



A marvel in space and time

Real-time PCR system Mastercycler® ep *realplex*

eppendorf



Small, fast, flexible!

Mastercycler® ep *realplex*—licensed real-time PCR solutions

Necessity is the mother of invention.

Incorporating the distinct advantages of the Mastercycler ep gradient PCR thermal cycler family, we have developed a fully licensed real-time PCR system that meets the requirements of the latest quantitative PCR applications. Comprehensive yet intuitive software paired with recent advances in real-time PCR technology make the Mastercycler ep *realplex* a critical contribution to modern science and research.

Maximum speed, minimum space.

Time and space are valuable resources in every lab. The Mastercycler ep *realplex* concept is, therefore,

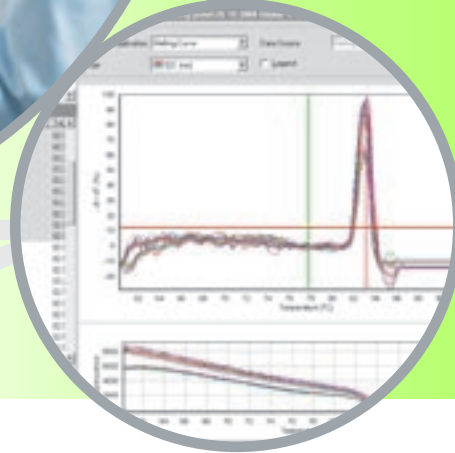
precisely aimed at saving valuable time otherwise spent awaiting results. Extremely fast temperature ramping, short detection times and intuitive assay programming reduce the overall time spent on real-time PCR, which translates to more experiments completed per day than ever before.

Its compactness enables the Mastercycler ep *realplex* to extend these benefits to virtually any laboratory bench-top space, no matter how limited in size.



licensed
for real-time
PCR

System concept



Two times two is four.

Depending upon your range of real-time PCR applications, select between two available detection systems — Mastercycler ep *realplex*² and Mastercycler ep *realplex*⁴. Both are operated by the same *realplex* software, and both are equipped with an LED array to excite the fluorescent dyes in each individual PCR sample. The differences between each system include the number of fluorescence filters and photo-multipliers and the different, resulting levels of multiplexing capacity.

Upgrade — any time.

The modular system concept of the *realplex* allows you to upgrade your Mastercycler ep gradient or Mastercycler ep gradient S PCR thermal cycler to a real-time PCR cycler at any time. Furthermore, if you have opted for a *realplex*², yet later find that expanded multiplexing has become essential, it is possible to upgrade to four wavelength capability at any time by replacing the *realplex*² optical detection module with *realplex*⁴.

Eppendorf engineering.

The Mastercycler ep *realplex* features the latest technology, united with good design and a quality finish. A minimal number of moving parts in the optical detection module minimizes the vulnerability of the overall system and promotes consistently high sensitivity in your real-time PCR experiments.

The Eppendorf TSP (Thermal Sample Protection) technology prevents premature heating of the samples and the associated amplification of nonspecific PCR products during the heating phase of the heated lid. The overall system has been solidly engineered to ensure quiet operation in the laboratory.

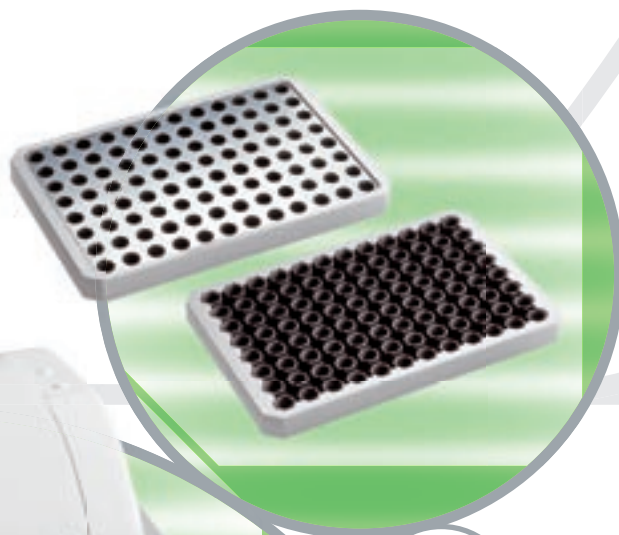
Mastercycler ep *realplex*: real-time PCR without limits

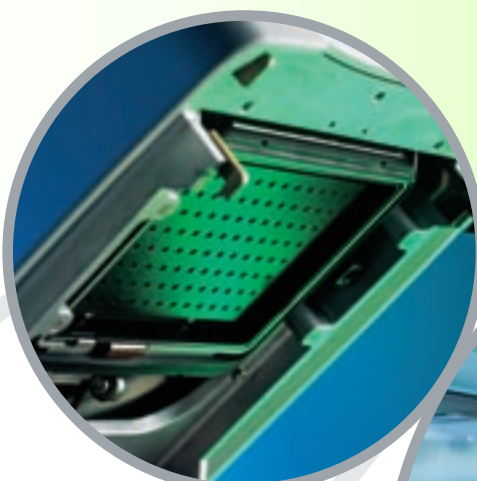
Quality in all forms.

The Mastercycler ep gradient thermal cycler is the basis of the *realplex*. Together with a sturdy yet highly sensitive optical module, it forms the new Eppendorf real-time PCR system Mastercycler ep *realplex*.

All *realplex* systems have special surface-treated thermal blocks in the 96-well format, and they are equipped as standard with the Mastercycler ep SteadySlope® gradient technology for assay optimization. Choose from two thermomodules, depending on your requirements. The “standard” variant is fitted with a specially finished 96-well aluminum block, while the “high-speed” variant has a 96-well silver block with Impulse PCR and extremely fast heating and cooling rates. Regardless of the thermomodule selected, reproducible results can be attributed to the highest accuracy and uniformity of Mastercycler ep thermal cycling.

Both thermomodules can be combined with two different optical modules, *realplex*² and *realplex*⁴, which permit the use of nearly all fluorescent dyes used in real-time PCR. The *realplex*² module has 2 fluorescence filters and one channel photo-multiplier (CPM), while the *realplex*⁴ module incorporates 4 filters and two CPMs for 4-fold multiplexing assays.

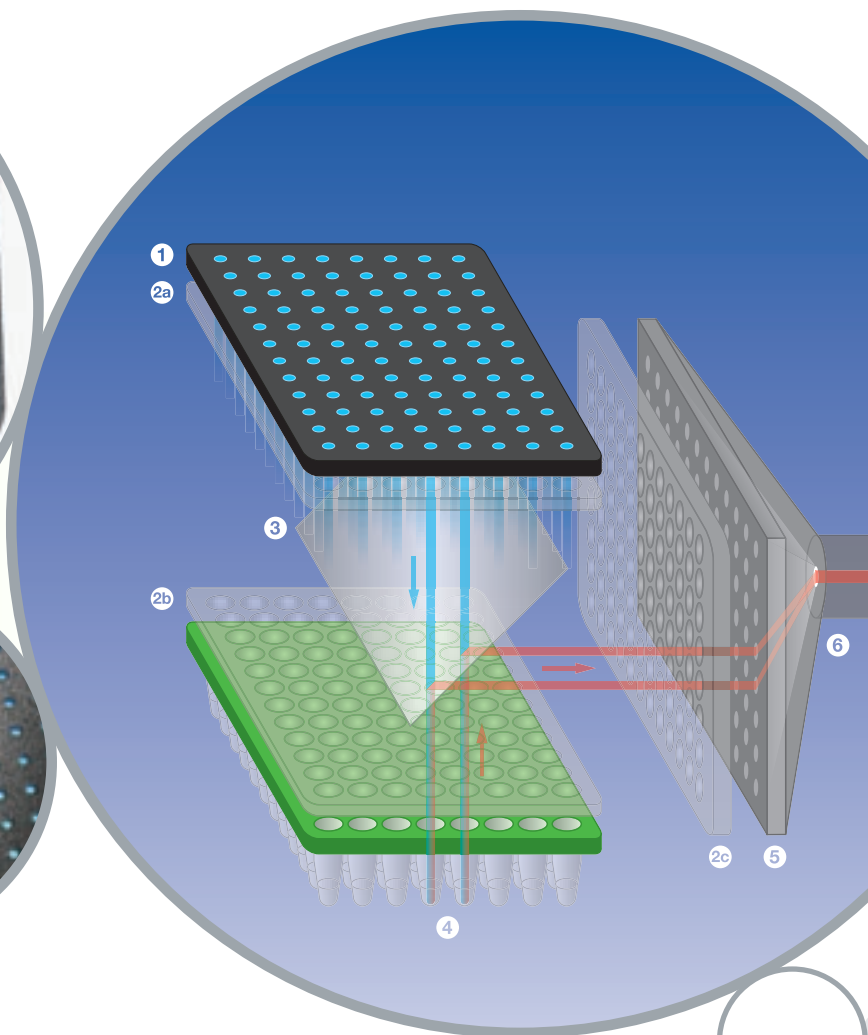




Unrestricted!

In order to give you the greatest possible flexibility in experimental design, Eppendorf's *realplex* offers a completely open system concept.

Pre-established assays optimized for real-time PCR reagents and your preferred tubes or plates can be easily transferred to the Mastercycler ep *realplex*. Even the enormous speed advantage of the silver S thermo-module can be fully utilized without restriction — use reagents, kits or sample tubes and plates of your choice.



Mastercycler ep *realplex*: optical highlight.

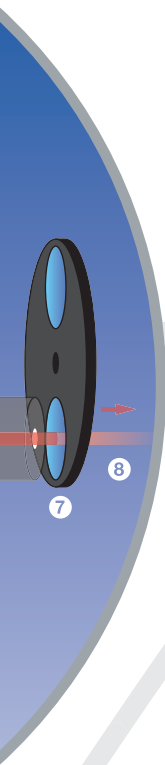
96-well excitation.

The fluorescent dyes chosen for each experiment are excited by an array of 96 individual LEDs, which have a substantially longer lifespan in contrast to halogen lamps. The result is a real-time PCR system that is less susceptible, minimizing equipment downtime as well as the frequency of maintenance and associated delays to your experiments.

The LEDs generate a blue light at a wavelength of ~470 nm, which can excite nearly all fluorophores used in real-time PCR (including SYBR®Green, FAM, VIC, TET, HEX, ROX, JOE, TAMRA). Emitted fluorescence is focused through an array of lenses and passed to the optical detection unit through 96 individual optical fibers.

Immediately upstream of the detectors, two high-quality emission filters with bandwidths of 10 nm to 30 nm are contained in a filter rotor.

New type channel photo-multipliers (CPM) serve as the detectors, which in contrast to conventional photo-multiplier tubes, are far less sensitive to magnetic field interference. The most important characteristic of the new CPMs is greatly increased sensitivity, which translates to higher sensitivity of reported results.



Mastercycler ep *realplex* light path

- ❶ Light emission from 96x LED array (470 nm)
- ❷a/b/c Focusing with 96x lens arrays
- ❸ Semipermeable beam splitter
- ❹ Light emission from excited fluorescent dyes in the reaction mixture (see representation in the graphic)
- ❺ Merging of the rays through a “96-in-1” optical fiber
- ❻ Focusing; passage through additional beam splitters
- ❼ Filter wheel with interference filters
- ❽ Advancing the light beam to the photo multiplier



System concept

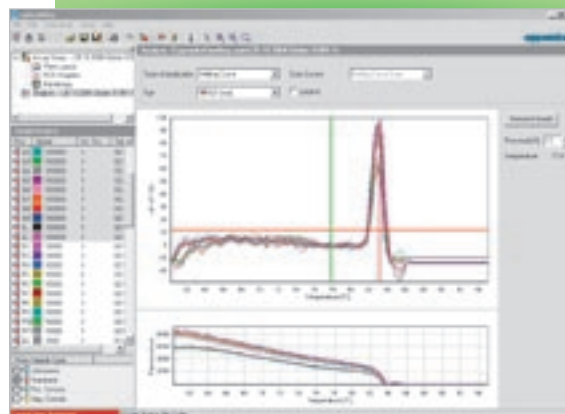
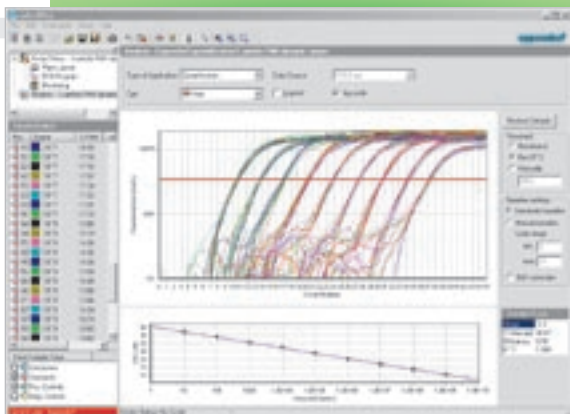
Dynamic range.

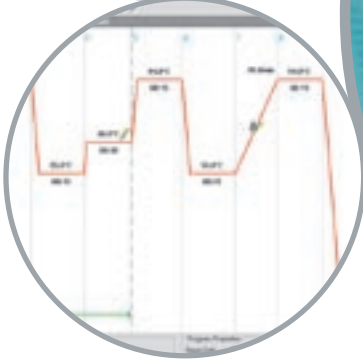
Generating the broadest range data points of quantification analyses and endpoint measurements requires amplification linearity across a wide dynamic range as well as high sensitivity. Over a linear dynamic range the Mastercycler ep *realplex* detects up to 9 levels of magnitude with a detection limit of ≤ 50 fM fluorescein. A high signal-to-noise ratio permits even single molecule detection in optimized assays.

Melting curves.

The Mastercycler ep *realplex* provides an optional melting curve analysis module. Thus, specific PCR products can be differentiated from nonspecific products such as primer-dimers. Melting curve analysis is also a key tool for the genotyping of SNPs and for detecting the smallest deletions.

System software





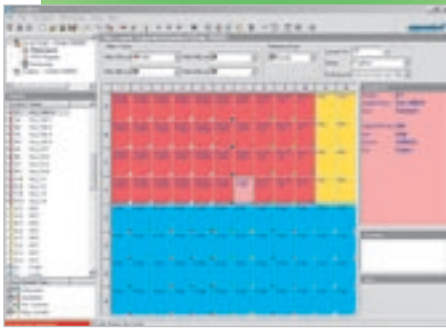
Programmed for success!

Results in real-time.

With a total of six different evaluation modules *realplex* software offers maximum flexibility for data processing. Numerical values are converted into amplification plots or into colored bar charts according to analysis type and objective, thus enabling quick and precise interpretation of the generated data. Data results can also be imported into Microsoft® Excel at any time to comply with established analysis and documentation procedures in your laboratory.

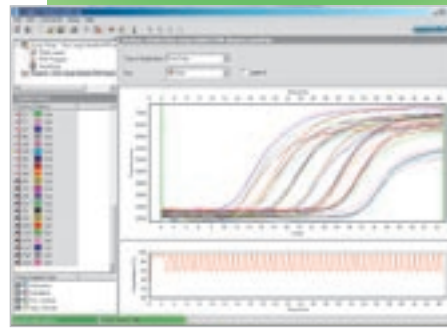
The complete *realplex* software package has been designed with “user-friendliness” in mind: the intuitive user interface ensures a simple, fast PCR and assay set-up, as well as simple transfer of PCR protocols that have been previously established on other real-time PCR systems.

Newly developed software algorithms aid in multiplex assays and ensure that interference of other dye emissions is mathematically compensated and subtracted. In this way, only the data for the selected wavelength range is recorded in the raw data and subsequent analyses.



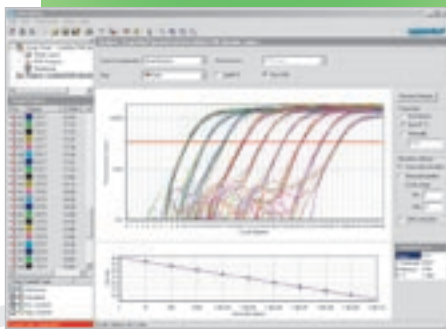
● Plate layout

The clear and comprehensive organization of multiple viewing windows as well as intuitive options for selection within each view enable easy and fast plate setup when creating assays.



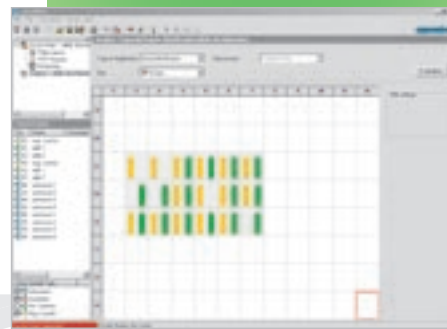
● Raw data

This Mastercycler ep *realplex* software module makes it possible to observe the generated raw data in real time, which means that you can immediately evaluate your real-time PCR and interrupt the reaction as soon as the desired result is obtained.



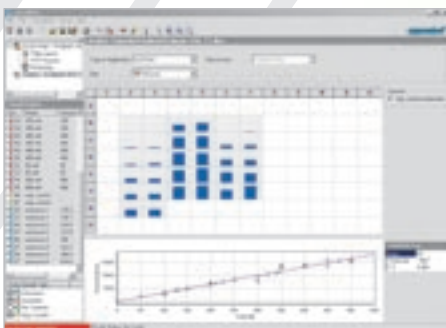
● Quantification/relative quantification

The analysis module performs calculations from raw data for a variety of assays, including absolute quantification of DNA or relative quantification of gene expression based on the $\Delta\Delta C_t$ method.



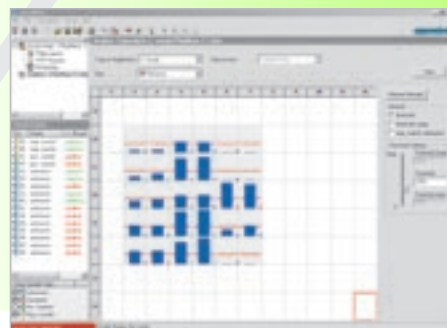
● Gene identification

The selection of this analysis module option generates allele-discrimination results. Data previously generated in melting curve analyses, endpoint measurements or C_t -value determinations serves as the basis.



● Endpoint

The analysis module option for endpoint measurements instructs the Mastercycler ep *realplex* to serve as a “plate fluorometer” and aids in the determination of absolute fluorescence intensities, regardless of previous method of DNA amplification.



● +/- Assay

Critical threshold values can be defined through this analysis module option to enable the differentiation between positive and negative samples (e.g., for the detection of pathogens). Data from previous endpoint measurements serves as the source for such determinations.

A broad spectrum of system solutions

A real system – with real-time PCR consumables and support plans

Safer science

Eppendorf remains committed to a long-standing policy of respect for intellectual property. In this effort to offer the highest value to our customers, we acquired a license for our real-time PCR products, including the Mastercycler® ep *realplex*. It is the Eppendorf belief that only licensed technologies can offer the level of safety and reliability to enable our customers to fully focus on their application objectives with utmost confidence.

Eppendorf's Top Performance Plans add further confidence to your results by periodical quality inspections of the instrument. Cleaning, calibration and validation of your Mastercycler ep *realplex* will be performed by authorized technical experts and thus provide total care for the best performance.

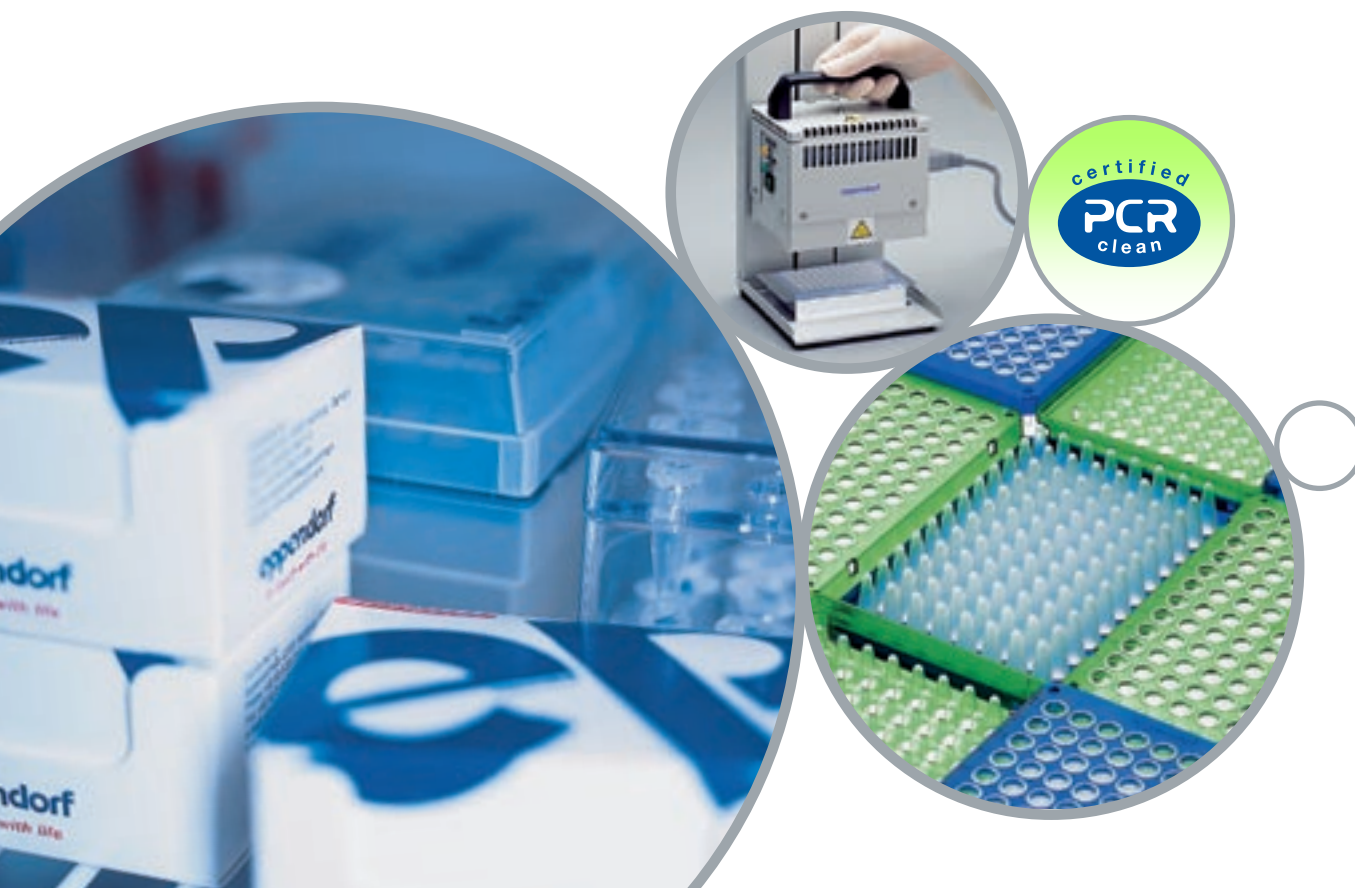
Well-for-well, a cleaner solution!

The highest reproducibility of results requires high quality consumables, such as Eppendorf® twin.tec PCR plates. Featuring a polycarbonate frame, this

two-component, injection-molded plate complies with robotic system requirements, while its 20% thinner well-walls ensure optimal heat transfer and an ideal, snug fit of the polypropylene wells to the thermoblock of the Mastercycler ep *realplex*.

In a sensitive application such as real-time PCR, it is essential to use the "cleanest possible" consumables to avoid contamination and PCR inhibition. Precisely for this reason twin.tec PCR plates are manufactured under "PCR clean" clean room conditions, and they are certified free of human DNA, DNase, RNase and PCR inhibitors.

The secure sealing of the reaction vessels is critically important as well: in addition to the prevention of contamination, effective protection against evaporation must be considered; therefore, we recommend that sample plates be heat-sealed with suitable heat-sealing film.





Eppendorf smart workstations epMotion® 5070 and 5075

“Extra hands” for increased precision.

Real-time PCR succeeds or fails by the degree of precision and accuracy in sample preparation and the prevention of cross-contamination in the pipetting process. Eppendorf smart workstations epMotion® 5070 and epMotion 5075 set the highest standards in this regard.

The affordable automation of real-time PCR setup using our workstations eliminates errors such as carry-over during pipetting and dispensing errors, which often occur during manual preparation. Additionally, the modular system concept of the epMotion family offers a variety of options for applications and retrofits. For example, through integration of a vacuum station, epMotion 5075 completely automates your entire nucleic acid purification.

epMotion 5070 and epMotion 5075

- Simple operation with a control panel
- Greatest operator convenience thanks to a comprehensive database of validated applications and consumables
- Precise, reproducible dispensing of 1 µl–1,000 µl volumes
- Innovative optical sensor detects tubes and plates, as well as their liquid levels, and pipette tips

For more information visit our websites at **www.epMotion.com** and **www.eppendorf.com/realplex**

Technical specifications: Mastercycler ep *realplex*

Optical module	
Excitation source	96 LEDs (470 nm)
Emission filters	520 / 550 nm (<i>realplex</i> ²) 520 / 550 / 580 / 605 nm (<i>realplex</i> ⁴)
Detector	1-channel photomultiplier tube (<i>realplex</i> ²) 2-channel photomultiplier tubes (<i>realplex</i> ⁴)
Dynamic range	9 orders of magnitude from starting copy number
Sensitivity	≤ 50 fmol fluorescein
Thermo module	
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–99 °C
Degree range of gradient, maximum	1 °C–20 °C (thermomodule Mastercycler ep) 1 °C–24 °C (thermomodule Mastercycler ep S)
Temperature control range of gradient	30 °C–99 °C
Temperature of lid	105 °C
Block homogeneity	35 °C ± 0.3 °C 90 °C ± 0.4 °C
Control accuracy	± 0.2 °C
Heating speed*	approx. 4 °C/s (thermomodule Mastercycler ep) approx. 6 °C/s (thermomodule Mastercycler ep S)
Cooling speed*	approx. 3 °C/s (thermomodule Mastercycler ep) approx. 4.5 °C/s (thermomodule Mastercycler ep S)
Complete system	
Dimensions (W x D x H)	26 cm x 41 cm x 39.6 cm (10.24" x 16.14" x 15.59")
Total weight	24 kg (52.91 lb)
Weight of thermomodule	17 kg (37.48 lb)
Weight of detection module	7 kg (15.43 lb)
Voltage requirements	100 V–130 V, 50 Hz–60 Hz, 200 V–240 V, 50 Hz–60 Hz
Power consumption	800 W

*Measured at block

Ordering information

Description	Int. order no.	Eppendorf North America
Mastercycler® ep <i>realplex</i> :		
Mastercycler® ep <i>realplex</i> ² , with aluminum block and two emission filters	6300 000.507	please inquire
Mastercycler® ep <i>realplex</i> ² S, with silver block and two emission filters	6300 000.604	
Mastercycler® ep <i>realplex</i> ⁴ , with aluminum block and four emission filters	6302 000.504	
Mastercycler® ep <i>realplex</i> ⁴ S, with silver block and four emission filters	6302 000.601	
Optical detection module to upgrade Mastercycler ep gradient / S		
<i>realplex</i> ²	please inquire	
<i>realplex</i> ⁴		
System accessories		
twin.tec PCR Plates		
epMotion 5070 and 5075, automated liquid handling workstations		For more information visit www.epMotion.com
Service		
Support Plans	please inquire	
Validation/calibration service		

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Eppendorf AG · 22331 Hamburg · Germany · Phone +49 40-53801-0 · Fax +49 40-53801-556 · E-Mail: eppendorf@eppendorf.com

Internet: www.eppendorf.com · Application Hotline: Phone +49 180 366 67 89 · E-Mail: application-hotline@eppendorf.com

Eppendorf North America, Inc. 800-645-3050

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