Dry the position with a new cotton swap.
- Repeating this process 3 to 5 times with new clean cotton swaps.
- Switch on the device and perform another background calibration (see chapter 3.8.1). Wells should be completely dry before using instrument.

If there are still positions marked as failed, repeat the procedure with new cotton swabs. In the case that the contamination can not removed by this procedure, please call the Eppendorf Service.

7.3 Validation and calibration of thermoblock

Validation and calibration of the thermoblock is performed according to the instruction manual of the Temperature-Validation System for the Mastercycler ep.

8 Troubleshooting

Errors in the programming or handling of the device can be corrected by eliminating the cause shown in the display.

Technical faults may be caused by malfunctions, e.g. power failure or power fluctuations. In most cases it is sufficient to briefly switch the device off and back on after approx.10 seconds. If the fault re-occurs, please note down the error code (e.g. 0x0B04) shown in the display and contact Eppendorf Service.

8.1 General Errors

Error	Cause	Solution
Background calibration was not successfull	Contamination in thermoblock	Decontamination Chapter 7.2
	Contamination of background plate	Use a new background plate
	Contamination of *realplex* modul	
Color calibration was not successfull	Evaporation in wells	Use a new color calibration plate
	Wrong background calibration was used during color calibration	Repeat with the right background calibration
	Contamination of color calibration plate	Use a new color calibration plate
	Wrong dye concentrations used or dye bleached	Use a new color calibration plate
No amplification can be detected	Component of reaction mix is missing	Check the reaction mix
	Reagents were handled wrong	• Store reagents at –20 °C • Protect the reagents from light • Avoid repeated thaw and freeze cycles
	Air bubbles	Centrifuge at 300 x g
	Reaction mix is not at the bottom of wells	Centrifuge at 300 x g
	Data acquisition has occured in wrong PCR step or at wrong temperature	• Check PCR Progam • Check measuring point
	Data acquisition in wrong positions	Check plate layout
	Wrong pipetting	• Check sample volume in wells • Use epMotion
	Non-optimized reagents	Titration of reagents ($MgCl_2$)
	Inhibition by contamination from nucleic acid purification	• Use kits for ultra pure nucleic acid purification • Do not use more than10 µl DNA in a total volume of 20 µl • Check PCR system with positive control
	Large amplicons may reduce the PCR efficiency	Use short amplicons for real-time PCR
	GC-rich primer or probe	• Add DMSO to reaction mix • Redesign primer and probe
	Primer or probe does not bind	Check primer and probe design
Logarithmic phase is not reached until end of PCR	Low template concentration	Increase template concentration
	PCR system not optimized	• Titration of reagents (e.g. $MgCl_2$) • Check primer and probe design
	Not enough cycles	Perform PCR with more cycles

8 Troubleshooting

Error	Cause	Solution
Amplification curve too flat	Low PCR efficiency	• Titration of reagents (e.g. $MgCl_2$) • Check primer and probe design • Check purity of template
Amplification curve decrease at the end of PCR	Due to the large amount of products at the end of PCR the re-annealing of these products prevents annealing of probes	The so-called "hook effect" has no influence on the analysis results
Negative control is positive	Primer-Dimer in SYBR Green assays	• Check primer design • PCR setup at 4 °C • Use Hotstart enzymes • Use higher amounts of template • Increase annealing temperature • Optimize $MgCl_2$ concentration
	Contamination	• Strict separation of nucleic acid purification, PCR setup and analysis • Use to avoid contamination from already finished assays
Poor reproducibility and accuracy	Wrong pipetting	• Use master mixes • Use epMotion or electronic pipettes • Pipette large volumes • Reduce amount of pipette steps
	Wrong pipetting	• Check sample volume in wells • Use epMotion
	Insufficient mixing	Vortex master mixes and samples for 5 s
	Air bubbles	Centrifuge at 300 x g
	Drops on sealing option	Centrifuge at 300 x g
	Drops on well	Centrifuge at 300 x g
	Contamination with flourogenic material	• Use powder-free gloves • Do not write on wells
	Contamination of *realplex* module	• Check with black plate • Call Eppendorf Service
	Low template concentration	unspecific binding of DNA to consumables can be avoided by the use of glycogen
Fluorescence signal too low	Probe or SYBR concentration too low	Check PCR setup
	Reagents were handled wrong	• Store reagents at – 20 °C • Protect the reagents from light • Avoid repeated thaw and freeze cycles
	PCR efficiency too low	• Titration of $MgCl_2$ • Check primer and probe design • Final concentration of primer and probe should be 300 – 900 nM • Optimize annealing and denaturing temperature with gradient function • Check PCR program
Fluorescence signal too high	Probe or SYBR concentration too high	Check PCR setup
	Signal enhancement is wrong	Call Eppendorf Service

8 Troubleshooting

8.2 Errors during SYBR Green assays

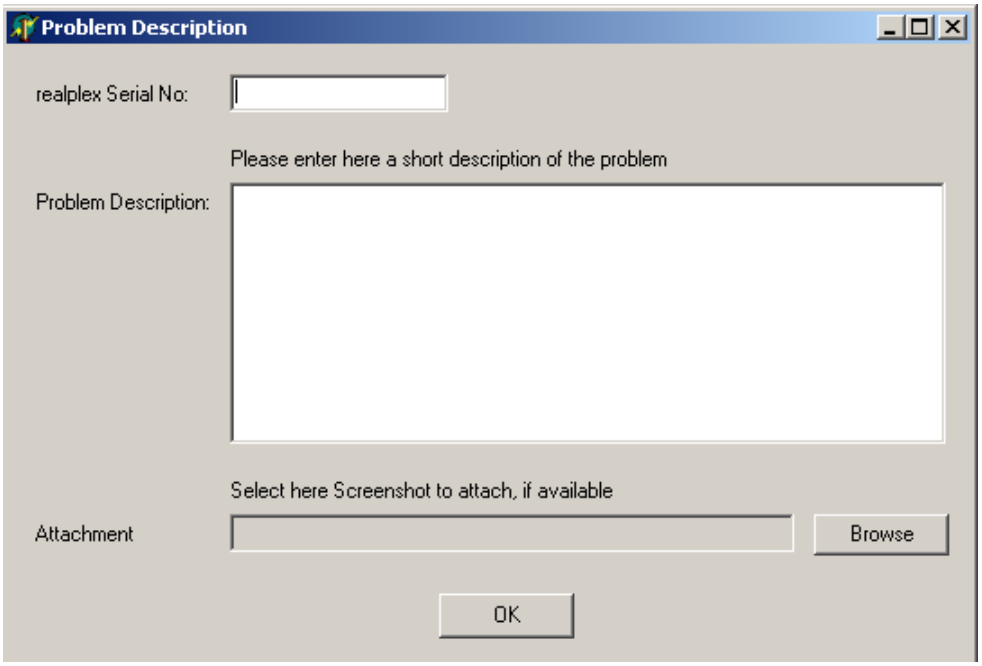
Error	Cause	Solution
Signals in stationary phase differ	High amount of bound dye due to a high initial template concentration	not use more than 50 ng genomic DNA or 100 ng RNA in a total volume of 20 µl
	Bleaching effect	• Protect SYBR from light • Avoid repeated thaw and freeze cycles
Negative control is positive	Primer-Dimer in SYBR Green assays	• Check primer design • PCR setup at 4 °C • Use Hotstart enzymes • Use higher amounts of template • Increase annealing temperature • Optimize $MgCl_2$ concentration • Perform melting curve analysis to detect primer dimers
No amplification	DNA is single stranded dye to the high temperature during data aquisition	Check PCR program
Standard curve not OK	PCR setup does not match plate layout	Check plate layout and PCR setup

8.3 Errors during qRT-PCR

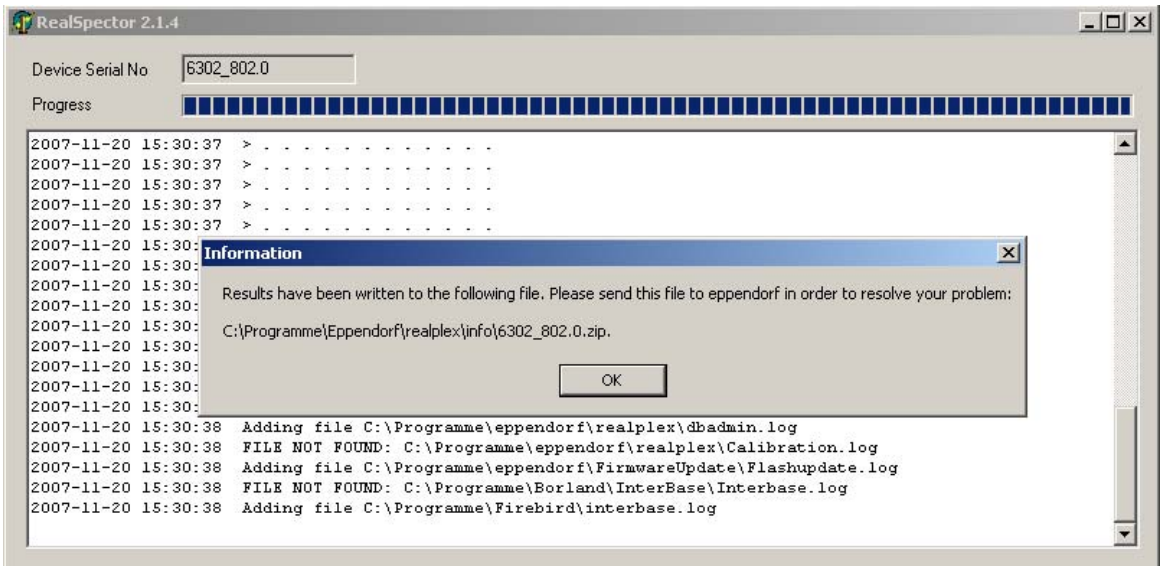
Error	Cause	Solution
Low fluorescence signals	Contamination with RNase	• Use powder-free gloves and RNase free H_2O • Store RNA at – 70 °C • Check integrity of RNA
	RT-PCR is inhibited	Use ultra pure RNA
	$MgCl_2$ concentration is not optimal	Titration of $MgCl_2$, concentration should be 3 – 8 mM
	RNA concentration not optimal	Titration of RNA, amount should be 1 – 500 pg
	cDNA concentration not optimal (not valid for two-step PCR)	Titration of cDNA
	Temperature too high during reverse transcription	Reduce temperature for reverse transcription
Amplification curve is arcuate during logarithmic phase	Primer not optimal	• Use pure primer • Check design of primers
Additional peak during melting curve analysis	Contamination with genomic DNA	• Use mRNA • Check purity of RNA
	Temperature too low during reverse transcription	Temperature during reverse transcription should be higher than 55 °C
Peak in negative control during melting curve analysis	DNA contamination	Check all reagents

8.4 RealSpector

In the case of a potential instrument malfunction it will be helpful to give as much information as possible to Eppendorf Service. For this purpose a special software tool was created called **RealSpector**. The RealSpector application is available in the start menu (Programs / Eppendorf / RealSpector). The following start screen appears:



The necessary measurement readings are recorded automatically after pressing **OK**. The panels ***realplex* No.** and **Problem Description** may be filled with relevant information, if available. It does not matter which plate is inserted in the thermoblock. All relevant data are stored automatically by the software and will be available in the directory C:\Program Files\Eppendorf\realplex\info as zipped data file (name: “serial numer of *realplex* module”.zip).



Note: The *realplex* software application has to be shut down prior to a RealSpector run. Cancellation of the measurement process is not made possible.

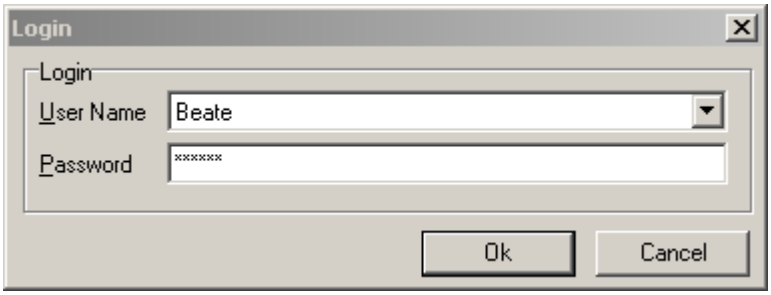
In very rare cases hardware and/or software communication between *realplex* and thermo module as well as the computer is not possible at all. Then, serial number and problem description have to be entered manually by the user. An attachment of a document to the zipped data file can also be made.

Please send the zipped data file to your Eppendorf Service Organization.

9 Short instructions

9.1 Login

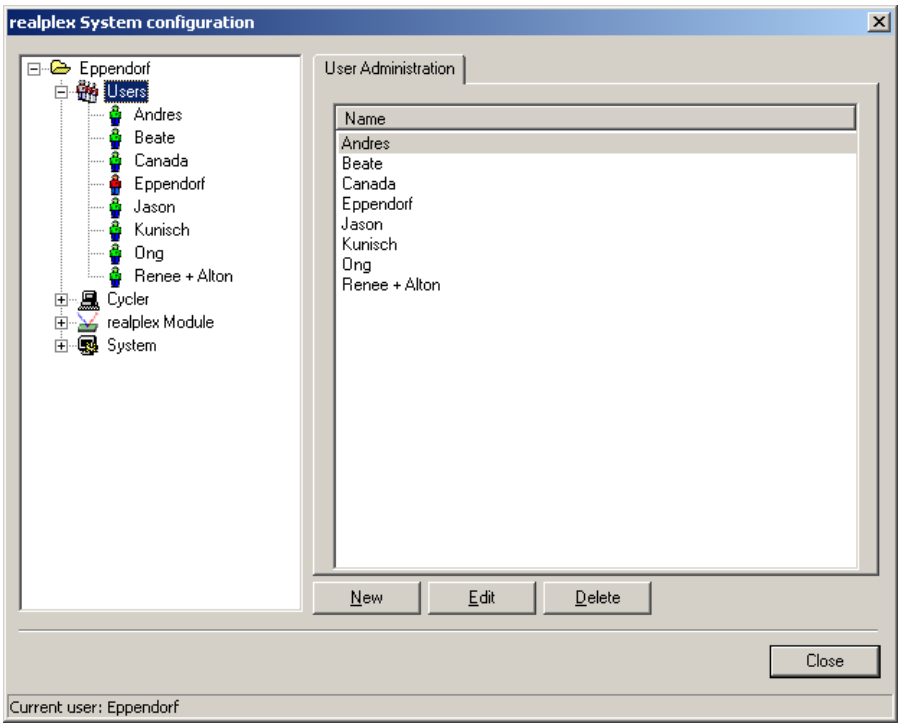
After opening the *realplex* software the Login is displayed.



Select the required user and enter the relevant password.

9.2 Setting up new users

New users can only be set up by the Administrator when he is logged in. To do so, the dialogue **realplex System configuration** is opened under the menu item **Setup**.



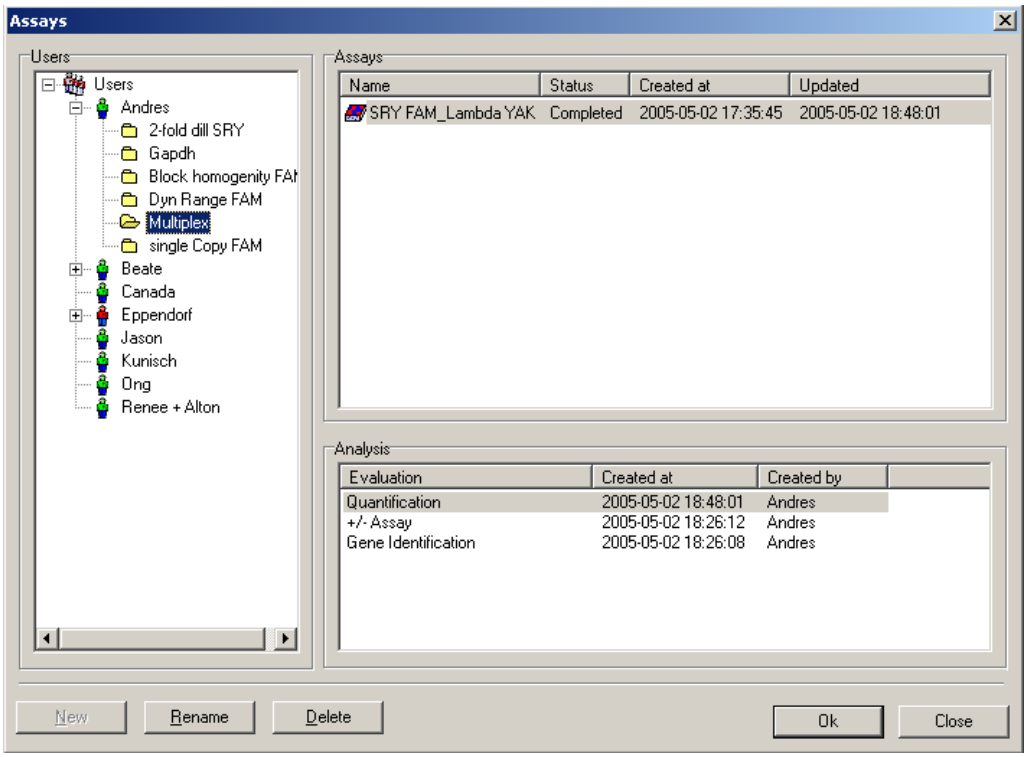
The function key **New** is used to set up a new user. Already existing users can be changed or deleted using the functions **Edit** and **Delete**.

9 Short instructions

9.3 Creating assays

If the icon **New Assay** is selected, a blank plate layout and a standard PCR program are loaded in the work area.

After selection of the icon **Open Assay**, the dialogue **Assays** is opened.



The assay marked **Setup** has not yet started. On opening it is loaded to **Assay Setup** as well as to **Analysis**. However, no data are available in the latter.

Assays which can be used as a template for the creation of new assays are marked **Template**.
See section 3.6.4 and 3.6.5 for further information about the creation and usage of templates.

In the case of assays marked **Complete** data acquisition has already taken place. If this assay is opened, the data are displayed under **Analysis**. In addition, the assay appears in **Assay Setup** unless another assay is currently underway (see section 6.1.1).
If individual assays are selected, all analyses of this assay saved to date are shown in the lower window **Analysis**. The date of creation and the name of the user who performed analysis are also listed here.

Assays which have been aborted e.g. due to a power outage or system failure are marked **Aborted**. They may also be analyzed if sufficient data were acquired before the assay was aborted.

9.4 Saving assays

A logged on user can save assays under his node or in one of his folders. The editing functions for these programs are only accessible to the logged-in user. Other users are only able to view and copy these assays.

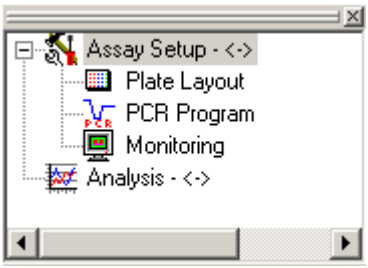
If the icon **Save Assay** is selected, the current assay is saved.

9 Short instructions

9.5 Performing an assay

9.5.1 General

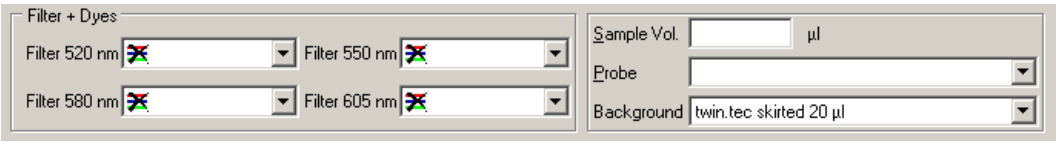
The software of the Mastercycler ep *realplex* basically consists of two modules **Assay Setup** and **Analysis**. The module **Assay Setup** is divided into the sub-items **Plate Layout**, **PCR Program** and **Monitoring** which are needed for creating and running an assay.



They are displayed in the work area on the right if individual sub-items are selected.
Alternatively, the different modules can also be loaded to the work area using the functions of the menu item **Commands**.

9.5.2 Plate Layout

The following settings are necessary depending on the assay type:



- Selection of the dyes in **Filters + Dyes**.
- Probe and Background can be selected in the appropriate combo boxes in the right panel. Moreover, the sample volume can be entered.
- Enter the sample types. To do so mark the position in the plate layout and use the following icons:

If the icon **Standard** is selected, the relevant dialogue is opened. This can be extended by the option **Auto Series** for simple creation of standard series and replicates.

The dialogue **New Positive Control** can be opened with the relevant icon.

If the icon **Negative Control** is selected, the relevant dialogue is opened.

If the icon **Unknown** is selected, the relevant dialogue is opened. A simple creation of replicates is possible by extending the dialogue by choosing **Auto Series**.

Note: If there are no dyes, probes or backgrounds available in the relevant combo boxes they have to be entered or created (see Chapter 3.6, 3.7 and 3.8).

9.5.3 PCR Program

After selecting the item **PCR Program** in the navigator a 3StepCycle is displayed in the work area. This PCR program can be modified with the following icons:

If the icon **Insert Step** is selected, the relevant dialogue is displayed and can be used to insert various program steps.

If other adjustment options are to be amended for program steps, the icon **Edit Step** can be used to call up the dialogue in which all other settings and changes can be made.

Individual temperature steps are deleted by first being highlighted and then deleted by selecting the icon **Delete Step**.

The measuring point is determined in a temperature step by highlighting a step and selecting **Set measuring point**.

9 Short instructions

9.5.4 Start Assay

After the created assay has been saved and the sample tubes inserted, the heated lid is slid forwards. The sealing clamp is then moved down and the program started.

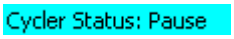
If the icon **Start Current Cycler Program** is selected, the assay is started.
Alternatively this function is also available under the menu item **Command**.

After the assay has started, the dialogue **Start PCR** is displayed and can be used to select the background plate with a combo box.

9.5.5 Interruption of an ongoing assay

The assay can be interrupted, e.g. to add a reagent. The current temperature of the block and the lid remain unchanged.

The icon **Pause Current Cycler Program** can be used to interrupt the ongoing assay.



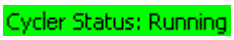
The status display then shows **Pause**.

The program can be continued at any time (see section 9.5.6) or aborted (see section 9.5.7).

9.5.6 Continue assay

If the assay is to be continued, the heated lid has to be closed first.

A program that has been stopped can be continued at any time with the icon **Start Current Cycler Program**.



After the assay has been continued, the status display then shows **Running**.

9.5.7 Abort assay

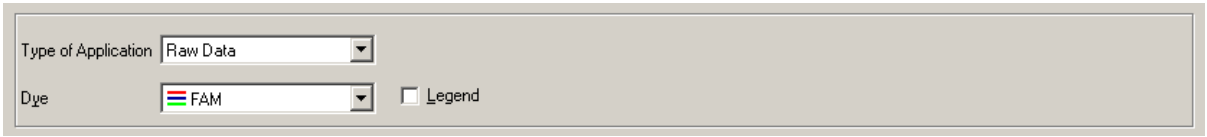
An assay is interrupted when the icon **Pause Current Cycler Program** is selected.

Afterwards the assay is aborted by selecting the icon **Abort Current Cycler Program**.

The information **Idle** is shown in the status display.

9.5.8 Analysis

In the top panel different analysis modules can be selected using the combo box **Type of Application**. These data are then displayed in the work field according to the module.



With multiplex assays, the combo box **Dye** can also be used to select individual dyes. It is possible to display the data selectively for one dye or all of the dyes at the same time via **all dyes**.

After selection of an application type and the threshold setting the results are displayed in the table **Samples / Analysis**. Further information concerning the threshold settings can be found in the chapter for each application type (see chapter 6).

10 Technical data

Mastercycler ep *realplex*

Excitation source	96 LEDs (470 nm)
Emission filters	520 ± 10 nm / 550 ± 10 nm (*realplex*^{2}) 520 ± 10 nm / 550 ± 10 nm / 580 ± 5 nm / 605 ± 15 nm (*realplex*^{4})
Photomultiplier	1 Channel photomultiplier (*realplex*^{2}) 2 Channel photomultiplier (*realplex*^{4})
Sample capacity	96 x 0.2 ml PCR tubes or one 96 PCR plate (unskirted, semi-skirted, skirted – as per SBS standard)
Temperature control range of block	4 °C to 99 °C
Range of gradient	1 °C to 20 °C (thermo module Mastercycler ep) 1 °C to 24 °C (thermo module Mastercycler ep S)
Temperature control range of gradient	30 °C to 99 °C
Temperature control range of lid	37 °C to 105 °C
Temperature of lid	105 °C
Block homogeneity	35 °C ± 0.3 °C 90 °C ± 0.4 °C
Control accuracy	± 0.2 °C
Heating rate*	approx. 4 °C/s (thermo module Mastercycler ep) approx. 6 °C/s (thermo module Mastercycler ep S)
Cooling rate*	approx. 3 °C/s (thermo module Mastercycler ep) approx. 4.5 °C/s (thermo module Mastercycler ep S)
Dimensions (W x D x H)	26 cm x 41 cm x 39.6 cm
Total weight	24 kg
Weight of thermo module	17 kg
Weight of *realplex* module	7 kg
Mains power connection	100 V – 130 V, 50 – 60 Hz, 200 V – 240 V, 50 – 60 Hz (Mains power deviation not greater than ±10 % of nominal mains power)
Power consumption	800 W
Over voltage category	II (IEC 610 10-1)
Contamination level	2
Protection class	I
IP protection	20
Ambient conditions (only indoors)	15 °C to 35 °C, 70 % rel. humidity, to 2000 m sl
Storage conditions	-20 °C to 50 °C, 85 % rel. humidity
Transport conditions	-20 °C to 50 °C, 85 % rel. humidity

* measured at block

Technical specifications subject to change!

Mains power connection supply and power consumption of *realplex* module via connected thermo module.
The device has been awarded the CE mark.

11 Ordering information

Please only use the original accessories recommended by ourselves. The use of spare parts or disposables other than those recommended by ourselves may impair the function and safety of the device. Any warranty and liability for damage thus caused will be excluded.

Description	Order no.
Mastercycler ep *realplex*^{2}, 100 V – 130 V	950020202
Mastercycler ep *realplex*^{2i}, 100 V – 130 V	6300 000.906
Mastercycler ep *realplex*^{2}, 200 V – 240 V	6300 000.507
Mastercycler ep *realplex*^{2i}, 200 V – 240 V	6300 000.957
Mastercycler ep *realplex*^{2} S, 100 V – 130 V	950020211
Mastercycler ep *realplex*^{2} S, 200 V – 240 V	6300 000.604
Mastercycler ep *realplex*^{4}, 100 V – 130 V	950020300
Mastercycler ep *realplex*^{4i}, 100 V – 130 V	6302 000.903
Mastercycler ep *realplex*^{4}, 200 V – 240 V	6302 000.504
Mastercycler ep *realplex*^{4i}, 200 V – 240 V	6302 000.954
Mastercycler ep *realplex*^{4} S, 100 V – 130 V	950020318
Mastercycler ep *realplex*^{4} S, 200 V – 240 V	6302 000.601
realplex^{2}, 100 V – 130 V	950020105
realplex^{2}, 200 V – 240 V	6300 000.000
realplex^{4}, 100 V – 130 V	950020113
realplex^{4}, 200 V – 240 V	6302 000.008
Accessories	
Operating instructions for Mastercycler ep *realplex* (english)	6302 900.378
Temperature-Validation System	5331 222.005
Filter 520 nm	6302 101.009
Filter 550 nm	6302 103.001
Filter 580 nm	6302 104.008
Filter 605 nm	6302 105.004
PC CAN Bus cable and USB/CAN adapter	6302 070.405
CAN Bus cable	6302 070.456
Software *realplex* 2.2	6302 861.500
Software *routine-realplex* 2.3	6302 862.506
Consumables	
twin.tec PCR Plate 96, semi-skirted, colorless (25 pieces)	0030 128.575
twin.tec PCR Plate 96, semi-skirted, yellow (25 pieces)	0030 128.583
twin.tec PCR Plate 96, semi-skirted, red (25 pieces)	0030 128.613
twin.tec PCR Plate 96, semi-skirted, green (25 pieces)	0030 128.591
twin.tec PCR Plate 96, semi-skirted, blue (25 pieces)	0030 128.605
twin.tec PCR Plate 96, skirted, colorless (25 pieces)	0030 128.648
twin.tec PCR Plate 96, skirted, yellow (25 pieces)	0030 128.656
twin.tec PCR Plate 96, skirted, red (25 pieces)	0030 128.680
twin.tec PCR Plate 96, skirted, green (25 pieces)	0030 128.664
twin.tec PCR Plate 96, skirted, blue (25 pieces)	0030 128.672
twin.tec *real-time* PCR Plate 96, semi-skirted, blue (25 pieces)	0030 132.530
twin.tec *real-time* PCR Plate 96, semi-skirted, white (25 pieces)	0030 132.548
twin.tec *real-time* PCR Plate 96, semi-skirted, black (25 pieces)	0030 132.556

11 Ordering information

Description	Order no.
twin.tec *real-time* PCR Plate 96, skirted, blue (25 pieces)	0030 132.505
twin.tec *real-time* PCR Plate 96, skirted, white (25 pieces)	0030 132.513
twin.tec *real-time* PCR Plate 96, skirted, black (25 pieces)	0030 132.521
Heat Sealing Film (10 x 10 pieces)	0030 127.650
Recommended aids	
Heat Sealer 230 V / 50 Hz	5390 000.024
PCR-Cooler 0.2 ml pink	3881 000.023
PCR-Cooler 0.2 ml blue	3881 000.031
PCR-Rack, sorted according to color (10 pieces)	0030 127.455
Liquid Handling Workstation® ep*Motion* 5070	
basic device incl. control panel, software, optical sensor, waste box, MMC and reader, operating instructions, 50/60 Hz, 200 – 240 V	5070 000.000

12 Ordering information North America

Please only use the original accessories recommended by ourselves. The use of spare parts or disposables other than those recommended by ourselves may impair the function and safety of the device. Any warranty and liability for damage thus caused will be excluded.

Description	Order no.
Mastercycler ep *realplex*^{2}, 100 V – 130 V	950020202
Mastercycler ep *realplex*^{2} S, 100 V – 130 V	950020211
Mastercycler ep *realplex*^{4}, 100 V – 130 V	950020300
Mastercycler ep *realplex*^{4} S, 100 V – 130 V	950020318
realplex^2, 100 V – 130 V	950020105
realplex^4, 100 V – 130 V	950020113
Accessories	
Operating instructions for Mastercycler ep *realplex* (english)	on request
Temperature-Validation System	950008008
Filter 520 nm	on request
Filter 550 nm	on request
Filter 580 nm	on request
Filter 605 nm	on request
Software *realplex* 2.2	950019010
Software *routine-realplex* 2.3	950019015
Consumables	
twin.tec PCR Plate 96, semi-skirted, colorless (25 pieces)	951020303
twin.tec PCR Plate 96, semi-skirted, yellow (25 pieces)	951020320
twin.tec PCR Plate 96, semi-skirted, red (25 pieces)	951020389
twin.tec PCR Plate 96, semi-skirted, green (25 pieces)	951020346
twin.tec PCR Plate 96, semi-skirted, blue (25 pieces)	951020362
twin.tec PCR Plate 96, skirted, colorless (25 pieces)	951020401
twin.tec PCR Plate 96, skirted, yellow (25 pieces)	951020427
twin.tec PCR Plate 96, skirted, red (25 pieces)	951020486
twin.tec PCR Plate 96, skirted, green (25 pieces)	951020443
twin.tec PCR Plate 96, skirted, blue (25 pieces)	951020460
twin.tec *real-time* PCR Plate 96, semi-skirted, blue (25 pieces)	951022043
twin.tec *real-time* PCR Plate 96, semi-skirted, white (25 pieces)	951022055
twin.tec *real-time* PCR Plate 96, semi-skirted, black (25 pieces)	951022067
twin.tec *real-time* PCR Plate 96, skirted, blue (25 pieces)	951022003
twin.tec *real-time* PCR Plate 96, skirted, white (25 pieces)	951022015
twin.tec *real-time* PCR Plate 96, skirted, black (25 pieces)	951022027
Heat Sealing Film (10 x 10 pieces)	951020060
Recommended aids	
Heat Sealer 230 V / 50 Hz	951023078
PCR-Cooler 0.2 ml pink	022510541
PCR-Cooler 0.2 ml blue	022510525
PCR-Rack, sorted according to color (10 pieces)	951010065
Liquid Handling Workstation® ep*Motion* 5070	
basic device incl. control panel, software, optical sensor, waste box, MMC and reader, operating instructions, 50 / 60 Hz, 200 – 240 V	960000005

EG-Konformitätserklärung
EC Conformity Declaration

Das bezeichnete Produkt entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen. Bei einer nicht mit uns abgestimmten Änderung des Produktes oder einer nicht bestimmungsgemäßen Anwendung verliert diese Erklärung ihre Gültigkeit.

The product named below fulfills the relevant fundamental requirements of the EC directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Produktbezeichnung, Product name:

Mastercycler® ep *realplex*^{2}, Mastercycler® ep *realplex*^{4}

Produkttyp, Product type:

real-time PCR Gerät / real-time PCR instrument

Einschlägige EG-Richtlinien/Normen, Relevant EC directives/standards:

2006/95/EG, EN 61010-1, EN 61010-2-010

2004/108/EG, EN 55011/B , EN 61000-6-1, EN 61000-3-2/3, EN 61000-4-14

Vorstand, Board of Management:

21.05.2007

Hamburg, Date:

Projektmanagement, Project Management:

ISO CERTIFIED 9001

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