

Fast real-time PCR without optimization on the Mastercycler® ep *realplex*

Advanced PCR kits from QIAGEN not only deliver fast results in real-time PCR on the Mastercycler ep *realplex*, but also eliminate the need to optimize reaction and cycling conditions, allowing immediate startup. QuantiFast™ Kits significantly reduce amplification times, enabling fastest possible PCR run times on the Mastercycler ep *realplex*. QuantiTect® Multiplex Kits allow reliable quantification of multiple targets in the same well without any need for assay optimization.

QuantiFast Kits — for fast amplification

QuantiFast Kits are ready-to-use master mixes that provide fast real-time quantification and avoid the need for PCR optimization. Kits are available for SYBR® Green or probe-based detection in real-time PCR, two-step RT-PCR, and one-step RT-PCR.

Benefits of QuantiFast Kits:

- Maximal speed on the Mastercycler ep *realplex*
- Specific, sensitive detection of low-copy targets
- Accurate detection of a wide range of template amounts
- Optimized master mixes and protocols

Fast amplification is achieved through a specially developed fast PCR buffer. A novel patent-pending additive in the buffer, Q-Bond, increases the affinity of DNA polymerase for short single-stranded DNA, reducing primer annealing time to a few seconds. In addition, the unique buffer composition supports the melting of DNA, reducing denaturation and extension times. The buffer also contains HotStarTaq® *Plus* DNA Polymerase, which requires only 3 or 5 minutes at 95°C for activation. For one-step RT-PCR, QuantiFast RT Mix enables cDNA synthesis in just 10 minutes.

High specificity and sensitivity with QuantiFast Kits are achieved through a balanced combination of KCl and NH₄Cl in the fast PCR buffer, which promotes specific primer annealing. In addition, HotStarTaq *Plus* DNA Polymerase provides a stringent hot start, preventing the formation of nonspecific products.

Optimized thermal cycling programs for use with QuantiFast Kits are available. To install these programs on your Mastercycler ep *realplex*, contact your Eppendorf sales representative or visit:

www.qiagen.com/goto/cyclingprogram .

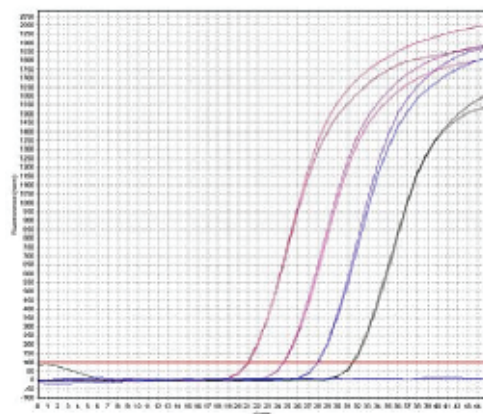
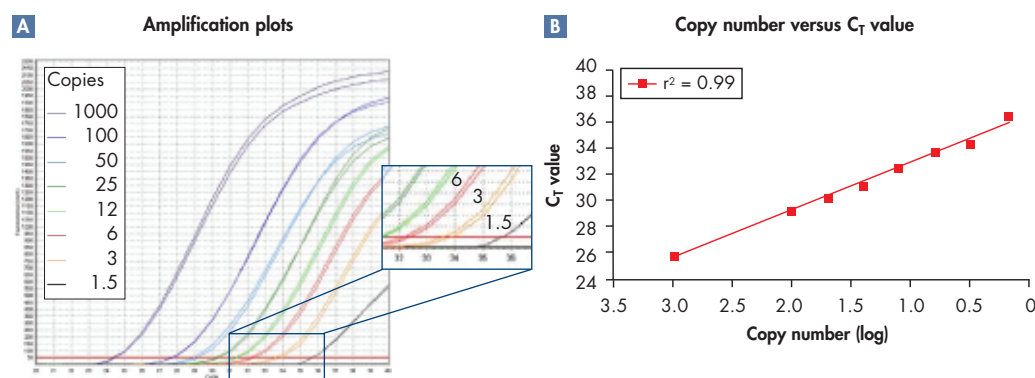


Figure 1. High sensitivity in real-time, one-step RT-PCR using SYBR Green I. Expression of MAP2K1 (a kinase) in HeLa cells was analyzed using the QuantiFast SYBR Green RT-PCR Kit and a QuantiTect Primer Assay for MAP2K1. Duplicate reactions (25 µl volume) were run using 10-fold dilutions of RNA. MAP2K1 transcript was detected in down to 10 pg RNA. NTC: no template control.

The QuantiFast SYBR Green PCR Kit (for genomic DNA and cDNA templates) and QuantiFast SYBR Green RT-PCR Kit (for RNA templates) deliver fast SYBR Green based quantification with high specificity and sensitivity (Figures 1 and 2). Specific detection in gene expression analysis is guaranteed when the kits are used in combination with QuantiTect Primer Assays. These are genomewide, bioinformatically validated primer sets for detecting transcripts from human, mouse, rat, and many other species.

Figure 2. Detection of low-copy numbers in real-time PCR using SYBR Green I.

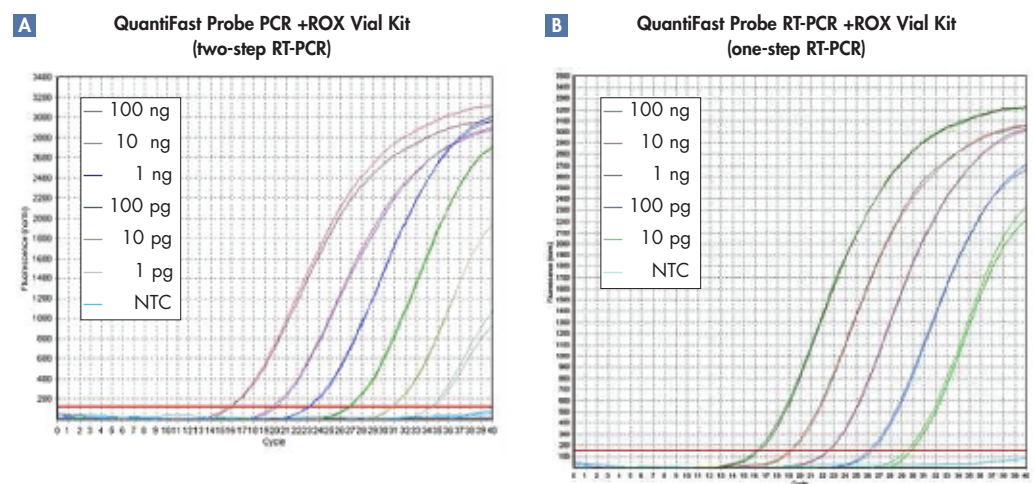
The QuantiFast SYBR Green PCR Kit was used to detect the Y-chromosome-specific, single-copy gene SRY in genomic DNA from a male donor. Reactions were run in duplicate (25 μ l volume) without melting-curve analysis. **A** Curves for 1000 down to 1.5 copies can be clearly distinguished from each other. **B** A plot of copy number (log) versus C_T value demonstrates high linearity.



The QuantiFast Probe PCR +ROX Vial Kit (for genomic DNA and cDNA templates) and QuantiFast Probe RT-PCR +ROX Vial Kit (for RNA templates) deliver fast and reliable quantification using sequence-specific probes.* The kits not only detect low-copy targets, but also provide accurate quantification over a wide dynamic range (Figure 3).

Figure 3. Wide dynamic range and high sensitivity in probe-based real-time RT-PCR.

QuantiFast Probe Kits provided accurate gene expression analysis from low to high template amounts. **A** Duplicate reactions (25 μ l volume) were performed using 10-fold dilutions of human leukocyte cDNA and a Primer Express[®] designed TaqMan[®] assay for ubiquitin (a regulatory protein). **B** Duplicate reactions (25 μ l volume) were performed using 10-fold dilutions of HeLa cell RNA and a Primer Express designed TaqMan assay for β -actin (a structural protein). **NTC**: no template control.



* The kits include ROX passive reference dye as a separate solution. ROX dye is not necessary for real-time PCR on the Mastercycler ep *realplex*, but is essential for certain cyclers from other suppliers.

QuantiTect Multiplex Kits — for amplification of multiple targets

QuantiTect Multiplex Kits enable multiplex, real-time analysis of up to 3 targets per well on the Mastercycler ep *realplex*. The ready-to-use master mixes eliminate the need to optimize reaction and cycling conditions, and can be used in PCR, two-step RT-PCR, and one-step RT-PCR.

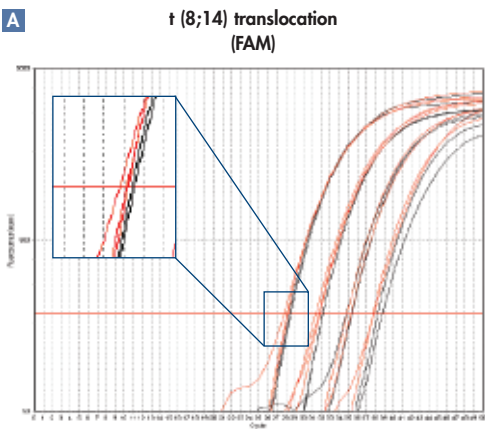
Benefits of QuantiTect Multiplex Kits:

- No optimization of PCR conditions required
- Efficient coamplification of targets and endogenous control
- Sensitive detection of as few as 10 copies of target

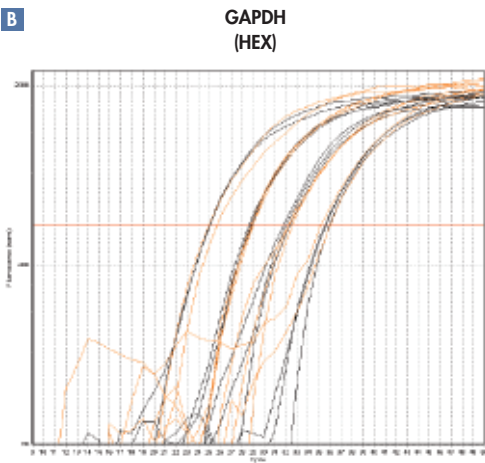
Multiplex analysis without any optimization steps is achieved through a special PCR buffer, which contains an optimized combination of KCl and (NH₄)₂SO₄ and unique synthetic factor MP. These buffer components promote stable and efficient primer annealing, enabling high PCR efficiencies. The buffer also contains HotStarTaq DNA Polymerase, which only becomes active after incubation at 95°C. This prevents the formation of nonspecific products and primer-dimers. For one-step RT-PCR, optimized QuantiTect Multiplex RT Mix allows cDNA synthesis from a wide range of RNA template amounts.

The QuantiTect Multiplex PCR NoROX Kit (for genomic DNA and cDNA templates) and QuantiTect Multiplex RT-PCR NR Kit (for RNA templates) provide reliable quantification of target and control genes in the same reaction, allowing precise relative quantification. In Figure 4, C_T values for targets in a duplex reaction are equivalent to those obtained in a control experiment where the targets were amplified in separate reactions. This demonstrates that each target in the duplex reaction was efficiently and sensitively amplified without affecting the other target.

For beginners in multiplex, real-time PCR, a quick startup guide including a dye selection table is available at www.qiagen.com/goto/MultiplexChecklist .



Ramos cell line cDNA	Mean C _T value of t(8;14)	
	Duplex PCR	Single PCR
100 ng	28.06	28.32
10 ng	31.61	31.89
1 ng	35.28	35.23
0.1 ng	38.09	39.38



Ramos cell line cDNA	Mean C _T value of GAPDH	
	Duplex PCR	Single PCR
100 ng	25.80	25.90
10 ng	28.91	28.75
1 ng	32.39	32.07
0.1 ng	36.23	35.72

Figure 4. Comparable amplification in duplex PCR and single PCR. Duplex, real-time, two-step RT-PCR was performed using the QuantiTect Multiplex PCR NoROX Kit and TaqMan probes. For comparison, single, real-time, two-step RT-PCR was also performed. Reactions were run in duplicate (20 µl volume). Curves for duplex PCR (colored) and single PCR (black) overlap, showing equivalent C_T values.

Ordering Information

Product	Contents	Cat. no.
QuantiFast SYBR Green PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204054
QuantiFast SYBR Green RT-PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204154
QuantiFast Probe PCR +ROX Vial Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 210 µl ROX Dye Solution, 2 x 2 ml RNase-Free Water	204354
QuantiFast Probe RT-PCR +ROX Vial Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 210 µl ROX Dye Solution, 100 µl RT Mix, 2 ml RNase-Free Water	204554
QuantiTect Multiplex PCR NoROX Kit (200)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204743
QuantiTect Multiplex RT-PCR NR Kit (200)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204843
QuantiTect Reverse Transcription Kit - for use in real-time, two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311

* Other kit sizes available; please inquire.

QuantiFast and QuantiTect products are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

To find out more, visit www.qiagen.com/goto/cyclingprogram or www.eppendorf.com/realplex !

Trademarks: QIAGEN®, HotStarTaq®, QuantiFast®, Quantiscript®, QuantiTect® (QIAGEN Group); Mastercycler® (Eppendorf AG); Primer Express® (Applied Biosystems); SYBR® (Molecular Probes, Inc.); TaqMan® (Roche Group).

Practice of the patented polymerase chain reaction (PCR) process requires a license. The Mastercycler ep realplex Thermal Cycler is an Authorized Thermal Cycler and may be used with PCR 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. This is a licensed Real-Time Thermal Cycler under Applied's United States Patent No. 6,814,934 and corresponding claims in non-U.S. counterparts thereof, for use in research and for all other applied fields except human in vitro diagnostics. No right is conveyed expressly, by implication or by estoppel under any other patent claim.

Purchase of QuantiFast SYBR Green Kits is accompanied by a limited, non-transferable immunity from suit to use it with detection by a dsDNA-binding dye as described in U.S. Patents Nos. 5,994,056 and 6,171,785 and corresponding patent claims outside the United States for the purchaser's own internal research. No real-time apparatus or system patent rights or any other patent rights, and no right to use this product for any other purpose are conveyed expressly, by implication or by estoppel.

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QuantiTect Primer Assays are compatible for use in the 5' nuclease process or the dsDNA-binding dye processes covered by patents owned by Roche or owned by or licensed to Applied Biosystems. No license under these patents to practice the 5' nuclease process or the dsDNA-binding dye processes are conveyed expressly or by implication to the purchaser by the purchase of this product.

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