The first universal kit for quantitative, real-time, multiplex PCR

The QuantiTect® Multiplex PCR Kit enables accurate real-time quantification of multiple cDNA or genomic DNA targets in a pre-optimized and easy-to-handle format. Sequence-specific probes compatible with the kit include TaqMan® probes and dual-labeled probes from Operon Biotechnologies. The kit also provides a complete solution for quantitative, real-time, duplex PCR when used together with QuantiTect Assays for target and housekeeping genes.

Benefits of the QuantiTect Multiplex PCR Kit:

- **No optimization required** — reagents and protocols are pre-optimized
- **High sensitivity** — detection of as few as 10 copies of each target sequence
- **Reliable quantification** — target and reference genes are quantified in the same well or tube
- **Easy handling** — ready-to-use master mix is compatible with a wide range of real-time block cyclers

Accurate quantification with minimal handling

By using state-of-the-art technology to amplify several targets in the same reaction, the kit avoids the variability in setting up separate reactions and provides accurate quantification of target and reference genes. In addition, pipetting tasks are minimized, valuable samples and reagents are conserved, and throughput is increased.

Pre-optimized reaction conditions

In contrast to current methods for quantitative, real-time, multiplex PCR, the kit eliminates the need for optimization of the concentrations of primers, Mg^{2+}, and *Taq* DNA polymerase. The master mix supplied with the kit is specifically pre-optimized for quantitative, real-time, multiplex PCR, unlike master mixes supplied with quantitative, real-time, non-multiplex PCR kits. The pre-optimized master mix ensures that the PCR products in a multiplex reaction are amplified with the same efficiency and sensitivity as the PCR products in the corresponding single amplification reactions (Figure 1).

High specificity and sensitivity are achieved through pre-optimization of the following components of the master mix:

- **QuantiTect Multiplex PCR Buffer:** This unique buffer was specifically developed to meet the demands of quantitative, real-time, multiplex PCR using sequence-specific probes. An optimized combination of KCl and $(NH_4)_2SO_4$ promotes a high ratio of specific to nonspecific primer binding during each annealing step. In addition, synthetic factor MP, which was developed for multiplex PCR, increases the local concentration of primers at the template and stabilizes specifically bound primers, allowing efficient primer annealing and extension. These buffer components therefore prevent different amplification reactions from affecting each other.
- **HotStarTaq® DNA Polymerase:** Since this polymerase requires incubation at 95°C for activation, misprimed products and primer–dimers, which can compete for reactants, are not formed during reaction setup. Reactions can therefore be set up at room temperature without any risk of generating nonspecific artifacts.

Superior Performance in Quantitative, Real-Time, Multiplex PCR

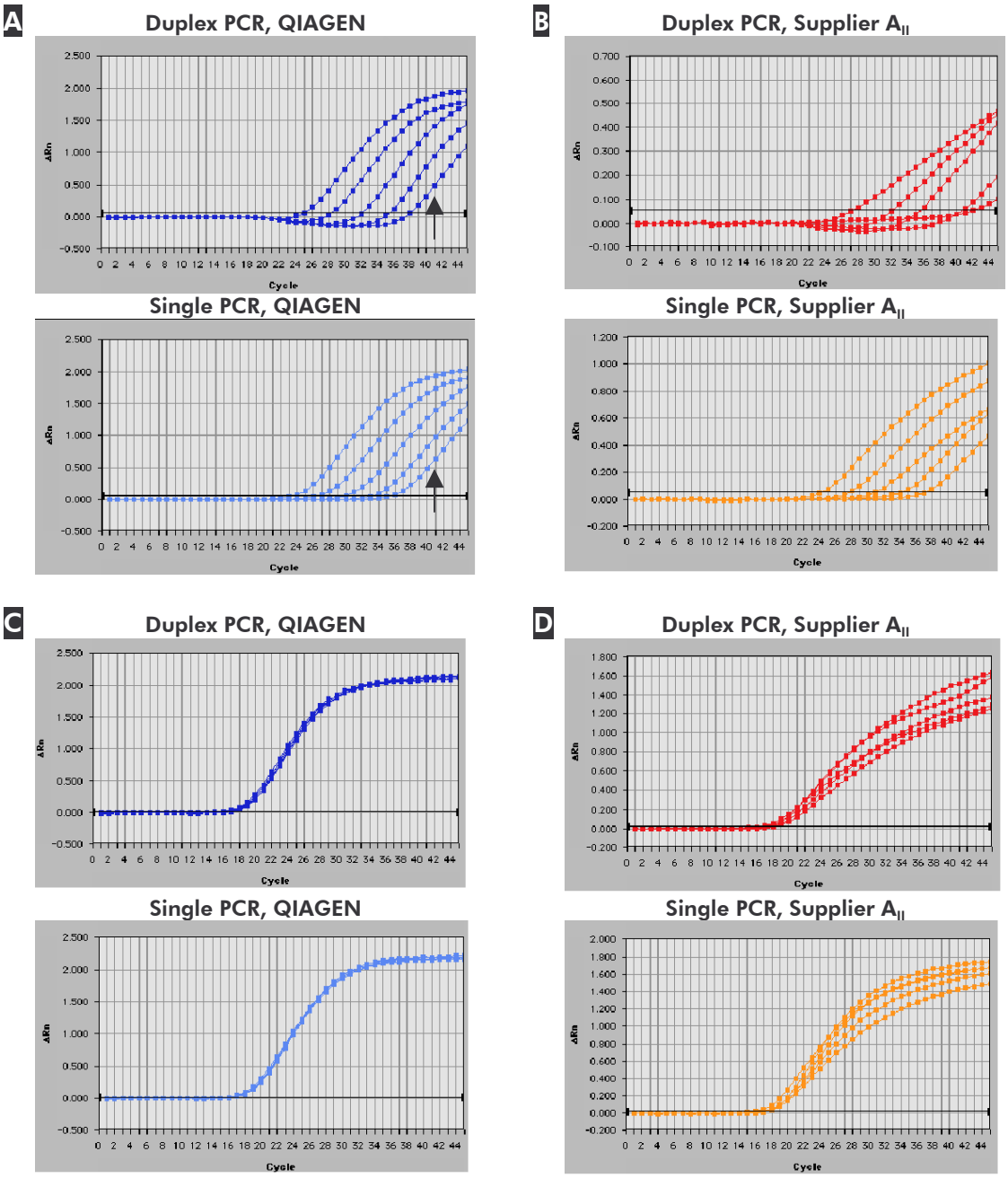

Figure 1 Two sequences were amplified either together in duplex PCR or in single PCRs on the ABI PRISM® 7700. The templates were genomic DNA from the Ramos cell line carrying a t(8;14) translocation (template amounts corresponding to about 50,000, 5000, 500, 50, or 10 copies of the target region), and 10^7 copies of a plasmid containing human GAPDH cDNA sequence. TaqMan probes labeled with FAM or HEX reporter dye plus BHQ™ quencher were used. Arrows on the amplification plots indicate detection of 10 copies of target sequence. **A** Duplex or single PCRs using the QuantiTect Multiplex PCR Kit; t(8;14) translocation sequence detected. **B** Duplex or single PCRs using reagents from Supplier A_{II}; t(8;14) translocation sequence detected. **C** Duplex or single PCRs using the QuantiTect Multiplex PCR Kit; GAPDH sequence detected. **D** Duplex or single PCRs using reagents from Supplier A_{II}; GAPDH sequence detected.

Ready-to-run protocols

The handbook supplied with the kit provides tested protocols for various real-time cyclers as well as valuable information on assay design, reporter dye selection, and data analysis (Table 1). The protocols provide universal conditions for reaction setup and dependable cycling conditions to ensure accurate results the first time.

Table 1. Comparable Threshold Cycle (C_T) Values Between Triplex PCR and Corresponding Single Amplification PCRs

	Detection of		
	t(8;14) translocation sequence (20 copies)	GAPDH cDNA sequence (10^6 copies)	NFκB cDNA sequence (see first column for copy number)
Triplex PCR with 10^5 copies of NFκB	34.31	20.37	21.92
Corresponding single PCRs	34.07	20.54	21.83
Triplex PCR with 10^4 copies of NFκB	34.61	20.62	25.03
Corresponding single PCRs	34.00	20.46	25.19
Triplex PCR with 10^3 copies of NFκB	35.17	19.94	28.38
Corresponding single PCRs	34.43	20.50	28.65

Three templates were coamplified in a triplex PCR: 10^6 copies of a linearized plasmid containing human GAPDH cDNA sequence; 140 pg of genomic DNA from the Ramos cell line carrying a t(8;14) translocation (approximately 20 copies of the target sequence); and 10^5, 10^4, or 10^3 copies of a plasmid containing human NFκB cDNA sequence. This simulates detection of high, low, and variable amounts of target genes in a single sample. These templates were also amplified in single PCRs. TaqMan probes labeled with FAM, HEX, or Bodipy® TMR reporter dye plus BHQ quencher were used. Reactions were performed using the ABI PRISM 7900.

New assays for quantitative, real-time, multiplex PCR

The kit is compatible with the expanding range of QuantiTect Gene Expression Assays (for target genes) and new QuantiTect Endogenous Control Assays (for housekeeping genes). Since QuantiTect Endogenous Control Assays use a QuantiProbe™ labeled with a distinct reporter dye, they can be combined in duplex PCR with other QuantiTect Assays that use a FAM labeled QuantiProbe. The combination of QuantiTect Assays with the QuantiTect Multiplex PCR Kit provides a complete solution for accurate quantitative real-time duplex PCR of target and reference genes (Figure 2).

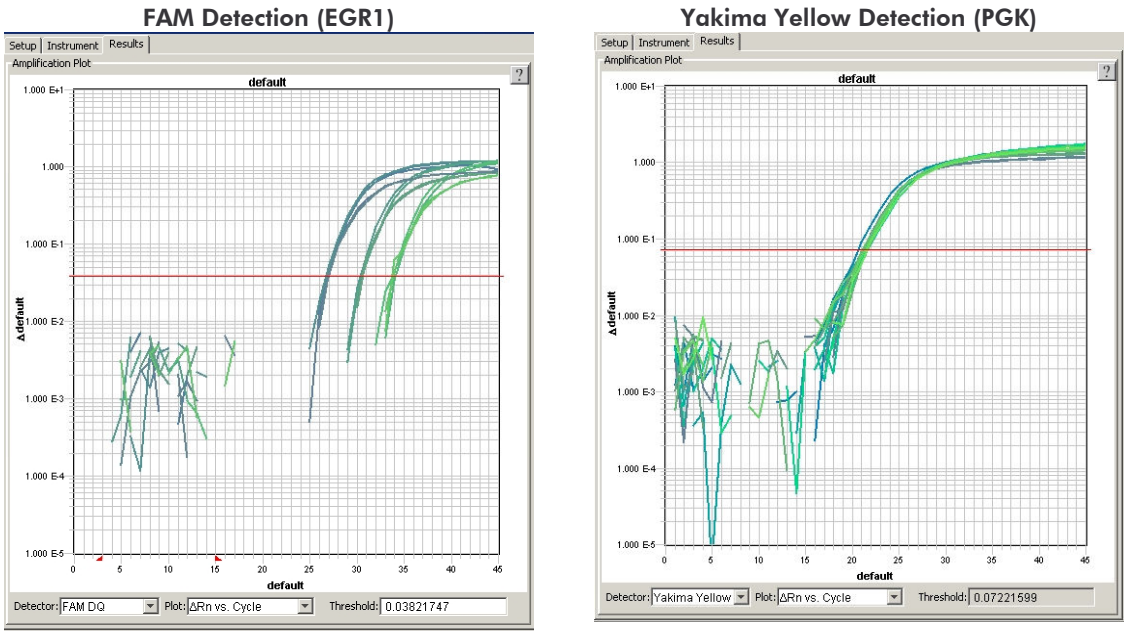

Figure 2 Variable amounts of human EGR1 sequence (50, 5, or 0.5 ng of cDNA from the 293 human cell line) mixed with constant amounts of human PGK sequence (10^6 copies of a synthetic nucleic acid containing the target sequence) were amplified. Duplex PCR and single PCRs of each template mixture were performed in duplicate using the QuantiTect Multiplex PCR Kit in combination with the QuantiTect Gene Expression Assay for EGR1, which uses a FAM labeled QuantiProbe, and the QuantiTect Endogenous Control Assay for PGK, which uses a Yakima Yellow™ labeled QuantiProbe. The amplification plots of the duplex and single reactions are overlaid to demonstrate identical C_T values between the 2 types of PCR. Reactions were performed using the ABI PRISM 7900.

Compatibility with many real-time cyclers

The kit is compatible with most real-time cyclers that use a 96- or 384-well block (Table 2). The master mix contains ROX dye, enabling the kit to be used with real-time cyclers that require ROX dye as passive reference dye, such as ABI™ Sequence Detection Systems. For other real-time cyclers or for assays that use probes labeled with Texas Red®, ROX, or other equivalent dye, the QuantiTect Multiplex PCR NoROX Kit (supplied with master mix containing no ROX dye) is also available.

Table 2. Real-Time Cyclers Compatible with QuantiTect Multiplex PCR Kits

	Compatible with master mix containing	
Real-time cycler	ROX dye	No ROX dye
ABI PRISM 7900	✔	–
ABI PRISM 7700*	✔	–
ABI PRISM 7000	✔	–
iCycler™†	✔	✔
Rotor-Gene™ 3000†	✔	✔
Mx4000®†	✔	✔
Mx3000P™†	✔	✔
DNA Engine Opticon® 2‡	✔	✔
Smart Cycler® II†	✔	✔

* Certain limitations with triplex assays due to the instrument itself.
† Duplex and triplex assays possible with master mix containing ROX dye; 4plex assays also possible with master mix containing no ROX dye.
‡ Only duplex assays possible with this instrument.

Conclusion

Using the QuantiTect Multiplex PCR Kit, accurate results in quantitative, real-time, multiplex PCR can be achieved with the minimum of effort.

Ordering Information

Product	Contents	Cat. no.
QuantiTect Multiplex PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Multiplex PCR Master Mix (contains ROX dye), RNase-Free Water	204543
QuantiTect Multiplex PCR Kit (1000)*	For 1000 x 50 µl reactions: 25 ml QuantiTect Multiplex PCR Master Mix (contains ROX dye), RNase-Free Water	204545
QuantiTect Multiplex PCR NoROX Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Multiplex PCR NoROX Master Mix (contains no ROX dye), RNase-Free Water	Inquire
QuantiTect Multiplex PCR NoROX Kit (1000)*	For 1000 x 50 µl reactions: 25 ml QuantiTect Multiplex PCR NoROX Master Mix (contains no ROX dye), RNase-Free Water	Inquire

* Visit www.qiagen.com/goto/assays to view the full range of QuantiTect Gene Expression Assays and QuantiTect Endogenous Control Assays.

Visit www.qiagen.com/goto/qmpcr to get quantitative, multiplex PCR without the pre-optimization!

Trademarks: QIAGEN®, HotStarTaq®, QuantiProbe™, QuantiTect® (QIAGEN Group); ABI™, ABI PRISM® (Applera Corporation or its subsidiaries); BHQ™ (Biosearch Technologies, Inc.); Bodipy®, Texas Red® (Molecular Probes, Inc.); DNA Engine Opticon® (MJ Research, Inc.); iCycler™ (Bio-Rad Laboratories, Inc.); Mx3000®, Mx4000P™ (Stratagene); Rotor-Gene™ (Corbett Research); Smart Cycler® (Cepheid); TaqMan® (Roche Group); Yakima Yellow™ (Epoch Biosciences).

Purchase of QIAGEN products for PCR containing *Taq* DNA Polymerase, HotStarTaq DNA Polymerase, or ProofStart DNA Polymerase is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

The 5' nuclease process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd.

Patents of third parties in certain countries may cover the process of multiplex PCR or of certain applications.

QuantiTect Gene Expression Assays, QuantiTect Endogenous Control Assays, and QuantiTect Custom Assays or portions hereof are subject to proprietary rights of Epoch Biosciences, Inc. and are made and sold under license from Epoch under the patents and patent applications as may be designated by Epoch from time to time set forth, including one or more of the following: U.S. Patent Nos. 5,801,155, 6,084,102, 6,312,894, 6,426,408, and 6,127,121, and applications currently pending. Purchase of this product carries with it a limited, non-transferable, non-exclusive (without the right to resell, repackage, or sublicense) license under U.S. Patent Nos. 6,030,787; 5,723,591; and 5,876,930, and corresponding foreign patents. Powered by innovation from Epoch. Manufactured for QIAGEN by Epoch Biosciences.