# 5

# PCR, real-time PCR, reverse transcription, and cloning

## 5.1 PCR and RT-PCR www.qiagen.com/PG/PCR

## 5.2 Real-time PCR and RT-PCR www.qiagen.com/PG/real-time



## 5.3 Cloning www.qiagen.com/PG/cloning

## QIAGEN products for PCR and RT-PCR

| Sample type | Application | Taq DNA Polymerase and Master Mix Kit* (pages 170–172) | HotStarTaq Plus DNA Polymerase/Master Mix Kit† (pages 173–174) | QIAGEN Fast Cycling PCR Kit† (page 181) | HotStarTaq DNA Polymerase/Master Mix Kit† (page 177) | ProofStart DNA Polymerase Kit† (page 178) | HotStar HiFidelity Polymerase Kit† (page 179) | QIAGEN LongRange DNA Polymerase Kit† (page 180) | QIAGEN Multiplex PCR Kit† (page 182) | QIAGEN OneStep RT-PCR Kit† (page 183) | QIAGEN LongRange 2Step RT-PCR Kit† (page 184) | Omniscript and Sensiscript RT Kits (pages 186–187) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DNA | Qualitative PCR | ■ | ■ | | ■ | | ■ | ■ | | | | |
| | | | ■ | | ■ | | ■ | ■ | | | | |
| | | ■ | | | | | | | | | | |
| | | ■ | | | ■‡ | | ■‡ | ■‡ | | | | |
| | | | | | | | | | ■ | | | |
| | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | |
| | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | |
| | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | |
| | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | |
| | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | |
| RNA/cDNA | Qualitative RT-PCR | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | Multiplex, two-step RT-PCR | | | | | | | | | | | |
| | One-step RT-PCR | | | | | | | | | | | |
| | Long-range, two-step RT-PCR | | | | | | | | | | | |
| | Single-cell, two-step RT-PCR | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | Single-cell, one-step RT-PCR | | | | | | | | | | | |
| | Virus detection | | | | | | | | | | | |
| | Probe generation for microarray analysis | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |

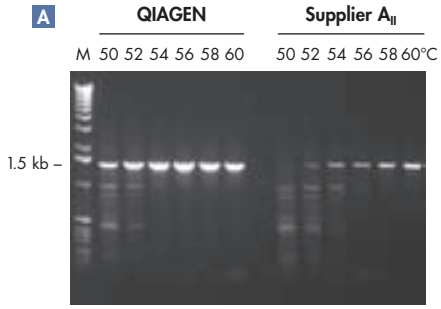
■: Recommended product.

* PCR without hot-start

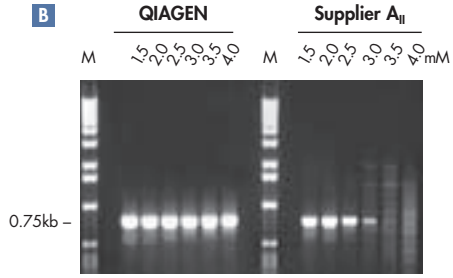
† PCR with hot-start.

‡ *Taq* DNA Polymerase or HotStarTaq *Plus* DNA Polymerase should be used in conjunction with HotStar HiFidelity Polymerase Kit or ProofStart DNA Polymerase for long-range PCR.

### Wide Annealing-Temperature Window

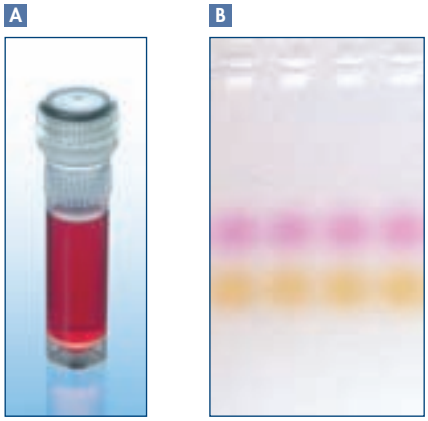



### Tolerance to Variable $Mg^{2+}$ Concentration




PCR amplification at the indicated annealing temperatures A and $Mg^{2+}$ concentrations B using QIAGEN PCR Buffer and *Taq* DNA Polymerase (**QIAGEN**). The same PCR was performed in parallel using PCR buffer and *Taq* DNA polymerase from another supplier (**Supplier A$_{II}$**). Amplification of A the single-copy human cystic fibrosis gene and B the single-copy human prion protein gene. **M**: markers.

### Ready-to-load PCR Buffer




A The novel CoralLoad PCR Buffer B containing 2 gel-tracking dyes allows the PCR product to be loaded immediately onto an analytical gel for increased speed and convenience.

## *Taq* DNA Polymerase and *Taq* PCR Core Kit

**For standard and specialized PCR applications**

- QIAGEN® PCR Buffer for minimal optimization
- Additional ready-to-load PCR buffer for faster and easier handling
- Q-Solution, an innovative PCR additive, for amplification of templates that are GC rich or that have extensive secondary structure
- Choice of formats for convenience and ease of handling

### Product description

*Taq* DNA polymerase is supplied with the unique QIAGEN PCR Buffer that minimizes the requirement for optimization and Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates. In addition, CoralLoad PCR Buffer (containing 2 gel-tracking dyes) is also provided, enabling immediate loading of PCR products. The *Taq* PCR Core Kit also includes a dNTP mix.

### Applications

*Taq* DNA Polymerase is suitable for standard and specialized applications including:

- General PCR
- RT-PCR
- Differential display
- PCR-based DNA fingerprinting (VNTR, STR, and RAPD)

*Taq* DNA Polymerase and the *Taq* PCR Core Kit are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| *Taq* DNA Polymerase (250 U)* | 250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 201203 |
| *Taq* DNA Polymerase (1000 U)* | 4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 201205 |
| *Taq* DNA Polymerase (5000 U)* | 20 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 201207 |
| *Taq* DNA Polymerase (25,000 U)* | 100 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 201209 |
| *Taq* PCR Core Kit (250 U) | 250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix‡ | 201223 |
| *Taq* PCR Core Kit (1000 U) | 4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix‡ | 201225 |

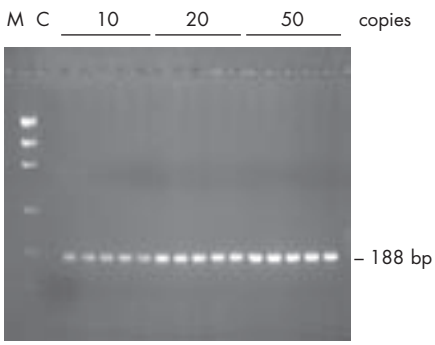
* For dNTPs, see page 180.

† Contains 15 mM $MgCl_2$.

‡ Contains 10 mM each dNTP.

**For further information: www.qiagen.com/PG/PCR**

**Reproducible PCR**




A fragment of the hepatitis B surface antigen gene (gene S) was amplified from 10, 20, and 50 copies of target template, using the *Taq* PCR Master Mix Kit. Five parallel amplifications were performed for each amount of starting template DNA. Equal volumes of the PCR products were analyzed on a 2% agarose gel. **C:** negative control; **M:** markers.

## *Taq* PCR Master Mix Kit

**Premixed solution for convenient PCR setup**

- Easy reaction setup
- Fewer pipetting steps
- Minimal optimization

### Product description

*Taq* PCR Master Mix contains *Taq* DNA Polymerase, the unique QIAGEN PCR Buffer that minimizes the requirement for optimization, and dNTPs. Providing all components in a master mix reduces pipetting steps, increasing throughput and reproducibility.

### Applications

The *Taq* PCR Master Mix Kit is suitable for standard and specialized applications including:

- General PCR
- RT-PCR
- Differential display
- PCR-based DNA fingerprinting (VNTR, STR, and RAPD)

The *Taq* PCR Master Mix Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| *Taq* PCR Master Mix Kit (250 U) | 3 x 1.7 ml *Taq* PCR Master Mix,* containing 250 units *Taq* DNA Polymerase total, 3 x 1.7 ml RNase-free Water | 201443 |
| *Taq* PCR Master Mix Kit (1000 U) | 12 x 1.7 ml *Taq* PCR Master Mix,* containing 1000 units *Taq* DNA Polymerase total, 12 x 1.7 ml RNase-free Water | 201445 |

* Providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP.

**For further information: www.qiagen.com/PG/PCR**

## HotStarTaq® *Plus* DNA Polymerase

**For fast and highly specific amplification in all applications**

- Fast 5-minute enzyme activation time
- High PCR specificity with minimal optimization
- Ready-to-load PCR buffer for faster and easier handling

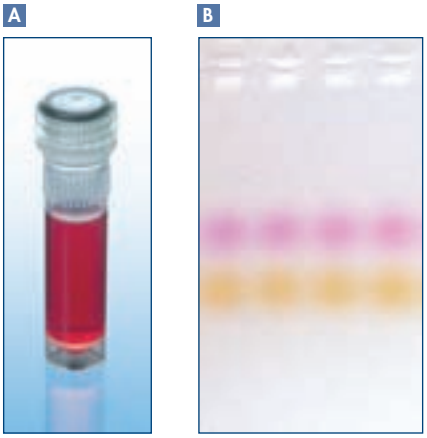
### Product description

The polymerase combines the high specificity, sensitivity, and minimal optimization of HotStarTaq DNA Polymerase with a fast 5-minute activation time. The novel CoralLoad PCR buffer containing gel-tracking dyes and room-temperature setup further streamlines the PCR procedure. Standard PCR buffer is also included for your convenience. In addition, Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates, is also provided.

### Applications

The polymerase is suitable for general PCR, complex genomic or cDNA templates, and very low-copy targets (e.g., single-cell PCR). The polymerase is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**Ready-to-load PCR Buffer**



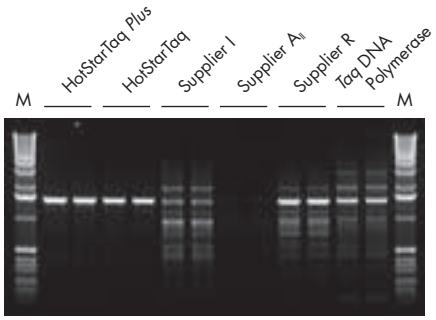

A The novel CoralLoad PCR Buffer B containing 2 gel-tracking dyes allows the PCR product to be loaded immediately onto an analytical gel for increased speed and convenience.

| Product | Contents | Cat. no. |
|---|---|---|
| HotStarTaq *Plus* DNA Polymerase (250 U)* | 1 x 250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203603 |
| HotStarTaq *Plus* DNA Polymerase (1000 U)* | 4 x 250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203605 |
| HotStarTaq *Plus* DNA Polymerase (5000 U)* | 1 x 5000 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203607 |
| HotStarTaq *Plus* DNA Polymerase (25,000 U)* | 100 x 250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203609 |

* dNTPs are available to order separately, see page 180. † Contains 15 mM $MgCl_2$.

**For further information: www.qiagen.com/PG/PCR**

**Highest Specificity with HotStarTaq *Plus* Polymerase**




PCR was carried out using QIAGEN HotStarTaq *Plus*, HotStarTaq, and *Taq* DNA Polymerases and 3 hot-start PCR enzymes from the indicated suppliers. Parallel reactions were performed following the suppliers' recommendations, using 50 ng human genomic DNA. A 1.5 kb fragment of the human CFTR gene was amplified in 35 PCR cycles.
**M**: Markers.

## New HotStarTaq *Plus* Master Mix Kit

**For fast and highly specific amplification in all applications**

- Fast 5-minute enzyme activation time
- Master mix format for added convenience with fewer pipetting steps, reducing the risk of contamination
- Higher PCR specificity and reduced non-specific amplification
- Optional ready-to-load buffer additive for easier handling

### Product description

HotStarTaq *Plus* Master Mix contains HotStarTaq *Plus* DNA Polymerase, the unique QIAGEN PCR Buffer that minimizes the requirement for optimization, and dNTPs. The HotStarTaq *Plus* Master Mix Kit provides the same unrivalled, highly specific and sensitive PCR as the HotStarTaq Master Mix Kit combined with a fast 5-minute enzyme activation time. In addition, CoralLoad Concentrate, containing 2 gel-tracking dyes, is also provided and can be added to the master mix to enable immediate loading of PCR products.

### Applications

The HotStarTaq *Plus* Master Mix Kit is suitable for general PCR, complex genomic templates, complex cDNA templates, and very low-copy targets (e.g., single-cell PCR).

The HotStarTaq *Plus* Master Mix Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| HotStarTaq *Plus* Master Mix Kit (250) | For 250 x 20 µl reactions: 3 x 0.85 ml HotStarTaq *Plus* Master Mix,* containing 250 units of HotStarTaq *Plus* DNA Polymerase total, 1 x 0.55 ml CoralLoad Concentrate, 2 x 1.9 ml RNase-Free Water | 203643 |
| HotStarTaq *Plus* Master Mix Kit (1000) | For 1000 x 20 µl reactions: 12 x 0.85 ml HotStarTaq *Plus* Master Mix,* containing 1000 units of HotStarTaq *Plus* DNA Polymerase total, 4 x 0.55 ml CoralLoad Concentrate, 8 x 1.9 ml RNase-Free Water | 203645 |

* Contains 3 mM $MgCl_2$ and 400 µM each dNTP

**For further information: www.qiagen.com/PG/PCR**

## HotStarTaq DNA Polymerase

**For highly specific amplification with minimal optimization**

- High PCR specificity with minimal optimization
- Reduced nonspecific amplification
- Easy handling and room-temperature setup

### Product description

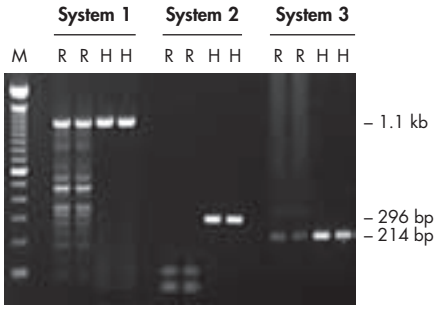
HotStarTaq DNA polymerase is supplied with the unique QIAGEN PCR Buffer that minimizes nonspecific amplification products, primer-dimers, and background. Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates is also provided.

### Applications

HotStarTaq DNA Polymerase is suitable for general PCR, complex genomic templates, complex cDNA templates, and very low-copy targets (e.g., single-cell PCR).

HotStarTaq DNA Polymerase is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**Higher Specificity with Different Primer–Template Systems**




Three different primer–template systems were amplified under the same conditions with either *Taq* DNA polymerase from Supplier R (**R**) or with HotStarTaq DNA Polymerase (**H**). **System 1**: A 1.1 kb fragment of a D-IgI homolog was amplified from human genomic DNA. **System 2**: A 296 bp fragment from the chromosomal region correlated with X-linked juvenile retinoschisis was amplified from human genomic DNA. **System 3**: A 214 bp fragment of the β-actin gene was amplified from cDNA synthesized from total RNA. **M**: markers.

| Product | Contents | Cat. no. |
|---|---|---|
| HotStarTaq DNA Polymerase (250 U)* | 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203203 |
| HotStarTaq DNA Polymerase (1000 U)* | 4 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203205 |
| HotStarTaq DNA Polymerase (5000 U)* | 1 x 5000 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203207 |
| HotStarTaq DNA Polymerase (25,000 U)* | 100 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$ | 203209 |

* For dNTPs, see page 180. † Contains 15 mM $MgCl_2$.

**For further information: www.qiagen.com/PG/PCR**

HotStarTaq Master Mix Kit

## HotStarTaq Master Mix Kit

**Premixed solution for high PCR specificity**

- Easy reaction setup at room temperature
- Ready to use with fewer pipetting steps
- Higher PCR specificity and reduced nonspecific amplification

### Product description

HotStarTaq Master Mix contains HotStarTaq DNA Polymerase, the unique QIAGEN PCR Buffer that minimizes the requirement for optimization, and dNTPs. Providing all components in a master mix reduces pipetting steps and risk of contamination, while increasing throughput and reproducibility.

### Applications

The HotStarTaq Master Mix Kit is suitable for general PCR and for use with complex genomic or cDNA templates, templates isolated from difficult sources, and projects such as genetic screening, in which large numbers of samples are amplified.

The HotStarTaq Master Mix Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| HotStarTaq Master Mix Kit (250 U) | 3 x 0.85 ml HotStarTaq Master Mix,* containing 250 units HotStarTaq DNA Polymerase total, 2 x 1.7 ml RNase-free water | 203443 |
| HotStarTaq Master Mix Kit (1000 U) | 12 x 0.85 ml HotStarTaq Master Mix,* containing 1000 units HotStarTaq DNA Polymerase total, 2 x 1.7 ml RNase-free water | 203445 |
| HotStarTaq Master Mix Kit (2500 U) | 1 x 25 ml HotStarTaq Master Mix,* containing 2500 units HotStarTaq DNA Polymerase total, 2 x 20 ml RNase-free water | 203446 |

* Providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP.

**For further information: www.qiagen.com/PG/PCR**

## HotStar HiFidelity Polymerase Kit

**For highly sensitive and reliable high-fidelity hot-start PCR**

- High sensitivity and specificity due to novel buffer additive and high-fidelity hot-start enzyme
- Unique UA/TA cloning feature
- 10-fold higher fidelity than *Taq* DNA Polymerase
- Room-temperature setup and fast 5-minute enzyme activation time for added convenience

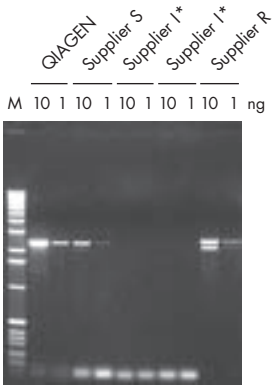
### Product description

The kit comes complete with enzyme, buffers, and dNTPs and is ready to use with minimal optimization required. HotStar HiFidelity DNA Polymerase is a new hot-start proofreading enzyme uniquely modified to produce A overhangs, enabling direct and streamlined UA/TA cloning. The buffer contains Factor SB to prevent degradation of primers and template during PCR setup, providing highly sensitive and reliable high-fidelity PCR. In addition, Q-Solution enables efficient amplification of "difficult" (e.g., GC-rich) templates.

### Applications

HotStar HiFidelity DNA Polymerase provides 10-fold higher fidelity than *Taq* DNA Polymerase combined with a unique UA/TA cloning feature, enabling use in highly sensitive applications, including RT-PCR of full-length transcripts, direct cloning, and mutation analysis. The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

### Highly Sensitive and Reliable PCR



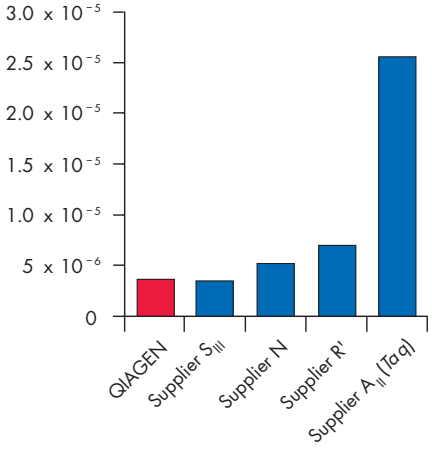

PCR was carried out using HotStar HiFidelity DNA Polymerase (QIAGEN) and 4 high-fidelity PCR enzymes from the indicated suppliers. A 2.3 kb fragment of the human IL9R gene was amplified from genomic DNA in 40 PCR cycles. **M**: Markers. *Two different high-fidelity enzymes from Supplier I.

| Product | Contents | Cat. no. |
|---|---|---|
| HotStar HiFidelity Polymerase Kit (100 U) | 100 units HotStar HiFidelity DNA Polymerase, 5x PCR Buffer (inc. dNTPs),* 5x Q-Solution, 25 mM $MgSO_4$, RNase-free water | 202602 |
| HotStar HiFidelity Polymerase Kit (1000 U) | 2x 500 units HotStar HiFidelity DNA Polymerase, 5x PCR Buffer (inc. dNTPs),* 5x Q-Solution, 25 mM $MgSO_4$, RNase-free water | 202605 |

* Contains dNTPs, Factor SB, and optimized concentration of $MgSO_4$

**For further information: www.qiagen.com/PG/PCR**

**Very Low Error Rate of ProofStart DNA Polymerase**




Error rates were determined for ProofStart DNA Polymerase, proofreading enzymes from Suppliers $S_{III}$ and N, a commercial *Taq*/proofreading polymerase mixture from supplier R (R'), and *Taq* DNA polymerase from Supplier $A_{II}$. Rates were determined using a β-galactosidase PCR mutation assay and are reported as the fraction of misincorporated bases per PCR product doubling.

## ProofStart® DNA Polymerase

**For high-fidelity hot-start PCR**

- Robust PCR due to hot-start enzyme
- Efficient proofreading activity for high fidelity
- Specially developed buffer for minimal PCR optimization

### Product description

ProofStart DNA Polymerase is a hot-start proofreading polymerase modified to prevent primer degradation during PCR setup, providing robust, high-fidelity PCR. ProofStart DNA Polymerase is supplied with the ProofStart PCR Buffer that minimizes the requirement for optimization and Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates.

### Applications

ProofStart DNA Polymerase provides very low error rates for applications such as cloning, site-directed mutagenesis, and mutation analysis.

ProofStart DNA Polymerase is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| ProofStart DNA Polymerase (100 U)* | For 40 high-fidelity amplification reactions: 100 units ProofStart DNA Polymerase, 10x ProofStart PCR Buffer,† 5x Q-Solution, 25 mM $MgSO_4$ | 202203 |
| ProofStart DNA Polymerase (500 U)* | For 200 high-fidelity amplification reactions: 500 units ProofStart DNA Polymerase, 10x ProofStart PCR Buffer,† 5x Q-Solution, 25 mM $MgSO_4$ | 202205 |

* For dNTPs, see page 180. † Contains 15 mM $MgSO_4$.

**For further information: www.qiagen.com/PG/PCR**

## New QIAGEN LongRange PCR Kit

**For sensitive and accurate long-range PCR**

- Amplification of extremely long PCR products (up to 40 kb)
- Low error rates, ensured by high-fidelity enzyme
- Minimal PCR optimization due to unique buffer system
- Amplification of low-copy targets and GC-rich templates

### Product description

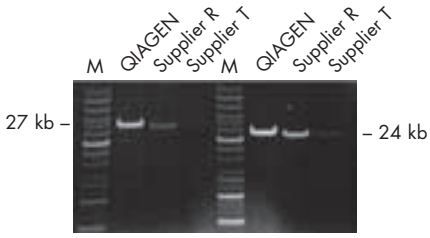
The ready-to-use QIAGEN LongRange PCR Kit contains a blend of *Taq* DNA polymerase and a powerful high-fidelity enzyme to ensure high amplification efficiency and maximum fidelity. The unique PCR buffer enhances extension rates and fidelity, even when amplifying complex templates. PCR products up to 40 kb can be reliably amplified. Q-Solution, for amplification of GC-rich templates, and high-purity dNTPs are also provided.

### Applications

The QIAGEN LongRange PCR Kit is suitable for high-fidelity long-range PCR of standard and complex templates for all applications including cloning and sequencing.

The QIAGEN LongRange PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**High Yields of Very Long PCR Products**




27 kb and 24 kb PCR products were amplified from complex DNA using the QIAGEN LongRange PCR Kit and 2 long-range PCR kits from the indicated suppliers according to the manufacturer's instructions. The QIAGEN LongRange PCR Kit provided much higher yields of specific product than the other kits tested.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN LongRange PCR Kit (20) | For 20 x 50 µl reactions: LongRange PCR Enzyme Mix (40 U), LongRange PCR Buffer, 5x Q-Solution, RNase-Free Water, 10 mM dNTPs | 206401 |
| QIAGEN LongRange PCR Kit (100) | For 100 x 50 µl reactions: LongRange PCR Enzyme Mix (200 U), LongRange PCR Buffer, 5x Q-Solution, RNase-Free Water, 10 mM dNTPs | 206402 |
| QIAGEN LongRange PCR Kit (250) | For 250 x 50 µl reactions: LongRange PCR Enzyme Mix (500 U), LongRange PCR Buffer, 5x Q-Solution, RNase-Free Water, 10 mM dNTPs | 206403 |

**For further information: www.qiagen.com/PG/PCR**

### Ideal for All Applications

| Sample type | Application |
|---|---|
| DNA | Standard PCR |
| | High-fidelity PCR |
| | Long-range PCR |
| | Multiplex PCR |
| | Genotyping |
| | Single-cell PCR |
| | Methylation-specific PCR (MSP) |
| | Nested PCR |
| RNA/ cDNA | Two-step RT-PCR |
| | Multiplex, two-step RT-PCR |
| | Single-cell, two-step RT-PCR |

dNTP Mix, PCR Grade can be used in a variety of sensitive PCR techniques.

## New dNTP Set, PCR Grade and dNTP Mix, PCR Grade

**For sensitive and reproducible PCR and RT-PCR**

- Individual dNTPs or premixed ready-to-use solution
- Highly pure
- Suitable even for highly sensitive PCR applications such as single-cell PCR, long-range PCR, multiplex PCR, and RT-PCR

### Product description

dNTP Set, PCR Grade is a complete set of individual, highly pure dNTPs. Each individual 100 mM dNTP is supplied in water and can be diluted and mixed with any other dNTP to the desired concentration. dNTP Mix, PCR Grade contains premixed dATP, dCTP, dGTP, and dTTP in water (pH 7.5), each at a concentration of 10 mM. Highly pure dNTPs are important for successful PCR, as the presence of contaminating impurities in PCR can result in a decrease in amplification sensitivity and product yield.

### Applications

QIAGEN dNTPs are suitable for use in all standard PCR techniques and all sensitive PCR techniques such as long-range PCR, multiplex PCR, and RT-PCR. In addition, these products are ideal for use in combination with all common PCR and RT-PCR enzymes, including QIAGEN *Taq* DNA Polymerase, HotStarTaq *Plus* DNA Polymerase, and reverse transcriptase enzymes.

| Product | Contents | Cat. no. |
|---|---|---|
| dNTP Mix, PCR Grade (200 µl) | Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP (1 x 200 µl) | 201900 |
| dNTP Mix, PCR Grade (800 µl) | Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP (4 x 200 µl) | 201901 |
| **New** dNTP Set, PCR Grade, 4 x 100 µl | 100 mM each dATP, dCTP, dGTP, dTTP for 1000 x 50 µl PCR reactions | 201912 |
| **New** dNTP Set, PCR Grade, 4 x 250 µl | 100 mM each dATP, dCTP, dGTP, dTTP for 2500 x 50 µl PCR reactions | 201913 |

**For further information: www.qiagen.com/PG/PCR**

## New QIAGEN Fast Cycling PCR Kit

**For ultrafast and specific PCR on any thermal cycler**

- Hot-start PCR amplification in less than 20 minutes (35 cycles)
- Ideally suited for use with any thermal cycler
- High specificity and sensitivity
- No primer redesign required
- Optional ready-to-load PCR dye for easier handling

### Product description

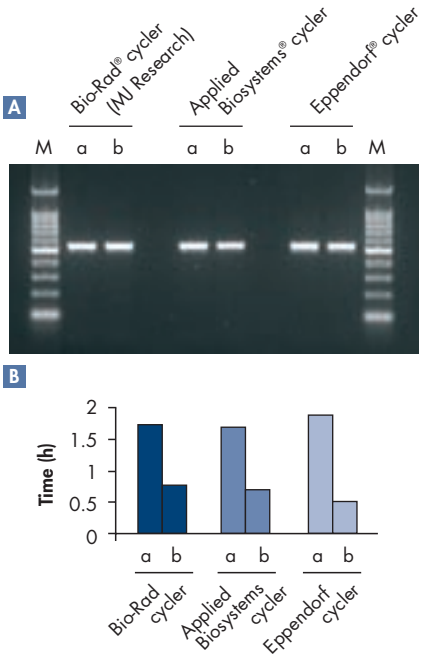
The kit contains HotStarTaq *Plus* DNA Polymerase for highly specific and sensitive PCR, and a unique buffer formulation for extremely short denaturation, annealing, and extension steps. The new patent-pending PCR buffer significantly reduces the time required to form the polymerase, primer, and template complex, reducing the total PCR cycling time from approximately 1.5 hours to just 20 minutes. The optional CoralLoad Fast Cycling Dye contains gel-tracking dyes for convenient analysis. In addition, Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates, is also provided.

### Applications

The kit is suitable for general PCR, complex genomic templates, and complex cDNA templates. The kit can be used with standard as well as fast ramping thermal cyclers.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**Save up to 75% of PCR Time**

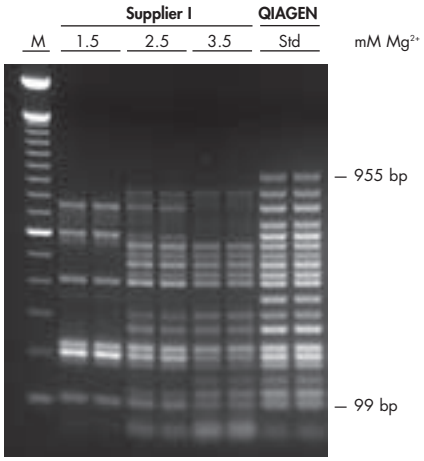



PCR was carried out using thermal cyclers with standard ramping times and fast ramping times from the indicated manufacturers. A 523 bp product was amplified using (a) HotStarTaq *Plus* DNA polymerase with standard cycling conditions and (b) the QIAGEN Fast Cycling PCR Kit with fast ramping times. A Agarose gel analysis of the PCR product showed identical, highly specific amplification for both techniques. B The QIAGEN Fast Cycling PCR Kit provides significant time savings (up to 1 h 25 min) compared to standard PCR amplification.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN Fast Cycling PCR Kit (200) | For 200 x 20 µl reactions: 2 x 1 ml QIAGEN Fast Cycling PCR Master Mix, 10x CoralLoad Fast Cycling Dye, 5x Q-Solution, RNase-Free Water | 203743 |
| QIAGEN Fast Cycling PCR Kit (1000) | For 1000 x 20 µl reactions: 10 x 1 ml QIAGEN Fast Cycling PCR Master Mix, 10x CoralLoad Fast Cycling Dye, 5x Q-Solution, RNase-Free Water | 203745 |

**For further information: www.qiagen.com/PG/PCR**

**Successful 19-plex PCR Without Optimization**




Multiplex PCR of 19 targets (99–955 bp) was carried out for 35 cycles using standard conditions (**Std**) for the QIAGEN Multiplex PCR Kit (**QIAGEN**) without optimization, or using the indicated $Mg^{2+}$ concentrations with a hot-start enzyme and supplied KCl-based buffer from Supplier I (**Supplier I**). Equal volumes of each multiplex PCR were analyzed on a 1.7% agarose gel stained with ethidium bromide. **M**: markers.

## QIAGEN Multiplex PCR Kit

**For highly specific and sensitive multiplex PCR without optimization**

- No optimization required
- High specificity and sensitivity with a built-in hot start
- Highly suited for many types of multiplex PCR applications
- Easy to use and cost-effective

### Product description

The QIAGEN Multiplex PCR Kit provides a QIAGEN Multiplex PCR Master Mix with HotStarTaq DNA Polymerase and a unique PCR buffer containing the novel synthetic factor MP. Together with optimized salt concentrations, this factor stabilizes specifically bound primers and enables efficient extension of all primers in the reaction without optimization. Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates, is also supplied.

### Applications

The QIAGEN Multiplex PCR Kit is suitable for use in many types of multiplex PCR applications such as microsatellite analysis, genotyping, GMO typing, and SNP amplification.

The QIAGEN Multiplex PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN Multiplex PCR Kit (100) | For 100 x 50 µl multiplex PCRs: 2x QIAGEN Multiple PCR Master Mix (3 x 0.85 ml),* 5x Q-Solution (1 x 2 ml), 2 x 1.7 ml RNase-Free Water | 206143 |
| QIAGEN Multiplex PCR Kit (1000) | For 1000 x 50 µl multiplex PCRs: 2x QIAGEN Multiple PCR Master Mix (1 x 25 ml),* 5x Q-Solution (1 x 10 ml), 1 x 20 ml RNase-Free Water | 206145 |

* Contains HotStartTaq DNA Polymerase, Multiplex PCR Buffer, $MgCl_2$ (final concentration 3 mM), and dNTPs.

**For further information: www.qiagen.com/PG/PCR**

## QIAGEN OneStep RT-PCR Kit

### For fast and successful one-step RT-PCR

- Fast and easy one-tube setup
- One-step RT-PCR of any RNA template without optimization
- Unique enzyme mix for high specificity and sensitivity
- Optimized buffer for efficient reverse transcription and amplification

### Product description

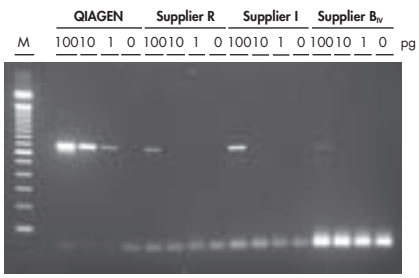
The QIAGEN One-Step RT-PCR Kit provides a blend of Sensiscript and Omniscript Reverse Transcriptases, HotStarTaq DNA Polymerase, QIAGEN OneStep RT-PCR Buffer, a dNTP mix, and Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates. The easy one-tube setup and optimized components result in highly sensitive and successful results.

### Applications

The QIAGEN OneStep RT-PCR Kit is suitable for highly sensitive RT-PCR applications such as gene expression analysis, virus detection, and single-cell RT-PCR.

The QIAGEN OneStep RT-PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

### Efficient, Sensitive RT-PCR




One-step RT-PCR was carried out using the indicated amounts of total RNA from HeLa cells and primers specific for α-catenin. All reactions were carried out following suppliers' instructions. **M**: 100 bp ladder.

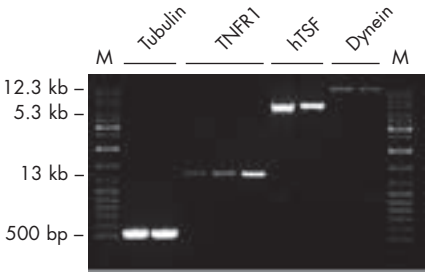
| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN OneStep RT-PCR Kit (25) | For 25 x 50 µl reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,* dNTP Mix,† 5x Q-Solution, RNase-Free Water | 210210 |
| QIAGEN OneStep RT-PCR Kit (100) | For 100 x 50 µl reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,* dNTP Mix,† 5x Q-Solution, RNase-Free Water | 210212 |

* Contains 12.5 mM $MgCl_2$.

† Contains 10 mM each dNTP.

**For further information: www.qiagen.com/PG/PCR**

**Highly Sensitive Amplification of Large and Rare Transcripts**




The QIAGEN LongRange 2Step RT-PCR Kit was used to amplify the indicated sequences from total RNA from mouse and human cell lines. The sensitivity of the kit is shown by the high yields of TNFR1 and dynein sequences, both of which are rare transcripts.

## New QIAGEN LongRange 2Step RT-PCR Kit

**For sensitive and accurate long-range two-step RT-PCR**

- Dedicated two-step solution for accurate amplification of long transcripts
- High-yield amplification of up to 12.5 kb cDNA
- Low error rates and extended read length ensured by high-fidelity PCR enzyme mix
- Minimal PCR optimization due to unique buffer system
- Amplification of low abundance targets and GC-rich templates

### Product description

The QIAGEN LongRange 2Step RT-PCR Kit combines the features and benefits of the QIAGEN LongRange PCR Kit with a high-yield reverse transcription step. The recombinant homodimeric viral reverse transcriptase (AMV) provides highly-sensitive, full-length cDNA synthesis up to 12.5 kb. The special buffer composition allows reverse transcription at elevated temperatures, resulting in reduced RNase H activity and improved denaturation of difficult templates. In combination with the components for long-range PCR, 3 powerful enzymes enable long and accurate two-step amplification, even for low-copy targets. The kit comes complete and ready to use with all components necessary for successful results. Preoptimized buffers reduce the need for lengthy protocol optimization.

### Applications

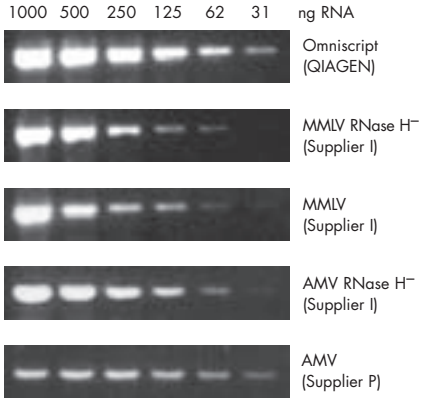
The QIAGEN LongRange 2Step RT-PCR Kit is suitable for dedicated long-range two-step RT-PCR applications such as cloning and gene expression analysis. The high sensitivity and accuracy make this kit highly suited for amplifying low abundance targets.

The QIAGEN LongRange 2Step RT-PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN LongRange 2Step RT-PCR Kit (20) | For 20 x 50 µl PCRs: Reverse transcription step (10 x 20 µl reactions) — LongRange Reverse Transcription Enzyme, Buffer, dNTPs, Oligo-dT, RNase-Free Water; PCR step — QIAGEN LongRange PCR Kit (see page 179) | 205920 |
| QIAGEN LongRange 2Step RT-PCR Kit (100) | For 100 x 50 µl PCRs: Reverse transcription step (50 x 20 µl reactions) — LongRange Reverse Transcription Enzyme, Buffer, dNTPs, Oligo-dT, RNase-Free Water; PCR step — QIAGEN LongRange PCR Kit (see page 179) | 205922 |

**For further information: www.qiagen.com/PG/PCR**

**Superior Sensitivity and Dynamic Range of Omniscript RT**




Reverse transcription was carried out with different reverse transcriptases according to suppliers' specifications, using the indicated amounts of total RNA from HeLa cells. 1/20 of the reverse-transcription reaction was used in a 25-cycle PCR amplification with QIAGEN *Taq* DNA Polymerase. A 1.7 kb β-actin fragment was amplified.

## Omniscript® RT Kit

**For efficient and sensitive reverse transcription using 50 ng to 2 µg RNA per reaction**

- High cDNA yields — high affinity enzyme with optimized buffer system
- High sensitivity — detect as few as 10 copies of template
- Fast and easy procedure — no tedious pipetting steps or additional RNase H digestion step required

### Product description

The kit is supplied with Omniscript Reverse Transcriptase, 10x Buffer RT, and dNTP Mix for efficient and sensitive reverse transcription of 50 ng to 2 µg RNA in a fast and easy procedure.

### Applications

The kit can be used in a wide variety of applications, including standard RT, real-time RT-PCR, microarray analysis, and linear RNA amplification.

The Omniscript RT Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| Omniscript RT Kit (10) | For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-Free Water | 205110 |
| Omniscript RT Kit (50) | For 50 reverse-transcription reactions: 200 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-Free Water | 205111 |
| Omniscript RT Kit (200) | For 200 reverse-transcription reactions: 800 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-Free Water | 205113 |

* Contains 5 mM each dNTP.

**For further information: www.qiagen.com/PG/PCR**

## Sensiscript® RT Kit

**For efficient and sensitive reverse transcription using less than 50 ng RNA per reaction**

- High cDNA yields — high affinity enzyme with optimized buffer system
- High sensitivity — detect as few as 10 copies of template
- Fast and easy procedure — no tedious pipetting steps or additional RNase H digestion step required

### Product description

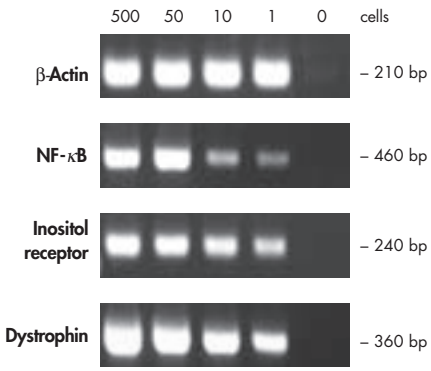
The kit is supplied with Sensiscript Reverse Transcriptase, 10x Buffer RT, and dNTP Mix for efficient and sensitive reverse transcription of <50 ng RNA in a fast and easy procedure.

### Applications

The kit can be used in a wide variety of applications, including standard RT, real-time RT-PCR, differential display RT-PCR, analysis of LMD samples, and linear RNA amplification.

The Sensiscript RT Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**RT-PCR with RNA Corresponding to 1 Cell**



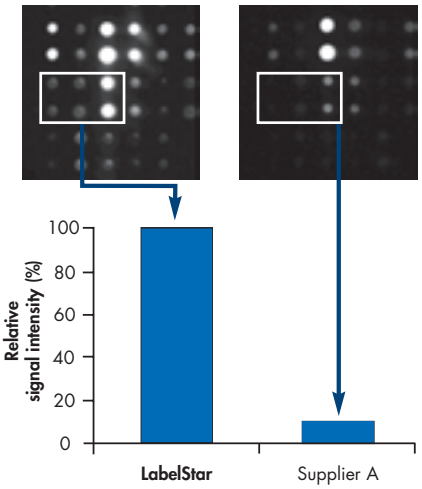

Total RNA was purified from 10 to 5000 HeLa cells using RNeasy® technology. 1/10 of each eluate (corresponding to RNA from 1 to 500 cells) was used for reverse transcription with Sensiscript Reverse Transcriptase. 1/2 of each RT reaction was then used in a 40-cycle PCR with QIAGEN *Taq* DNA Polymerase and primers specific for genes encoding β-actin, NF-κB, inositol-1,4, 5-triphosphate receptor, or dystrophin. Sizes of the amplicons are as indicated.

| Product | Contents | Cat. no. |
|---|---|---|
| Sensiscript RT Kit (50) | For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-Free Water | 205211 |
| Sensiscript RT Kit (200) | For 200 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-Free Water | 205213 |

* Contains 5 mM each dNTP.

**For further information: www.qiagen.com/PG/PCR**

**Higher Signal Intensities Using the LabelStar Array Kit**




cDNA labeling (with Cy®5) and purification were performed using 2 µg total RNA from mouse brain using the LabelStar Array Kit and a kit from Supplier A, following the manufacturers' instructions. The signal intensities of six spots were compared. Mean values of relative signal intensities were calculated (see bar graph). On average, signal intensities were ninefold higher using the LabelStar Array Kit.

## LabelStar™ Array Kit

**For efficient cDNA labeling and cleanup, prior to labeled cDNA array hybridization**

- High signal intensity and low background — identification of true positives at low expression levels
- High specificity — labeling using 0.2–50 µg RNA
- Flexibility in choice of label — incorporation of any commonly used modified nucleotide
- Optimized labeling and cleanup procedure — reproducible, high-quality results
- Fast and easy method — provides reliable performance

### Product description

The LabelStar Array Kit is supplied with LabelStar Reverse Transcriptase for reverse transcription of RNA and incorporation of modified nucleotides (supplied by user), and with MinElute® spin columns for cleanup of the labeled cDNA.

### Applications

The LabelStar Array Kit is suitable for different labeling strategies in microarray analysis, including direct cDNA labeling, indirect cDNA labeling, radioactive labeling, and direct labeling after RNA amplification.

The LabelStar Array Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| LabelStar Array Kit (12) | For 12 labeling reactions: LabelStar Reverse Transcriptase, dNTPs,* RNase Inhibitor, Oligo-dT Primer, 12 MinElute Spin Columns, RNase-Free Reagents, Buffers | 28902 |
| LabelStar Array Kit (50) | For 50 labeling reactions: LabelStar Reverse Transcriptase, dNTPs,* RNase Inhibitor, Oligo-dT Primer, 50 MinElute Spin Columns, RNase-Free Reagents, Buffers | 28904 |

* 20 mM solutions each; labeled nucleotides to be supplied by user.

**For further information: www.qiagen.com/PG/PCR**

## Real-time PCR and RT-PCR — SYBR Green detection

| Sample type | Procedure | FastLane Cell cDNA Kit (page 208) | FastLane Cell RT-PCR Kits (page 209) | QuantiTect† Reverse Transcription Kit (page 207) | QuantiFast§ SYBR Green PCR Kit* (page 193) | QuantiTect‡ SYBR Green PCR Kit (page 194) | QuantiFast§ SYBR Green RT-PCR Kit* (page 195) | QuantiTect‡ SYBR Green RT-PCR Kit (page 196) | QuantiTect‡ Primer Assays (page 192) |
|---|---|---|---|---|---|---|---|---|---|
| Cells | Two-step RT-PCR | ■ | | | | ■ | | | ■ |
| | One-step RT-PCR | | ■ | | | | | | ■ |
| RNA/cDNA | Two-step RT-PCR, fast-cycling | | | ■ | ■ | | | | ■ |
| | Two-step RT-PCR | | | ■ | | ■ | | | ■ |
| | One-step RT-PCR, fast-cycling | | | | | | ■ | | ■ |
| | One-step RT-PCR | | | | | | | ■ | ■ |
| DNA | PCR, fast-cycling† | | | | ■ | | | | |
| | PCR† | | | | | ■ | | | |

■: Recommended product.

* For faster results on standard real-time cyclers and on real-time cyclers with fast cycling capabilities.

† Suitable for genotyping.

‡ QuantiTect Kits are supplied with a master mix containing dATP, dCTP, dGTP, and dTTP/dUTP.

§ QuantiFast Kits are supplied with a master mix containing dATP, dCTP, dGTP, and dTTP.

## Real-time PCR and RT-PCR — probe detection

| Sample type | Procedure | FastLane Cell cDNA Kit (page 208) | FastLane Cell RT-PCR Kits (page 209) | QuantiTect® Reverse Transcription Kit (page 207) | QuantiFast® Probe PCR Kits*† (page 197) | QuantiTect® Probe PCR Kit (page 199) | QuantiFast® Probe RT-PCR Kits*† (page 200) | QuantiTect® Probe RT-PCR Kit (page 202) |
|---|---|---|---|---|---|---|---|---|
| Cells | Two-step RT-PCR | ■ | | | | ■ | | |
| | One-step RT-PCR | | ■ | | | | | |
| RNA/ cDNA | Two-step RT-PCR, fast-cycling | | | ■ | ■ | | | |
| | Two-step RT-PCR | | | ■ | | ■ | | |
| | One-step RT-PCR, fast-cycling | | | | | | ■ | |
| | One-step RT-PCR | | | | | | | ■ |
| DNA | PCR, fast-cycling‡ | | | | ■ | | | |
| | PCR‡ | | | | | ■ | | |

■: Recommended product.

* For faster results on standard real-time cyclers and on real-time cyclers with fast cycling capabilities.

† Two kit formats available: kit with ROX dye in the master mix, and kit with ROX dye as separate solution.

‡ Suitable for genotyping and SNP analysis.

§ QuantiTect Kits are supplied with a master mix containing dATP, dCTP, dGTP, and dTTP/dUTP.

¶ QuantiFast Kits are supplied with a master mix containing dATP, dCTP, dGTP, and dTTP.

## Real-time PCR and RT-PCR — multiplex probe detection

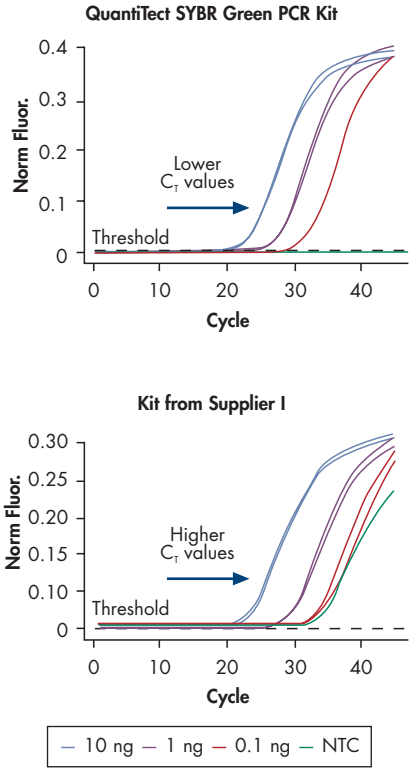
| Sample type | Procedure | FastLane Cell cDNA Kit (page 208) | FastLane Cell RT-PCR Kits (page 209) | QuantiTect† Reverse Transcription Kit (page 207) | QuantiTect† Multiplex PCR Kit (page 203) | QuantiTect† Multiplex PCR NoROX Kit (page 203) | QuantiTect† Multiplex RT-PCR Kit (page 205) | QuantiTect† Multiplex RT-PCR NR Kit (page 205) |
|---|---|---|---|---|---|---|---|---|
| Cells | Two-step RT-PCR, with ROX dye | ■ | | | ■ | | | |
| | Two-step RT-PCR, no ROX dye | ■ | | | | ■ | | |
| | One-step RT-PCR, with ROX dye | | ■ | | | | | |
| | One-step RT-PCR, no ROX dye | | ■ | | | | | |
| RNA/ cDNA | Two-step RT-PCR, with ROX dye | | | ■ | ■ | | | |
| | Two-step RT-PCR, no ROX dye | | | ■ | | ■ | | |
| | One-step RT-PCR, with ROX dye | | | | | | ■ | |
| | One-step RT-PCR, no ROX dye | | | | | | | ■ |
| DNA | PCR, with ROX dye* | | | | ■ | | | |
| | PCR, no ROX dye* | | | | | ■ | | |

■: Recommended product.

* Suitable for genotyping and SNP analysis.

† QuantiTect Kits are supplied with a master mix containing dATP, dCTP, dGTP, and dTTP/dUTP.

**Sensitive Quantification of Target Gene**




Dilutions of human leukocyte cDNA (10 ng to 100 pg) were analyzed on the Rotor-Gene™ 3000 using the QuantiTect Primer Assay for human BAX and the indicated kits. Only the combination of QuantiTect Kit and Assay provided more sensitive quantification, as demonstrated by the lower $C_T$ values. **NTC:** no template control.

## QuantiTect® Primer Assays

**For use in real-time RT-PCR with SYBR® Green detection**

- Guaranteed performance — no need to design primers
- PCR efficiencies of ~100% — reliable relative quantification data
- High sensitivity and specificity — accurate quantification over a wide linear range
- Cost savings — detection by SYBR Green instead of by more expensive sequence-specific probes

### Product description

QuantiTect Primer Assays are genomewide, bioinformatically validated primer sets for SYBR Green based real-time RT-PCR. Assays are available for any gene from human, rat, mouse, and many other species. Each assay is supplied as a lyophilized mix of forward and reverse primers for a specific gene.*

### Applications

QuantiTect Primer Assays are ideal for gene expression analysis applications, such as validation of RNAi or of microarray results. When used in combination with QuantiFast and QuantiTect SYBR Green Kits (pages 193–196), QuantiTect Primer Assays guarantee highly specific and sensitive results in real-time RT-PCR comparable to probe-based detection. QuantiTect Primer Assays can be searched for and ordered online at GeneGlobe ( www.qiagen.com/PG/GeneGlobe ).

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

*Reaction components for real-time PCR or RT-PCR are not included.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Primer Assay (200) | For 200 x 50 µl reactions (in 96 wells or single tubes) or 500 x 20 µl reactions (in 384 wells or single capillaries): 10x QuantiTect Primer Assay (lyophilized) | Varies |

**For further information: www.qiagen.com/PG/GeneGlobe**
**Tel: 02-33430411 ■ Fax: 02-33430426 ■ Technical Information: 800 787980**

## New QuantiFast SYBR Green PCR Kit

**For fast, quantitative, real-time PCR and two-step RT-PCR using SYBR Green I**

- Reduced cycling times — significant time savings of 50–65%
- High sensitivity — precise and reliable detection of 10 copies of target
- Wide linear range — accurate quantification over several logs of template dilution
- Easy setup — master mix compatible with any cycler

### Product description

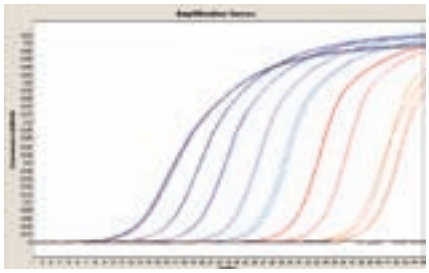
The kit is specially designed for fast cycling real-time PCR. It reduces cycling times on all cyclers and is particularly suited for fast cyclers with short ramping times. The ready-to-use master mix, which can be stored at 2–8°C, contains SYBR Green I dye, ROX passive reference dye (optimized concentration), HotStarTaq *Plus* DNA Polymerase, and dNTP mix in a unique PCR buffer.

### Applications

The kit provides accurate real-time quantification of DNA and cDNA targets* on any real-time cycler. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**Reproducible Results over a Wide Linear Range**



Different amounts of a plasmid containing GAPDH sequence ($10^8$, $10^7$, $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, 10, and 5 copies) were analyzed in duplicate on the LightCycler® 2.0 using the QuantiFast SYBR Green PCR Kit.

* cDNA can be rapidly synthesized using the QuantiTect Reverse Transcription Kit or FastLane Cell cDNA Kit (pages 207 and 208).

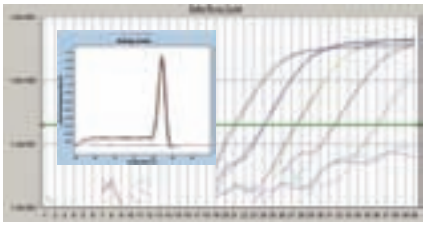
| Product | Contents | Cat. no. |
|---|---|---|
| QuantiFast SYBR Green PCR Kit (80)† | For 80 x 25 µl reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water | 204052 |
| QuantiFast SYBR Green PCR Kit (400)† | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water | 204054 |
| QuantiFast SYBR Green PCR Kit (2000)† | For 2000 x 25 µl reactions: 25 ml 2x Master Mix, 20 ml RNase-Free Water | 204056 |

† Genomewide, validated primer sets for use with the kit are available; see page 192.

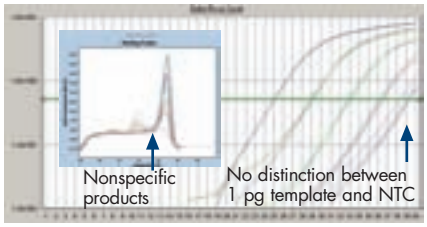
**For further information: www.qiagen.com/PG/real-time**
**Tel: 02-33430411 ■ Fax: 02-33430426 ■ Technical Information: 800 787980**

**Specific and Sensitive Quantification in Two-Step RT-PCR**

**QuantiTect SYBR Green PCR Kit**



***Power* SYBR Green PCR Master Mix**




Dilutions of human leukocyte cDNA (equivalent to 10 ng, 1 ng, 100 pg, 10 pg, and 1 pg RNA) were analyzed on the ABI PRISM 7000 using the QuantiTect Primer Assay for human IL8 and the indicated kits. Insets show melting curve analysis performed on the LightCycler 480. **NTC**: no template control.

## QuantiTect SYBR Green PCR Kit

**For quantitative, real-time PCR and two-step RT-PCR using SYBR Green I**

- Guaranteed performance — unique PCR system with integrated hot start
- Immediate startup — no need to optimize reaction and cycling conditions
- Sensitive detection — quantification of low-abundance transcripts
- Wide linear range — accurate quantification over several logs of template dilution on any cycler

### Product description

The QuantiTect SYBR Green PCR Kit is supplied with a ready-to-use master mix, which can be stored at 2–8°C, contains SYBR Green I dye, ROX passive reference dye, HotStarTaq DNA Polymerase, and dNTP mix in an optimized buffer.

### Applications

The kit provides accurate real-time quantification of DNA and cDNA targets* on any real-time cycler. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

* cDNA can be rapidly synthesized using the QuantiTect Reverse Transcription Kit or FastLane Cell cDNA Kit (pages 207 and 208).

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect SYBR Green PCR Kit (40)† | For 40 x 50 µl reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water | 204141 |
| QuantiTect SYBR Green PCR Kit (200)† | For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water | 204143 |
| QuantiTect SYBR Green PCR Kit (1000)† | For 1000 x 50 µl reactions: 25 ml 2x Master Mix, 20 ml RNase-Free Water | 204145 |

† Genomewide, validated primer sets for use with the kit are available; see page 192.

**For further information: www.qiagen.com/PG/real-time**

## New QuantiFast SYBR Green RT-PCR Kit

**For fast, quantitative, real-time, one-step RT-PCR using SYBR Green I**

- Reduced cycling times — significant time savings of 50–65%
- High sensitivity — precise and reliable detection of down to 10 copies of target
- Wide linear range — accurate quantification over several logs of template dilution
- Easy setup — master mix compatible with any cycler

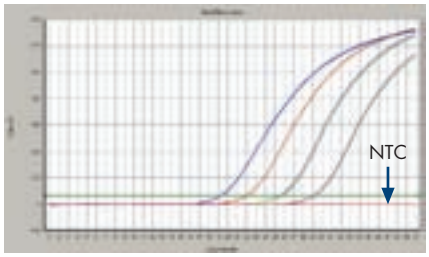
### Product description

The QuantiFast SYBR Green RT-PCR Kit is specially designed for fast cycling real-time PCR. It reduces cycling times on all cyclers and is particularly suited for fast cyclers with short ramping times. The supplied master mix, which can be stored at 2–8°C, contains SYBR Green I dye, ROX passive reference dye (optimized concentration), HotStarTaq *Plus* DNA Polymerase, and dNTP mix in a unique RT-PCR buffer. The supplied RT mix (storage at –20°C) contains an optimized blend of reverse transcriptases that allow cDNA synthesis in only 10 minutes.

### Applications

The kit provides accurate real-time quantification of RNA targets on any real-time cycler. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid. The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

### Sensitive and Specific Results




Different amounts of RNA from human leukocytes (100 ng, 10 ng, 1 ng, and 0.1 ng) were analyzed in duplicate on the Applied Biosystems 7500 Fast Real-Time PCR System using the QuantiFast SYBR Green RT-PCR Kit and the QuantiTect Primer Assay for human interleukin 8. **NTC:** no template control.

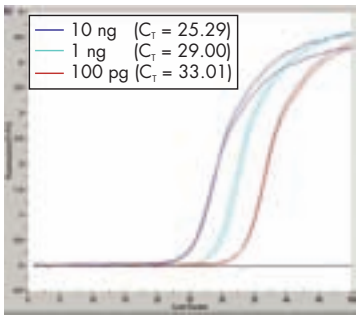
| Product | Contents | Cat. no. |
|---|---|---|
| QuantiFast SYBR Green RT-PCR Kit (400)* | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water | 204154 |
| QuantiFast SYBR Green RT-PCR Kit (2000)* | For 2000 x 25 µl reactions: 25 ml 2x Master Mix, 0.5 ml RT Mix, 20 ml RNase-Free Water | 204156 |

* Genomewide, validated primer sets for use with the kit are available; see page 192.

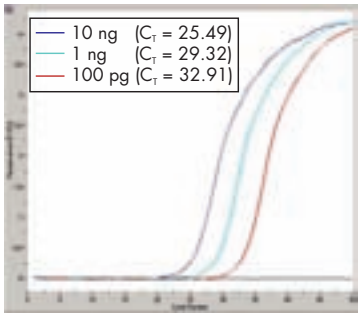
**For further information: www.qiagen.com/PG/real-time**

### Comparable Results in One-Step and Two-Step RT-PCR

**QuantiTect SYBR Green RT-PCR Kit (One-Step RT-PCR)**




**QuantiTect SYBR Green PCR Kit (Two-Step RT-PCR)**




Total RNA (10 ng to 100 pg) or the equivalent amounts of cDNA from HeLa cells were analyzed on the LightCycler 2.0 using the QuantiTect Primer Assay for human MAPK14 and the indicated kits. Similar $C_T$ values were achieved in one-step and two-step RT-PCR.

## QuantiTect SYBR Green RT-PCR Kit

**For quantitative, real-time, one-step RT-PCR using SYBR Green I**

- Guaranteed performance — unique RT-PCR system with integrated hot start
- Immediate startup — no need to optimize reaction and cycling conditions
- Sensitive detection — quantification of low-abundance transcripts
- Wide linear range — accurate quantification over several logs of template dilution on any cycler

### Product description

The supplied master mix, which can be stored at 2–8°C, contains SYBR Green I dye, ROX passive reference dye, HotStarTaq DNA Polymerase, and dNTP mix in an optimized buffer. The supplied RT mix (storage at –20°C) contains an optimized blend of Omniscript and Sensiscript Reverse Transcriptases.

### Applications

The kit provides accurate real-time quantification of RNA targets on any real-time cycler. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect SYBR Green RT-PCR Kit (200)* | For 200 x 50 µl reactions:<br>3 x 1.7 ml 2x Master Mix, 100 µl RT Mix,<br>2 x 2 ml RNase-Free Water | 204243 |
| QuantiTect SYBR Green RT-PCR Kit (1000)* | For 1000 x 50 µl reactions:<br>25 ml 2x Master Mix, 0.5 ml RT Mix,<br>20 ml RNase-Free Water | 204245 |

* Genomewide, validated primer sets for use with the kit are available; see page 192.

**For further information: www.qiagen.com/PG/real-time**

## New QuantiFast Probe PCR Kits

**For fast, quantitative, real-time PCR and two-step RT-PCR using sequence-specific probes**

- Reduced cycling times — significant time savings of 50–65%
- High sensitivity — precise and reliable detection of 10 copies of target
- Wide linear range — accurate quantification over several logs of template dilution
- Easy setup — master mix format compatible with any real-time cycler

### Product description

The QuantiFast Probe PCR Kit is specially designed for fast cycling real-time PCR. It reduces cycling times on all real-time cyclers and is particularly suited for use on fast cyclers (i.e., cyclers with short ramping times). The supplied, ready-to-use master mix, which can be stored at 2–8°C, contains HotStarTaq *Plus* DNA Polymerase and dNTP mix in a unique PCR buffer. The master mix also contains ROX dye, whose concentration is optimized for real-time cyclers that require a high ROX concentration in the amplification reaction.

The QuantiFast Probe PCR +ROX Vial Kit is similar to the QuantiFast Probe PCR Kit. The only difference is that ROX dye is not present in the master mix, but in a separate vial.

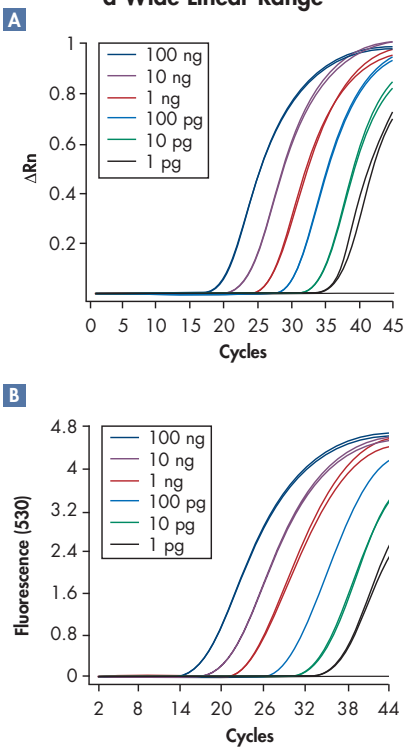
### Applications

The kits provide accurate real-time quantification of DNA and cDNA targets* on any real-time cycler. The QuantiFast Probe PCR Kit is for all cyclers from Applied Biosystems except the Applied Biosystems 7500. The QuantiFast Probe PCR +ROX Vial Kit is for all other cyclers, including the Applied Biosystems 7500 and instruments from Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

The kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

* cDNA can be rapidly synthesized using the QuantiTect Reverse Transcription Kit or FastLane Cell cDNA Kit (pages 207 and 208).

**Reliable Results over a Wide Linear Range**




**A** 10-fold dilutions of human leukocyte cDNA (from 100 ng to 0.001 ng) were analyzed in duplicate on the Applied Biosystems 7900 (ROX dye in master mix; gene expression assay for human interleukin 8). **B** 10-fold dilutions of human HeLa cDNA (from 100 ng to 0.001 ng) were analyzed in duplicate on the LightCycler 2.0 (no ROX dye in reaction; gene expression assay for human ubiquitin).

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiFast Probe PCR Kit (80)* | For 80 x 25 µl reactions: 1 ml 2x Master Mix (contains ROX dye), 2 ml RNase-Free Water | 204252 |
| QuantiFast Probe PCR Kit (400)* | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water | 204254 |
| QuantiFast Probe PCR Kit (2000)* | For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 20 ml RNase-Free Water | 204256 |
| QuantiFast Probe PCR +ROX Vial Kit (80)† | For 80 x 25 µl reactions: 1 ml 2x Master Mix (contains no ROX dye), 45 µl ROX dye solution, 2 ml RNase-Free Water | 204352 |
| QuantiFast Probe PCR +ROX Vial Kit (400)† | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains no ROX dye), 210 µl ROX dye solution, 2 x 2 ml RNase-Free Water | 204354 |
| QuantiFast Probe PCR +ROX Vial Kit (2000)† | For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains no ROX dye), 1.05 ml ROX dye solution, 20 ml RNase-Free Water | 204356 |

* For use with all instruments from Applied Biosystems except the Applied Biosystems 7500.

† For use with the Applied Biosystems 7500 and instruments from Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

**For further information: www.qiagen.com/PG/real-time**

## QuantiTect Probe PCR Kit

**For quantitative, real-time PCR and two-step RT-PCR using sequence-specific probes**

- High sensitivity — accurate analysis of low-copy targets
- Wide linear range — accurate quantification over several logs of template dilution
- Immediate setup — no need to optimize reaction and cycling conditions
- Flexible experimental design — use of any sequence-specific probe on any real-time cycler

### Product description

The kit is supplied with a ready-to-use master mix for accurate real-time quantification of DNA and cDNA targets.* The mix, which can be stored at 2–8°C, contains HotStarTaq DNA Polymerase, ROX passive reference dye, and dNTP mix in an optimized buffer.

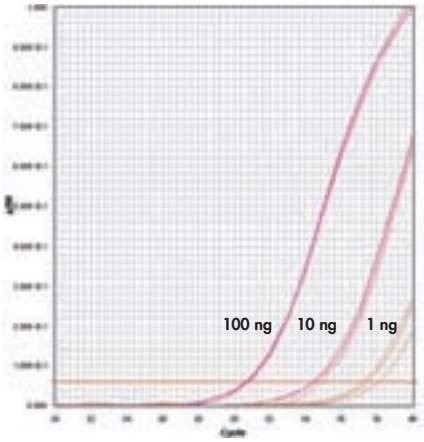
### Applications

The kit provides accurate real-time quantification of DNA and cDNA targets on any real-time cycler. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

* cDNA can be rapidly synthesized using the QuantiTect Reverse Transcription Kit or FastLane Cell cDNA Kit (pages 207 and 208).

### Accurate Analysis of Low-Copy Targets

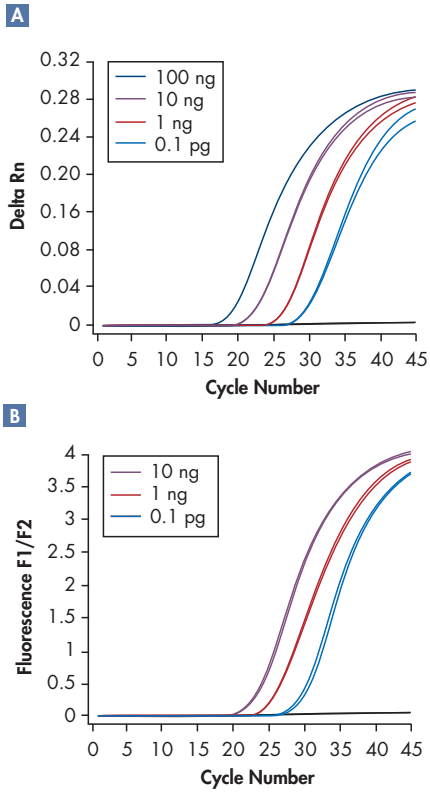



Human interleukin 4 gene expression levels were analyzed using 100, 10, and 1 ng cDNA. Reactions were carried out on the ABI PRISM® 7900, using the QuantiTect Probe PCR Kit and a gene expression assay for Hs_IL4.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Probe PCR Kit (40) | For 40 x 50 µl reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water | 204341 |
| QuantiTect Probe PCR Kit (200) | For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water | 204343 |
| QuantiTect Probe PCR Kit (1000) | For 1000 x 50 µl reactions: 25 ml 2x Master Mix, 20 ml RNase-Free Water | 204345 |

**For further information: www.qiagen.com/PG/real-time**

**Reliable Results over a Wide Linear Range**




A 10-fold dilutions of human leukocyte RNA (from 100 ng to 0.1 ng) were analyzed in duplicate on the Applied Biosystems 7500 Fast Real-Time PCR System (ROX dye in master mix; gene expression assay for human interleukin 8). B 10-fold dilutions of human leukocyte RNA (from 10 ng to 0.1 ng) were analyzed in duplicate on the LightCycler 2.0 (no ROX dye in reaction; gene expression assay for human interleukin 8).

## New QuantiFast Probe RT-PCR Kits

**For fast, quantitative, real-time, one-step RT-PCR using sequence-specific probes**

- Reduced cycling times — significant time savings of 50–65%
- High sensitivity — precise and reliable detection of down to 10 copies of target
- Wide linear range — accurate quantification over several logs of template dilution
- Easy setup — master mix format compatible with any real-time cycler

### Product description

The QuantiFast Probe RT-PCR Kit is specially designed for fast cycling real-time PCR. It reduces cycling times on all real-time cyclers and is particularly suited for use on fast cyclers (i.e., cyclers with short ramping times). The supplied master mix, which can be stored at 2–8°C, contains HotStarTaq *Plus* DNA Polymerase and dNTP mix in a unique RT-PCR buffer. The master mix also contains ROX dye, whose concentration is optimized for real-time cyclers that require a high ROX concentration in the amplification reaction. The supplied RT mix (storage at –20°C) contains an optimized blend of reverse transcriptases that allow cDNA synthesis in only 10 minutes.

The QuantiFast Probe RT-PCR +ROX Vial Kit is similar to the QuantiFast Probe RT-PCR Kit. The only difference is that ROX dye is not present in the master mix, but in a separate vial.

### Applications

The kit provides accurate real-time quantification of RNA targets on any real-time cycler. The QuantiFast Probe RT-PCR Kit is for all cyclers from Applied Biosystems except the Applied Biosystems 7500. The QuantiFast Probe RT-PCR +ROX Vial Kit is for all other cyclers, including the Applied Biosystems 7500 and instruments from Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

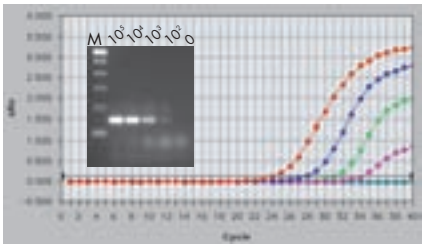
The kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiFast Probe RT-PCR Kit (400)* | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water | 204454 |
| QuantiFast Probe RT-PCR Kit (2000)* | For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water | 204456 |
| QuantiFast Probe RT-PCR +ROX Vial Kit (400)† | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contain no ROX dye), 210 µl ROX dye solution, 100 µl RT Mix, 2 ml RNase-Free Water | 204554 |
| QuantiFast Probe RT-PCR +ROX Vial Kit (2000)† | For 2000 x 25 µl reactions: 25 ml 2x Master Mix (contain no ROX dye), 1.05 ml ROX dye solution, 0.5 ml RT Mix, 20 ml RNase-Free Water | 204556 |

* For use with all instruments from Applied Biosystems except the Applied Biosystems 7500.
† For use with the Applied Biosystems 7500 and instruments from Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

**For further information: www.qiagen.com/PG/real-time**

### Highly Sensitive Quantification Using the QuantiTect Probe RT-PCR Kit




Amplification plots of real-time RT-PCR analysis using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). One-step, quantitative RT-PCR was carried out using the QuantiTect Probe RT-PCR Kit. Reactions were performed with the indicated number of copies of an in vitro transcript of the TATA-box binding protein (TBP). **Inset**: Agarose-gel analyses of end-point PCR results. **M**: markers.

## QuantiTect Probe RT-PCR Kit

**For quantitative, real-time, one-step RT-PCR using sequence-specific probes**

- High sensitivity — accurate analysis of low-copy targets
- Wide linear range — accurate quantification over several logs of template dilution
- Immediate setup — no need to optimize reaction and cycling conditions
- Flexible experimental design — use of any sequence-specific probe on any real-time cycler

### Product description

The supplied master mix, which can be stored at 2–8°C, contains HotStarTaq DNA Polymerase, ROX passive reference dye, and dNTP mix in an optimized buffer. The supplied RT mix (storage at –20°C) contains an optimized blend of Omniscript and Sensiscript Reverse Transcriptases.

### Applications

The kit provides accurate real-time quantification of RNA targets on any real-time cycler. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Probe RT-PCR Kit (200) | For 200 x 50 µl reactions:<br>3 x 1.7 ml 2x Master Mix, 100 µl RT Mix,<br>2 x 2 ml RNase-Free Water | 204443 |
| QuantiTect Probe RT-PCR Kit (1000) | For 1000 x 50 µl reactions:<br>25 ml 2x Master Mix, 0.5 ml RT Mix,<br>20 ml RNase-Free Water | 204445 |

**For further information: www.qiagen.com/PG/real-time**

## QuantiTect Multiplex PCR Kits

**For quantitative, multiplex, real-time PCR and two-step RT-PCR using sequence-specific probes**

- No optimization required — reagents and protocols are preoptimized for multiplex analysis of up to 5 targets per well
- 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
- Easy handling — ready-to-use master mix is compatible with a wide range of real-time cyclers

### Product description

The QuantiTect Multiplex PCR Kit is supplied with a ready-to-use master mix for accurate real-time quantification of multiple DNA and cDNA targets.* The master mix, which can be stored at 2–8°C, contains HotStarTaq DNA Polymerase, synthetic factor MP, ROX passive reference dye, and dNTP mix in a unique buffer.

The QuantiTect Multiplex PCR NoROX Kit is similar to the QuantiTect Multiplex PCR Kit. The only difference is that the master mix contains no ROX dye.

### Applications

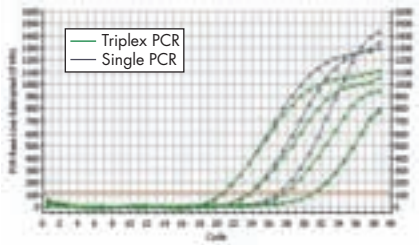
The kits provide accurate real-time quantification of up to 5 DNA or cDNA targets per well on a wide range of real-time cyclers. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid. The kits are compatible with many probe chemistries, including TaqMan probes.

The kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

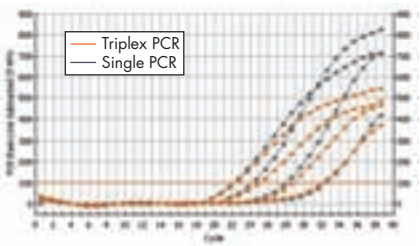
* cDNA can be rapidly synthesized using the QuantiTect Reverse Transcription Kit or FastLane Cell cDNA Kit (pages 207 and 208).

### Equivalent $C_T$ Values in Triplex PCR and Single PCR

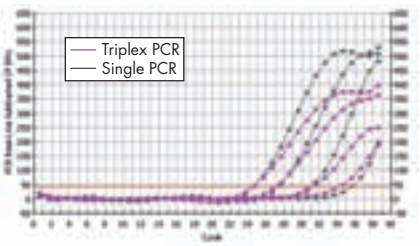
**Target 1 (FAM)**




**Target 2 (HEX)**




**Target 3 (Cy5)**




Dilutions of cDNA template (100 ng, 11.11 ng, 1.23 ng, and 0.14 ng) were analyzed by triplex PCR (colored curves) and by single PCR (gray curves) on the iCycler iQ®. TaqMan® probes labeled with FAM, HEX, or Cy5 dye were used. Data kindly provided by the University of Minnesota, Minneapolis, MN, USA.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Multiplex PCR Kit (40)* | For 40 x 50 µl reactions: 1 ml 2x QuantiTect Multiplex PCR Master Mix (contains ROX dye), 2 ml RNase-Free Water | 204541 |
| QuantiTect Multiplex PCR Kit (200)* | For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect Multiplex PCR Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water | 204543 |
| QuantiTect Multiplex PCR Kit (1000)* | For 1000 x 50 µl reactions: 25 ml 2x QuantiTect Multiplex PCR Master Mix, (contains ROX dye), 20 ml RNase-Free Water | 204545 |
| QuantiTect Multiplex PCR NoROX Kit (40)† | For 40 x 50 µl reactions: 1 ml 2x QuantiTect Multiplex PCR NoROX Master Mix (contains no ROX dye), 2 ml RNase-Free Water | 204741 |
| QuantiTect Multiplex PCR NoROX Kit (200)† | For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect Multiplex PCR NoROX Master Mix (contains no ROX dye), 2 x 2 ml RNase-Free Water | 204743 |
| QuantiTect Multiplex PCR NoROX Kit (1000)† | For 1000 x 50 µl reactions: 25 ml 2x QuantiTect Multiplex PCR NoROX Master Mix (contains no ROX dye), 20 ml RNase-Free Water | 204745 |

* Recommended for ABI PRISM and Applied Biosystems cyclers.

† Recommended for all other cyclers.

**For further information: www.qiagen.com/PG/real-time**

## QuantiTect Multiplex RT-PCR Kits

**For quantitative, multiplex, real-time, one-step RT-PCR using sequence-specific probes**

- No optimization required — reagents and protocols are preoptimized for multiplex analysis of up to 5 targets per well
- 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
- Easy handling — ready-to-use master mix is compatible with a wide range of real-time cyclers

### Product description

The QuantiTect Multiplex RT-PCR Kit is supplied with a master mix and a RT mix for accurate real-time quantification of multiple RNA targets. The master mix, which can be stored at 2–8°C, contains HotStarTaq DNA Polymerase, synthetic factor MP, ROX passive reference dye, and dNTP mix in a unique buffer. The RT mix (storage at –20°C) mix contains an optimized blend of Omniscript and Sensiscript Reverse Transcriptases.

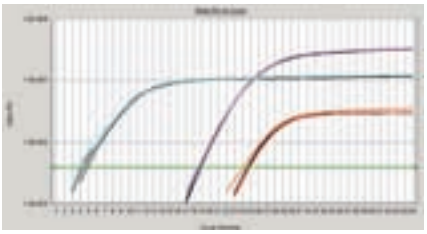
The QuantiTect Multiplex RT-PCR NR Kit is similar to the QuantiTect Multiplex RT-PCR Kit. The only difference is that the master mix contains no ROX dye.

### Applications

The kits provide accurate real-time quantification of up to 5 RNA targets per well on a wide range of real-time cyclers. These include instruments from Applied Biosystems, Roche, Bio-Rad, Eppendorf, Stratagene, Corbett, and Cepheid. The kits are compatible with many probe chemistries, including TaqMan probes.

The kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

### Comparable Results between Triplex PCR and Corresponding Single PCRs



28S rRNA (triplex PCR)
POLD3 (triplex PCR)
CDK2 (triplex PCR)
28S rRNA, POLD3, or CDK2 (single PCR)

Triplex, real-time, one-step RT-PCR was performed on the Applied Biosystems 7500 using the QuantiTect Multiplex RT-PCR Kit and TaqMan probes (labeled with FAM, HEX, or Cy5 dye). The template was 20 ng total RNA from the Ramos cell line, and the targets were 28S rRNA, POLD3, and CDK2. Reactions were performed in triplicate. The resulting $C_T$ values are equivalent to those obtained when the targets were amplified and detected individually (black curves).

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Multiplex RT-PCR Kit (200)* | For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect Multiplex RT-PCR Master Mix (contains ROX dye), 100 µl QuantiTect RT Mix, 2 x 2 ml RNase-Free Water | 204643 |
| QuantiTect Multiplex RT-PCR Kit (1000)* | For 1000 x 50 µl reactions: 25 ml 2x QuantiTect Multiplex RT-PCR Master Mix (contains ROX dye), 0.5 ml QuantiTect RT Mix, 20 ml RNase-Free Water | 204645 |
| QuantiTect Multiplex RT-PCR NR Kit (200)† | For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect Multiplex RT-PCR NoROX Master Mix (contains no ROX dye), 100 µl QuantiTect RT Mix, 2 x 2 ml RNase-Free Water | 204843 |
| QuantiTect Multiplex RT-PCR NR Kit (1000)† | For 1000 x 50 µl reactions: 25 ml 2x QuantiTect Multiplex RT-PCR NoROX Master Mix (contains no ROX dye), 0.5 ml QuantiTect RT Mix, 20 ml RNase-Free Water | 204845 |

* Recommended for ABI PRISM and Applied Biosystems cyclers.

† Recommended for all other cyclers.

**For further information: www.qiagen.com/PG/real-time**

## QuantiTect Reverse Transcription Kit

**For fast cDNA synthesis for sensitive real-time two-step RT-PCR**

- Fast and convenient procedure — cDNA synthesis and removal of genomic DNA in only 20 minutes
- Effective genomic DNA removal — no need to design RNA-specific primers or probes
- High sensitivity — high cDNA yields even from low-abundance transcripts
- cDNA synthesis from all regions — reliable detection of any target in real-time RT-PCR, even those from 5' regions

### Product description

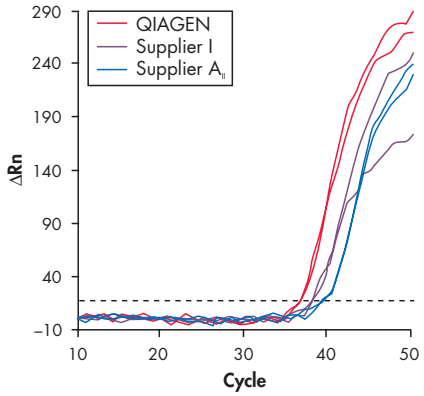
The QuantiTect Reverse Transcription Kit provides a fast and convenient procedure for cDNA synthesis and genomic DNA removal. Genomic DNA contamination in RNA samples is effectively eliminated by gDNA Wipeout Buffer. Fast and efficient reverse transcription from all regions of mRNA transcripts is provided by Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, and unique RT Primer Mix.

### Applications

The QuantiTect Reverse Transcription Kit is dedicated for use in real-time, two-step RT-PCR,* and provides high cDNA yields for sensitive quantification of even low-abundance transcripts. The kit is suitable for use with RNA from all types of starting material, including small amounts of cells and tissues.

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

**Higher Sensitivity in Real-Time, Two-Step RT-PCR**




Real-time, two-step RT-PCR analysis of TGFB2 (low expression). cDNA was synthesized from 1 µg human blood RNA using the QuantiTect Reverse Transcription Kit, a kit from Supplier A, or a kit from Supplier I. Real-time PCR was performed in duplicate on the ABI PRISM 7900 using the QuantiTect Probe PCR Kit and a gene expression assay for TGFB2. The $C_T$ values were lowest with the QuantiTect Reverse Transcription Kit, demonstrating that even low-abundance genes can be efficiently reverse transcribed and sensitively detected in real-time PCR.

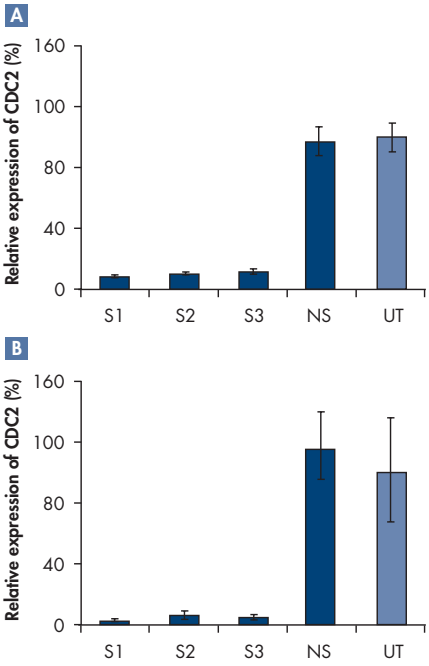
* Kits for real-time, two-step RT-PCR are available; see pages 193, 194, 197–199, and 203.

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Reverse Transcription Kit (50) | Components for 50 x 20 µl reverse-transcription reactions† | 205311 |
| QuantiTect Reverse Transcription Kit (200) | Components for 200 x 20 µl reverse-transcription reactions† | 205313 |

† gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer (with dNTPs), RT Primer Mix, and RNase-Free Water.

**For further information: www.qiagen.com/PG/real-time**

CDC2 Knockdown in Cultured Cells




A MCF-7 cells were transfected with various CDC2 siRNAs (**S1**, **S2**, or **S3**) or with nonsilencing siRNA (**NS**). After 48 hours, the relative expression of CDC2 was determined by real-time RT-PCR. The relative expression of CDC2 in untreated cells (**UT**) was set to 100%. cDNA was prepared using the FastLane Cell cDNA Kit. B The experiment was repeated on HeLa cells.

* Kits for real-time, two-step RT-PCR are available; see pages 193, 194, 197–199, and 203.

## FastLane Cell cDNA Kit

**For high-speed preparation of cDNA without RNA purification for use in real-time RT-PCR**

- No RNA purification — from cells to cDNA in only 4 steps and less than 45 minutes
- Short and simple workflow — easy parallel processing of several samples
- High sensitivity — detection of even low-abundance transcripts
- Detection of RNA only — accurate gene expression analysis with less effort

### Product description

The FastLane Cell cDNA Kit provides a fast and simple procedure for preparing first-strand cDNA directly from cultured cells without RNA purification. The kit is supplied with wash and lysis buffers for preparing lysates containing intact RNA, with gDNA Wipeout Buffer for eliminating genomic DNA contamination, and with reaction components for fast and efficient cDNA synthesis.

### Applications

cDNA synthesized by the FastLane Cell cDNA Kit gives highly sensitive and reproducible results in real-time, two-step RT-PCR.* The kit is ideal for experiments which require a snapshot of individual transcript levels, such as:

- Validation of siRNA-mediated gene knockdown
- Selection of potential drugs
- Detection of stimulated gene regulation

The kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

| Product | Contents | Cat. no. |
|---|---|---|
| FastLane Cell cDNA Kit (50) | Buffer FCW, Buffer FCP, and components for 50 x 20 µl reverse-transcription reactions† | 215011 |

† gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer (with dNTPs), RT Primer Mix, and RNase-Free Water.

**For further information: www.qiagen.com/PG/real-time**

## New FastLane Cell RT-PCR Kits

**For real-time, one-step RT-PCR analysis directly from cultured cells without RNA purification**

- No RNA purification — from cells to real-time RT-PCR results in only a few steps
- Short and simple workflows — easy parallel processing of several samples
- High sensitivity — detection of even low-abundance transcripts
- Detection of RNA only — accurate gene expression analysis with less effort

### Product description

FastLane Cell RT-PCR Kits contain a set of buffers for preparing FastLane lysates from cultured cells.* In addition to lysing cells, the buffers also stabilize cellular RNA and eliminate genomic DNA. FastLane lysates are used directly in real-time, one-step RT-PCR. A variety of detection formats are available: detection with SYBR Green or sequence-specific probes, or multiplex detection with sequence-specific probes (with or without ROX passive reference dye).

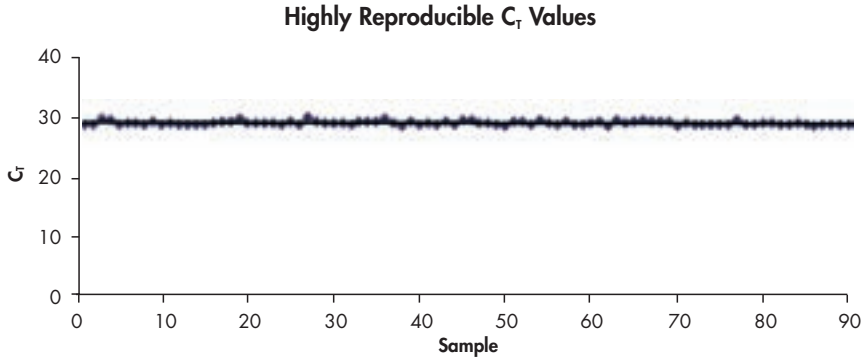
### Applications

The kits are ideal for experiments which require a snapshot of individual transcript levels, such as:

- Validation of siRNA-mediated gene knockdown
- Screening of potential drugs
- Detection of stimulated gene regulation

The kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

* For primary cells, please contact QIAGEN Technical Services.




HeLa cells were grown for two days in a 96-well plate (4 x $10^4$ cells per well after two days). Real-time, one-step RT-PCR with SYBR Green detection was performed using the FastLane Cell SYBR Green Kit and the QuantiTect Primer Assay for INPP5D. The mean $C_T$ value was 29.33 with a standard deviation of 0.30.

| Product | Contents | Cat. no. |
|---|---|---|
| FastLane Cell SYBR Green Kit (200) | FastLane Cell One-Step Buffer Set and QuantiTect SYBR Green RT-PCR Kit (200) | 216213 |
| FastLane Cell Probe Kit (200) | FastLane Cell One-Step Buffer Set and QuantiTect Probe RT-PCR Kit (200) | 216413 |
| FastLane Cell Multiplex Kit (200) | FastLane Cell One-Step Buffer Set and QuantiTect Multiplex RT-PCR Kit (200) | 216513 |
| FastLane Cell Multiplex NR Kit (200) | FastLane Cell One-Step Buffer Set and QuantiTect Multiplex RT-PCR NR Kit (200) | 216713 |

**For further information: www.qiagen.com/PG/real-time**

## QIAGEN PCR Cloning$^{plus}$ Kit

### For direct cloning of PCR products

- Just 40 minutes from PCR product to plated cells
- Ready-to-use Ligation Master Mix and immediate plating of transformed QIAGEN EZ Competent Cells
- High-specificity UA hybridization for efficient cloning

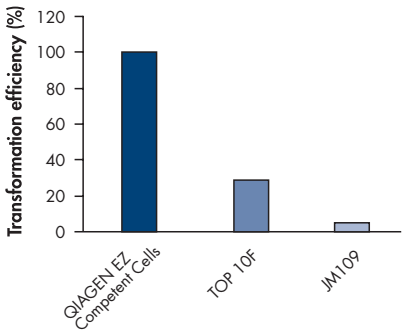
### Product description

QIAGEN PCR Cloning Kits provide ready-to-use ligation reactions, which contain linearized cloning vectors that carry U overhangs at each 3' end, allowing PCR products containing 3'-end A overhangs to be directly ligated and cloned with high efficiency. QIAGEN PCR Cloning$^{plus}$ Kits provide competent *E. coli* cells and SOC medium for efficient transformation.

### Applications

QIAGEN PCR Cloning Kits are suitable for cloning of any PCR product that has a single A overhang at each 3' end. PCR products generated using *Taq* and other non-proofreading DNA polymerases can be directly cloned without any preparation. QIAGEN also offers the QIA*express*® UA Cloning Kit (page 261), for direct cloning of PCR products into the pQE-30 UA vector for high-level expression of 6xHis-tagged proteins.

**Robust QIAGEN EZ Competent Cells Do Not Require Recovery Incubation**

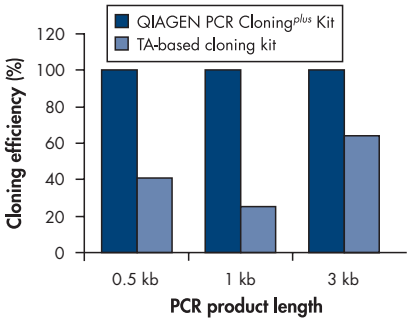



QIAGEN EZ Competent Cells (>$10^8$ cfu/µg DNA), TOP 10F (Supplier I; >$10^9$ cfu/µg DNA), and JM109 (Supplier P; >$10^8$ cfu/µg DNA) competent cells were transformed with pUC18 plasmid DNA. The recommended protocol from each supplier was followed, except that all cells were plated immediately onto agar/ampicillin plates without a recovery incubation in SOC medium. Colony numbers were converted to relative percentages, with QIAGEN EZ Competent Cells set at 100%. Colony numbers were not normalized for transformation efficiency. Normalization would result in an even higher transformation efficiency for QIAGEN EZ Competent Cells.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN PCR Cloning$^{plus}$ Kit (10) | For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml), QIAGEN EZ Competent Cells (10 tubes, 50 µl each), SOC medium (2 x 1.9 ml) | 231222 |
| QIAGEN PCR Cloning$^{plus}$ Kit (40) | For 40 reactions: 2x Ligation Master Mix (200 µl), pDrive Cloning Vector (2.0 µg), distilled water (1.7 ml), QIAGEN EZ Competent Cells (40 tubes, 50 µl each), SOC medium (6 x 1.9 ml) | 231224 |

**For further information: www.qiagen.com/PG/cloning**

**Highly Specific Cloning with a Short 30-Minute Ligation Time**




The effect of ligation time on cloning efficiency was compared for the QIAGEN PCR Cloning$^{plus}$ Kit and a TA-based cloning kit (Supplier I) using PCR products of different length (0.5 kb, 1 kb, and 3 kb). Colony numbers were converted to relative percentages, with the QIAGEN PCR Cloning$^{plus}$ Kit procedure set at 100% for each comparison. 30 min ligation (QIAGEN PCR Cloning$^{plus}$ Kit recommendation).

## QIAGEN PCR Cloning Kit

**For direct cloning of PCR products generated by *Taq* DNA polymerases**

- Fast and simple procedure
- Ready-to-use Ligation Master Mix
- High-specificity UA hybridization for efficient cloning

### Product description

QIAGEN PCR Cloning Kits provide ready-to-use ligation reactions, which contain linearized cloning vectors that carry U overhangs at each 3' end, allowing PCR products containing 3'-end A overhangs to be directly ligated and cloned with high efficiency.

### Applications

QIAGEN PCR Cloning Kits are suitable for cloning of any PCR product that has a single A overhang at each 3' end. PCR products generated using *Taq* DNA polymerase, other non-proofreading DNA polymerases, or the HotStar HiFidelity Polymerase Kit (page 177) can be directly cloned without any preparation. QIAGEN also offers the QIA*express* UA Cloning Kit (page 261), for direct cloning of PCR products into the pQE-30 UA expression vector for high-level expression of 6xHis-tagged proteins.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN PCR Cloning Kit (10) | For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml) | 231122 |
| QIAGEN PCR Cloning Kit (40) | For 40 reactions: 2x Ligation Master Mix (200 µl), pDrive Cloning Vector (2.0 µg), distilled water (1.7 ml) | 231124 |

**For further information: www.qiagen.com/PG/cloning**

## QIAGEN A-Addition Kit

**For easy and efficient modification of blunt-ended PCR products for use in UA- and TA-cloning procedures**

- Ease of use — simply add blunt-ended PCR products to the master mix and incubate for 30 minutes
- Fast procedure — no PCR product purification or precipitation required
- High efficiency of UA-/TA-cloning — large numbers of colonies contain desired insert

### Product description

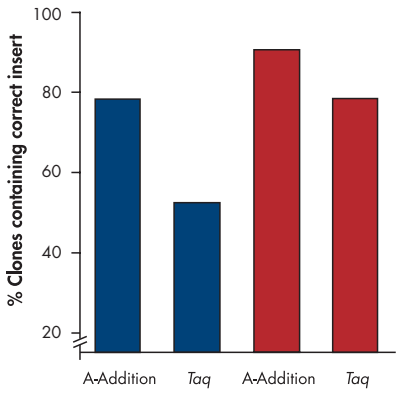
The QIAGEN A-Addition Kit contains ready-to-use enzymatic reactions that add 3'-end A overhangs to blunt-ended PCR products. These PCR products can then be cloned into UA-/TA-cloning vectors (e.g., the pDrive Cloning Vector in QIAGEN PCR Cloning Kits).

### Applications

The QIAGEN A-Addition Kit is suitable for processing any blunt-ended PCR fragment, such as PCR products generated by ProofStart DNA Polymerase (page 178). The processed PCR products can then be cloned using UA-/TA-cloning procedures, such as the QIAGEN PCR Cloning procedure (pages 211 and 212).

**More Colonies Containing the Desired Insert**

A




B

| Method | Colonies | Colonies |
|---|---|---|
| Proofreading polymerase + QIAGEN A-Addition | 421 | 4287 |
| *Taq* DNA polymerase | 150 | 441 |

Two different PCR products (**blue**: 750 bp; **red**: 1000 bp) were cloned into the pDrive UA-cloning vector using the QIAGEN PCR Cloning$^{plus}$ Kit. PCR products were amplified using either a proofreading DNA polymerase followed by the QIAGEN A-Addition procedure or using *Taq* DNA polymerase. All reactions were performed in parallel. A Cloning efficiency is given as a percentage of colonies containing the correct insert. B The total number of transformed colonies is shown.

| Product | Contents | Cat. no. |
|---|---|---|
| QIAGEN A-Addition Kit | For 40 A-addition reactions:<br>5x QIAGEN A-Addition Master Mix (80 µl),<br>distilled water (1.7 ml) | 231994 |

**For further information: www.qiagen.com/PG/cloning**