August 2011

FastLane® Cell RT-PCR Handbook

FastLane Cell SYBR® Green Kit
FastLane Cell Probe Kit
FastLane Cell Multiplex Kit
FastLane Cell Multiplex NR Kit

For real-time, one-step RT-PCR analysis of cultured cells without RNA purification

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Contents

Kit Contents

FastLane Cell SYBR Green Kit	**(200)**
Catalog no.	**216213**
Number of 50 µl reactions	**200**
FastLane Cell One-Step Buffer Set (Part 1 of 2):	
■ Buffer FCW	25 ml
■ Buffer FCPL	10 ml
■ Buffer FCPM	100 µl
■ gDNA Wipeout Buffer 2 (lyophilized)	1 vial
■ 5x Q-Solution®	2 ml
■ RNase-Free Water	1.9 ml
FastLane Cell SYBR Green Kit (Part 2 of 2):	
■ 2x QuantiTect® SYBR Green RT-PCR Master Mix	3 x 1.7 ml
■ QuantiTect RT Mix	100 µl
■ RNase-Free Water	2 x 2 ml
Handbooks:	
■ *FastLane Cell RT-PCR Handbook*	1
■ *QuantiTect SYBR Green RT-PCR Handbook*	1

FastLane Cell Probe Kit	**(200)**
Catalog no.	**216413**
Number of 50 µl reactions	**200**
FastLane Cell One-Step Buffer Set (Part 1 of 2):	
■ Buffer FCW	25 ml
■ Buffer FCPL	10 ml
■ Buffer FCPM	100 µl
■ gDNA Wipeout Buffer 2 (lyophilized)	1 vial
■ 5x Q-Solution	2 ml
■ RNase-Free Water	1.9 ml
FastLane Cell Probe Kit (Part 2 of 2):	
■ 2x QuantiTect Probe RT-PCR Master Mix	3 x 1.7 ml
■ QuantiTect RT Mix	100 µl
■ RNase-Free Water	2 x 2 ml
Handbooks:	
■ *FastLane Cell RT-PCR Handbook*	1
■ *QuantiTect Probe RT-PCR Handbook*	1

FastLane Cell Multiplex Kit	**(200)**
Catalog no.	**216513**
Number of 50 µl reactions	**200**
FastLane Cell One-Step Buffer Set (Part 1 of 2):	
■ Buffer FCW	25 ml
■ Buffer FCPL	10 ml
■ Buffer FCPM	100 µl
■ gDNA Wipeout Buffer 2 (lyophilized)	1 vial
■ 5x Q-Solution	2 ml
■ RNase-Free Water	1.9 ml
FastLane Cell Multiplex Kit (Part 2 of 2):	
■ 2x QuantiTect Multiplex RT-PCR Master Mix	3 x 1.7 ml
■ QuantiTect Multiplex RT Mix	100 µl
■ RNase-Free Water	2 x 2 ml
Handbooks:	
■ *FastLane Cell RT-PCR Handbook*	1
■ *QuantiTect Multiplex RT-PCR Handbook*	1

FastLane Cell Multiplex NR Kit	(200)
Catalog no.	216713
Number of 50 µl reactions	200
FastLane Cell One-Step Buffer Set (Part 1 of 2):	
■ Buffer FCW	25 ml
■ Buffer FCPL	10 ml
■ Buffer FCPM	100 µl
■ gDNA Wipeout Buffer 2 (lyophilized)	1 vial
■ 5x Q-Solution	2 ml
■ RNase-Free Water	1.9 ml
FastLane Cell Multiplex NR Kit (Part 2 of 2):	
■ 2x QuantiTect Multiplex RT-PCR NoROX™ Master Mix	3 x 1.7 ml
■ QuantiTect Multiplex RT Mix	100 µl
■ RNase-Free Water	2 x 2 ml
Handbooks:	
■ *FastLane Cell RT-PCR Handbook*	1
■ *QuantiTect Multiplex RT-PCR NR Handbook*	1

Shipping and Storage

Part 1 of 2 (FastLane Cell One-Step Buffer Set) is shipped at ambient temperature, and should be stored immediately upon receipt at 2–8°C. After resuspension, gDNA Wipeout Buffer 2 should be stored at –20°C.

Part 2 of 2 (containing RT-PCR master mix, RT mix, and RNase-free water) is shipped on dry ice and should be stored immediately upon receipt at –20°C in a constant-temperature freezer.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of FastLane Cell RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

FastLane Cell RT-PCR Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding FastLane Cell RT-PCR Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

The following risk and safety phrases apply to the components of FastLane Cell RT-PCR Kits.

Buffer FCPM

Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

gDNA Wipeout Buffer 2

Contains deoxyribonuclease: sensitizer. Risk and safety phrases:* R42/43, S22-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R36/37/38: Irritating to eyes, respiratory system and skin; R42/43: May cause sensitization by inhalation and skin contact; S22: Do not breathe dust; S23: Do not breathe vapor; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves.

Product Description

Part 1 of 2 (FastLane Cell One-Step Buffer Set) contains:

Buffer FCW:	Cell wash buffer for effective removal of extracellular contaminants
Buffer FCPL:	Component of cell processing mix to allow efficient lysis of cultured cells and RNA stabilization
Buffer FCPM:	Component of cell processing mix to enhance performance
gDNA Wipeout Buffer 2:	Component of cell processing mix to eliminate genomic DNA contamination
5x Q-Solution	Optional additive for real-time, one-step RT-PCR using 384-well plates on the Applied Biosystems® 7900HT
RNase-Free Water:	Ultrapure quality, PCR-grade

The product description for Part 2 of 2 (containing RT-PCR master mix, RT mix, and RNase-free water) is in the QuantiTect Handbook supplied with the FastLane Cell RT-PCR Kit.

Introduction

FastLane Kits accelerate and streamline real-time RT-PCR analysis of cultured cells. By eliminating the need for RNA purification, the kits allow you to carry out real-time RT-PCR directly from cell lysates. The kits are ideal for experiments requiring rapid, high-throughput gene expression analysis, such as validation of siRNA-mediated gene knockdown. FastLane Cell RT-PCR Kits are optimized for use in real-time, one-step RT-PCR, and are not suitable for qualitative RT-PCR.

Principle and procedure

The FastLane Cell RT-PCR procedure comprises only 3 steps: cell wash, cell processing, and real-time, one-step RT-PCR (see flowchart, next page).

Cell wash

Cultured cells are briefly washed with Buffer FCW to remove cell-culture medium, extracellular material released by living cells, and intracellular material released by any dead, lysed cells. Removal of such materials is important, since they can interfere with quantification by real-time RT-PCR.

Cell processing

After the wash with Buffer FCW, the cultured cells are lysed for 5 minutes using a cell processing mix (Buffer FCPL supplemented with Buffer FCPM and gDNA Wipeout Buffer 2). During cell lysis:

- **Cellular RNA is stabilized**: This ensures that the RNA accurately reflects the in vivo gene expression profile.
- **Genomic DNA is eliminated**: Accurate measurement of transcript levels by real-time RT-PCR depends on the elimination of false-positive results caused by genomic DNA contamination. During cell processing using the cell processing mix, contaminating genomic DNA is effectively removed. Thus, it is not necessary to specially design primers or probes that prevent or minimize detection of genomic DNA.
- **Reverse transcription inhibitors are blocked**: This allows efficient cDNA synthesis in real-time, one-step RT-PCR.

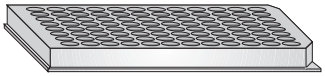
After cell lysis, the FastLane lysate is incubated at 75°C for 5 minutes.

Real-time, one-step RT-PCR

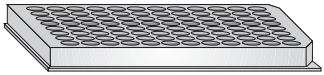
The FastLane lysate is used directly as a template in real-time, one-step RT-PCR. The lysate is suitable for use in real-time, one-step RT-PCR with SYBR Green or probe detection and for use in multiplex, real-time, one-step RT-PCR.

FastLane Cell RT-PCR Procedure

Cells seeded in
96-well plate

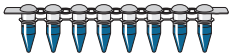


Wash cells



Lyse cells, stabilize RNA, and eliminate genomic DNA using cell processing mix (5 min at room temperature)

FastLane lysate



Pretreat FastLane lysates
(5 min at 75°C, then on ice)

Perform quantitative, real-time, one-step RT-PCR

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For preparation of FastLane lysate from cultured cells

- RNase-free plastic tubes (up to 1.5 ml for storing FastLane lysates, and greater than 20 µl for real-time, one-step RT-PCR)
- Ice
- Heating block, thermal cycler, or water bath (capable of reaching 75°C)
- Vortexer
- Microcentrifuge
- Optional: gene-specific primers
- Optional: 8-channel pipettor

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

We recommend using QuantiTect Primer Assays, which are genomewide, predesigned primer sets for highly specific transcript quantification. Assays are available for human, rat, mouse, and many other species, and can be easily ordered online at www.qiagen.com/GeneGlobe.

Protocol: High-Speed Setup of Real-Time, One-Step RT-PCR from Cultured Cells

Important points before starting

- **This protocol has been developed for use with adherent cultured cells grown in 96-well plates.** When using a 96-well plate, ≤1 x 10^4 cells typically need to be seeded per well. However, other cell numbers can be used, depending on the cell type and the culture conditions. In general, cells can be grown until confluent.

  If growing cells in other types of plates, follow this protocol and also refer to Appendix B (page 20) to find out the appropriate number of cells to seed and the appropriate buffer volumes to use.

  If growing cells in suspension, follow this protocol, replacing steps 1–8 with steps C1–C7 in Appendix C (page 21).
- If working with RNA for the first time, read Appendix A (page 19).
- Set up the RT-PCR on ice for optimal results.
- RNase inhibitor and dNTPs do not need to be added to the RT-PCR, as they are already present in the reaction components supplied with QuantiTect RT-PCR Kits.
- Separate denaturation and annealing steps are not necessary before starting the RT-PCR.
- For details on performing real-time, one-step RT-PCR and on using appropriate controls, refer to the QuantiTect Handbook supplied with the FastLane Cell RT-PCR Kit. Always be sure to:
  - Set up all reaction mixtures in an area separate from that used for DNA preparation or RT-PCR product analysis.
  - Use reagents and pipets set aside only for the setup of reverse transcription and PCR.
  - Use disposable pipet tips containing hydrophobic filters to minimize the risk of cross-contamination.
- **If performing SYBR Green based real-time RT-PCR using 384-well plates on the Applied Biosystems 7900HT**, read Appendix D (page 22).
- **Note**: 384-well cell-culture plates require either a reduction of PCR volume from 50 µl to at least 25 µl, or an additional QuantiTect RT-PCR Kit. Depending on the detection method, a QuantiTect SYBR Green RT-PCR Kit, QuantiTect Probe RT-PCR Kit, QuantiTect Multiplex RT-PCR Kit, or QuantiTect Multiplex RT-PCR NR Kit is required. For ordering information, see pages 24–25.

Things to do before starting

- Add 2.5 µl Buffer FCPM to Buffer FCPL, mix well, and store at 2–8°C. Tick the check box on the Buffer FCPL bottle to indicate that Buffer FCPM has been added. Be sure to briefly shake or vortex the Buffer FCPL/FCPM mix before each use.
- Add 750 µl RNase-free water to lyophilized gDNA Wipeout Buffer 2, mix by gently inverting the vial, divide into single-use aliquots, and store at –20°C. To avoid loss of lyophilized gDNA Wipeout Buffer 2, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe.

Procedure

1. **Seed an appropriate number of cells (e.g., 1 x 10^4 cells) per well of a 96-well plate.**

   Note: If using another type of plate, see Appendix B, page 20.

2 **Incubate the cells according to your experimental procedure or until confluent.**

3. **Prepare cell processing mix according to Table 1.**

   Note: Briefly mix gDNA Wipeout Buffer 2 by gently inverting the tube before adding it to Buffer FCPL.

Table 1. Components of Cell Processing Mix for 96-Well Plates*

Component	Volume/well (µl)
Buffer FCPL†	47
gDNA Wipeout Buffer 2‡	3
Total volume	**50**

* If using another type of plate, see Appendix B, page 20.

† Before using Buffer FCPL for the first time, add Buffer FCPM as described in "Things to do before starting".

‡ Before using gDNA Wipeout Buffer 2 for the first time, reconstitute it in RNase-free water as described in "Things to do before starting".

4. **Aspirate the cell-culture medium using a pipet, and discard.**

   Note: No enzymatic treatment of cells (e.g., by trypsin) is required.

5. **Add 125 µl Buffer FCW per well of the 96-well plate.**

   Note: Do not incubate the cells with Buffer FCW for long periods of time. Be careful when handling semi-adherent cells.

   Note: If using another type of plate, see Appendix B, page 20.

6. **Aspirate Buffer FCW using a pipet, and discard.**

   Note: Be sure to completely aspirate Buffer FCW.

7. **Add 50 µl cell processing mix (see Table 1) per well of the 96-well plate. Incubate for 5 min at room temperature (15–25°C).**

   For convenience, the incubation time can be increased up to 10 min.

   Note: After adding cell processing mix to the well, do not agitate the cells by pipetting the mix up and down several times. Agitation of the lysed cells could cause carryover of genomic DNA and cell debris.

   Note: If using another type of plate, see Appendix B, page 20.

8. **Transfer the FastLane lysates (containing stabilized RNA) into appropriately sized tubes. Incubate for 5 min at 75°C, and briefly centrifuge. Proceed immediately to step 9.**

   Note: If a pause in the procedure is required, store the tubes containing the FastLane lysates at –80°C.

9. **Set up real-time, one-step RT-PCR according to the QuantiTect Handbook supplied with the FastLane Cell RT-PCR Kit. Use 1–4 µl FastLane lysate per reaction.**

   If necessary, thaw FastLane lysates from step 8 at room temperature (15–25°C). Mix by flicking the tubes, and centrifuge briefly to collect residual liquid from the sides of the tubes.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
No product, or product detected late in real-time RT-PCR (problems occurring during reverse transcription)		
a)	Inappropriate cell numbers seeded	Seed your multiwell plate with different numbers of cells per well. Carry out the FastLane Cell RT-PCR procedure. Determine which cell number gives optimal RT-PCR results.
b)	Cells not washed with Buffer FCW	Be sure to wash cells using Buffer FCW to remove extracellular contaminants.
c)	Cells treated with incorrect volume of cell processing mix	Refer to Appendix B, page 20, to find the appropriate volume of cell processing mix to add per well of your multiwell plate.
d)	Incorrect setup of RT-PCR	Be sure to set up the reaction on ice. Keep the reaction on ice until ready to start the reaction.
e)	Volume of FastLane lysate used in RT-PCR too high	Adding a high volume of FastLane lysate to the RT-PCR mix may reduce amplification efficiency and the linearity of the reaction. Generally, the volume of FastLane lysate added should not exceed 20% of the final RT-PCR volume.
f)	Incomplete removal of Buffer FCW	Be sure to completely remove Buffer FCW from each well prior to adding cell processing mix. Residual Buffer FCW can inhibit RT-PCR.
g)	Pipetting error or missing reagent when setting up RT-PCR	Check the pipets used for experimental setup. Mix all reagents well after thawing, and repeat the RT-PCR.
h)	RNA denatured	Denaturation of the template RNA is not necessary. If denaturation was performed, the integrity of the RNA may be affected.

	Comments and suggestions
No product, or product detected late in real-time RT-PCR, or only primer–dimers detected	
Problems occurring during real-time RT-PCR	Refer to the QuantiTect Handbook supplied with the FastLane Cell RT-PCR Kit.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, use RNase-free plasticware or glassware. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during the FastLane Cell RT-PCR procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Appendix B: Seeding and Processing Cells for Different Plate Formats

The protocol on page 14 is for use with 96-well plates seeded with ≤1 x 10^4 cells per well. If using another type of plate, refer to Table 2 for the preparation of cell processing mix, and to Table 3 for the number of cells to seed per well and the volumes of Buffer FCW and cell processing mix to add per well. Grow the cells according to your experimental procedure or until confluent.

Table 2. Preparation of Cell Processing Mix for Different Plate Formats

	Volume/well (µl)					
Component	**384-well**	**96-well**	**48-well**	**24-well**	**12-well**	**6-well**
Buffer FCPL*	11.75	47	94	188	376	952
gDNA Wipeout Buffer 2†	0.75	3	6	12	24	48
Total volume	**12.5**	**50**	**100**	**200**	**400**	**1000**

* Before using Buffer FCPL for the first time, add Buffer FCPM as described in "Things to do before starting" (page 14).

† Before using gDNA Wipeout Buffer 2 for the first time, reconstitute it in RNase-free water as described in "Things to do before starting" (page 14).

Table 3. Cell Number and Buffer Volumes for Different Plate Formats

Plate format	**Number of cells to seed per well***	**Volume of Buffer FCW to add per well (µl)**	**Volume of cell processing mix to add per well (µl)**
384-well plate	5 x 10^3	25	12.5
96-well plate	1 x 10^4	125	50
48-well plate	2 x 10^4	250	100
24-well plate	4 x 10^4	500	200
12-well plate	8 x 10^4	1000	400
6-well plate	1 x 10^5	2000	1000

* The values given are only suggestions. The number of cells to seed per well depends on factors such as cell type and culture conditions. For optimal results in real-time RT-PCR, it may be necessary to optimize the number of cells.

Appendix C: Processing Suspended Cells

The protocol on page 14 has been developed for use with adherent cultured cells grown in 96-well plates. If growing cells in suspension in a 96-well plate, replace steps 1–8 of the protocol with steps C1–C7 below.

Important points before starting

- When using a 96-well plate, ≤1 x 10^4 cells need to be seeded per well. However, other cell numbers can be used, depending on the cell type and the culture conditions.
- If growing cells in other types of plate, refer to Appendix B, page 20, for the number of cells to seed per well and the volumes of Buffer FCW and cell processing mix to add per well.
- If working with frozen cell pellets, start the procedure at step C3. If cells are already washed with Buffer FCW, start the procedure at step C5.

Procedure

C1. Pellet the cells by centrifugation in an appropriate vessel according to your experimental procedure.

For example, centrifuge at 250 x *g* for 5 min.

C2. Aspirate the cell-culture medium using a pipet, and discard.

C3. Add 125 µl Buffer FCW per sample.

Note: Do not incubate the cells with Buffer FCW for long periods of time.

C4. Pellet cells by centrifugation in an appropriate vessel according to your experimental procedure.

For example, centrifuge at 250 x *g* for 5 min.

C5. Aspirate Buffer FCW using a pipet, and discard.

If desired, the cell pellet can be stored frozen after removal of Buffer FCW. Be sure to thaw the pellet before proceeding to step C6.

C6. Add 50 µl cell processing mix per sample (see Table 1, page 15). Incubate for 5 min at room temperature (15–25°C).

For convenience, the incubation time can be increased up to 10 min.

C7. Transfer FastLane lysates (containing stabilized RNA) into appropriately sized tubes. Incubate for 5 min at 75°C, and briefly centrifuge. Proceed immediately to step 9 on page 16.

Note: If a pause in the procedure is required, store the tubes containing FastLane lysates at –80°C.

Appendix D: Alternative Reaction Setup for SYBR Green Based Real-Time RT-PCR in 384-Well Format

When performing real-time RT-PCR using 384-well plates, temperature gradients in the small wells may in some cases result in unspecific primer annealing. This problem can be overcome by including Q-Solution in the reaction. Q-Solution is a PCR additive that changes the melting behavior of nucleic acids. When working with a particular primer–template combination for the first time, we recommend running parallel reactions with and without Q-Solution in order to find out whether Q-Solution is required.

When performing real-time RT-PCR in a 384-well format on the Applied Biosystems 7900HT using the FastLane Cell SYBR Green Kit, follow the protocol in the *QuantiTect SYBR Green RT-PCR Handbook* (pages 10–13) with one modification: **add 5x Q-Solution to a final concentration of 0.25x during reaction setup** as shown in Table 4.

Table 4. 384-Well Reaction Setup for the Applied Biosystems 7900HT

Component	Volume/reaction	Final concentration
2x QuantiTect SYBR Green RT-PCR Master Mix*	10 µl	1x
Primer A	Variable	0.5 µM†
Primer B	Variable	0.5 µM†
QuantiTect RT Mix	0.2 µl	0.2 µl/reaction
5x Q-Solution	**1 µl**	**0.25x**
FastLane Lysate (added at step 4 of QuantiTect SYBR Green RT-PCR protocol)	1–4 µl	≤500 ng/reaction
RNase-free water	Variable	–
Total volume	**20 µl**	**–**

* Provides a final concentration of 2.5 mM $MgCl_2$.

† A final primer concentration of 0.5 µM is usually optimal. However, for individual determination of best concentration, a primer titration from 0.4 µM to 1 µM can be performed.

Ordering Information

Product	Contents	Cat. no.
FastLane Cell SYBR Green Kit (200)	FastLane Cell One-Step Buffer Set, 2x QuantiTect SYBR Green RT-PCR Master Mix, QuantiTect RT Mix, and RNase-Free Water	216213
FastLane Cell Probe Kit (200)	FastLane Cell One-Step Buffer Set, 2x QuantiTect Probe RT-PCR Master Mix, QuantiTect RT Mix, and RNase-Free Water	216413
FastLane Cell Multiplex Kit (200)	FastLane Cell One-Step Buffer Set, 2x QuantiTect Multiplex RT-PCR Master Mix, QuantiTect Multiplex RT Mix, and RNase-Free Water	216513
FastLane Cell Multiplex NR Kit (200)	FastLane Cell One-Step Buffer Set, 2x QuantiTect Multiplex RT-PCR NoROX Master Mix, QuantiTect Multiplex RT Mix, and RNase-Free Water	216713
Accessories		
QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at www.qiagen.com/GeneGlobe)		
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions or 500 x 20 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies
Related products		
FastLane Cell cDNA Kit — for high-speed preparation of cDNA without RNA purification for use in real-time RT-PCR		
FastLane Cell cDNA Kit (50)	Buffer FCW, Buffer FCP, and components for 50 x 20 µl reverse-transcription reactions (gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water)	215011

Ordering Information

Product	Contents	Cat. no.
QuantiTect SYBR Green RT-PCR Kit — for real-time, one-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204243
QuantiTect SYBR Green RT-PCR Kit (1000)	For 1000 x 50 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water	204245
QuantiTect Probe RT-PCR Kit — for real-time, one-step RT-PCR using sequence-specific probes		
QuantiTect Probe RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204443
QuantiTect Probe RT-PCR Kit (1000)	For 1000 x 50 µl reactions: 25 ml 2x Master Mix (contains ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water	204445
QuantiTect Multiplex RT-PCR Kits — for multiplex, real-time, one-step RT-PCR using sequence-specific probes		
For instruments from Applied Biosystems:		
QuantiTect Multiplex RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl 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 Master Mix (contains ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water	204645
For instruments from other suppliers:		
QuantiTect Multiplex RT-PCR NR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204843

Ordering Information

Product	Contents	Cat. no.
QuantiTect Multiplex RT-PCR NR Kit (1000)	For 1000 x 50 µl reactions: 25 ml 2x Master Mix (without ROX dye), 0.5 ml RT Mix, 20 ml RNase-Free Water	204845
FlexiPlate siRNA — for highly flexible, economical RNAi screening		
FlexiPlate siRNA	Custom siRNA set for customer-specified genes and siRNA controls; minimum order 36 siRNAs; 0.1 nmol, 0.25 nmol, or 1 nmol scale; plate layout chosen by the customer at GeneGlobe	Varies*
Human Whole Genome siRNA Set V1.0 — for screening the human genome from the experts in high-throughput RNAi		
Human Whole Genome siRNA Set V1.0	siRNAs targeting ~17,000 known human genes (NM genes) from the RefSeq database; available with 2 or 4 siRNAs per gene (0.25 nmol or 1 nmol) or pools of 2 (0.5 nmol total) or 4 siRNAs (1 nmol total)	Varies
Human Druggable Genome siRNA Set V3.0 — for RNAi screening of human druggable genes		
Human Druggable Genome siRNA Set V3.0	siRNAs targeting ~7000 human druggable genes; subsets available for druggable, phosphatase, kinase, and GPCR genes; available with 2 or 4 siRNAs per gene (0.25 nmol or 1 nmol) or pools of 2 (0.5 nmol total) or 4 siRNAs (1 nmol total)	Varies

* Find out more at www.qiagen.com/GeneGlobe .

Ordering Information

Product	Contents	Cat. no.
Mouse Whole Genome siRNA Set V1.0 — for accessible high-throughput RNAi screening of the mouse genome		
Mouse Whole Genome siRNA Set V1.0	siRNAs targeting ~17,000 mouse genes from the RefSeq database; subsets available for druggable, phosphatase, kinase, and GPCR genes; available with 2 or 4 siRNAs per gene (0.25 nmol or 1 nmol) or pools of 2 (0.5 nmol total) or 4 siRNAs (1 nmol total)	Varies
HiPerFect® Transfection Reagent — for transfection of eukaryotic cells with siRNA, especially suitable for transfection using low siRNA concentrations		
HiPerFect Transfection Reagent (0.5 ml)*	HiPerFect Transfection Reagent for up to 166 transfections in 24-well plates or up to 666 transfections in 96-well plates	301704

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Find more products for gene expression analysis and gene silencing at www.qiagen.com/geneXpression and www.qiagen.com/siRNA.

* Larger and smaller sizes and bulk quantities available; please inquire.

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