October 2010

FastLane Cell cDNA Handbook

For high-speed preparation of first-strand cDNA directly from cultured cells without RNA purification

For use in real-time, two-step RT-PCR

QIAGEN

Contents

Kit Contents

FastLane Cell cDNA Kit	**(50)**
Catalog no.	**215011**
Number of 20 µl reverse-transcription reactions	**50**
Buffer set (part 1 of 2):	
■ Buffer FCW	25 ml
■ Buffer FCP	10 ml
Reverse-transcription set (part 2 of 2):	
■ gDNA Wipeout Buffer, 7x	100 µl
■ Quantiscript® Reverse Transcriptase*	50 µl
■ Quantiscript RT Buffer, 5x†	200 µl
■ RT Primer Mix	50 µl
■ RNase-Free Water	1.1 ml
Handbook	1

* Also contains RNase inhibitor.

† Includes Mg^{2+} and dNTPs.

Shipping and Storage

Buffer FCW and Buffer FCP are shipped at ambient temperature, and should be stored at room temperature (15–25°C). All other components of the FastLane Cell cDNA Kit are 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 cDNA Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The FastLane Cell cDNA Kit is intended for molecular biology applications. This product is 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).

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 the FastLane Cell cDNA Kit 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).

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.

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

Product Description

The FastLane Cell cDNA Kit contains:

Buffer FCW:	Cell wash buffer for effective removal of extracellular contaminants
Buffer FCP:	Cell processing buffer for efficient lysis of cultured cells and RNA stabilization
gDNA Wipeout Buffer, 7x:	Buffer for effective elimination of genomic DNA contamination.
Quantiscript Reverse Transcriptase:	An optimized blend of enzymes developed for use in real-time, two-step PCR, comprising Quantiscript Reverse Transcriptase and an RNase inhibitor. Quantiscript Reverse Transcriptase is a unique mix of Omniscript® and Sensiscript® Reverse Transcriptases, which are recombinant heterodimeric enzymes expressed in *E. coli*. The RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, and C as well as human placental RNases.
Quantiscript RT Buffer, 5x:	Buffer optimized for reverse transcription with Quantiscript Reverse Transcriptase; contains dNTPs.
RT Primer Mix:	Optimized blend of oligo-dT and random primers dissolved in water. RT Primer Mix allows high cDNA yields from all regions of RNA transcripts, even from 5' regions.
RNase-Free Water:	Ultrapure quality, PCR-grade

Introduction

The FastLane Cell cDNA Kit provides a high-speed procedure for generating first-strand cDNA directly from cultured cells. No RNA purification or RNase H digestion steps are necessary, minimizing pipetting tasks. The cDNA synthesized is free of genomic DNA and accurately represents the cellular gene expression profile, making it highly suited for use in quantitative, real-time, two-step RT-PCR. The FastLane Cell cDNA Kit is optimized for use in real-time, two-step RT-PCR, and is not suitable for qualitative PCR.

The FastLane Cell cDNA Kit is part of QIAGEN's range of products for RNAi and gene expression analysis, which are closely integrated to provide highly reproducible and sensitive results. Products include optimized PCR master mixes, ready-to-run assays, siRNAs, and transfection reagents. To find out more about standardization of RNAi and gene expression analysis workflows, visit www.qiagen.com/siRNA and www.qiagen.com/geneXpression .

Principle and procedure

The FastLane Cell cDNA procedure comprises 4 steps: removal of extracellular contaminants, cell lysis with RNA stabilization, elimination of genomic DNA, and reverse transcription.

Removal of extracellular contaminants

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 lysis with RNA stabilization

After the wash with Buffer FCW, the cultured cells are then lysed for 5 minutes using Buffer FCP. This buffer also stabilizes the cellular RNA and blocks inhibitors of reverse transcription. This allows efficient synthesis of first-strand cDNA from RNA that accurately reflects the in vivo gene expression profile.

Elimination of genomic DNA

The FastLane lysate is briefly incubated in gDNA Wipeout Buffer at 42°C for 5 minutes to effectively eliminate contaminating genomic DNA. The FastLane lysate is then used directly in reverse transcription. In contrast, other methods require DNase-treatment of RNA samples followed by RNA cleanup, which involve more time and effort.

Accurate results in real-time RT-PCR depend on the use of primers or probes designed to eliminate or minimize detection of genomic DNA. If such primers or probes are not available, then genomic DNA contamination must be eliminated.

Reverse transcription

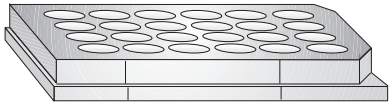
After genomic DNA elimination, the FastLane lysate is ready for reverse transcription using a master mix prepared from Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, and RT Primer Mix. The entire reaction takes place at 42°C and is then inactivated at 95°C. In contrast to other methods, additional steps for RNA denaturation, primer annealing, and RNase H digestion are not necessary.

Quantiscript Reverse Transcriptase has a high affinity for RNA and is optimized for efficient and sensitive cDNA synthesis from 10 pg to 1 µg of RNA. This high RNA affinity, in combination with Quantiscript RT Buffer, enables high cDNA yields, even from templates with high GC-content or complex secondary structure.

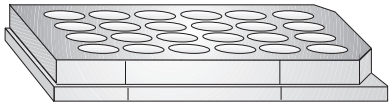
RT Primer Mix ensures cDNA synthesis from all regions of RNA transcripts, even from 5' regions. This allows high yields of cDNA template for real-time PCR analysis regardless of where the target region is located on the transcript.

FastLane Cell cDNA Procedure

Cells seeded in 24-well plate

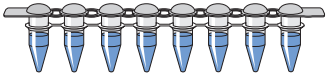


Remove cell-culture medium and contaminants using Buffer FCW

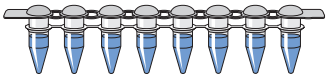


Lyse cells and stabilize RNA using Buffer FCP (5 min at room temperature)

FastLane lysate



Eliminate genomic DNA using gDNA Wipeout Buffer (5 min at 42°C, then on ice)



Synthesize first-strand cDNA using Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, and Primer RT Mix (30 min at 42°C, then 3 min at 95°C)

Perform quantitative, real-time PCR

Enzymatic activities of reverse transcriptase

Reverse transcriptase enzymes are generally derived from RNA-containing retroviruses such as avian myeloblastosis virus (AMV), Moloney murine leukemia virus (MMLV), or human immunodeficiency virus (HIV). Quantiscript Reverse Transcriptase is from a new source.

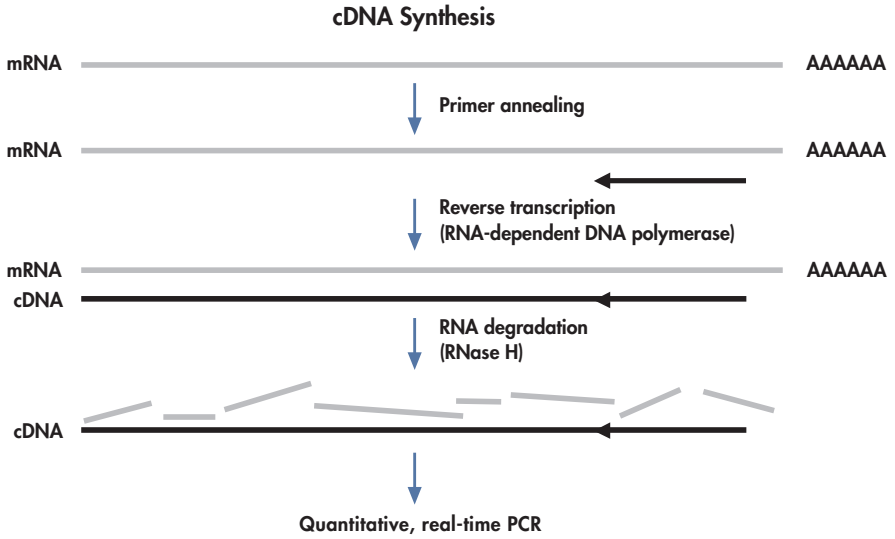


Figure 1 Quantiscript Reverse Transcriptase in first-strand cDNA synthesis.

In general, reverse transcriptase is a multifunctional enzyme with 3 distinct enzymatic activities: an RNA-dependent DNA polymerase, a hybrid-dependent exoribonuclease (RNase H), and a DNA-dependent DNA polymerase. In vivo, the combination of these 3 activities allows transcription of the single-stranded RNA genome into double-stranded DNA for retroviral infection. For reverse transcription in vitro (Figure 1), the first 2 activities are utilized to produce single-stranded cDNA:

- RNA-dependent DNA-polymerase activity (reverse transcription) transcribes cDNA from an RNA template. This activity of Quantiscript Reverse Transcriptase allows synthesis of cDNA for use in quantitative, real-time PCR.
- **RNase H activity** of Quantiscript Reverse Transcriptase specifically degrades only the RNA in RNA:DNA hybrids. Therefore, this RNase H activity affects RNA hybridized to cDNA, but has no effect on pure RNA. A separate RNA degradation step using RNase H enzyme is not necessary prior to real-time PCR. Furthermore, the Quantiscript RNase H activity, acting during reverse transcription, may improve the sensitivity of subsequent real-time 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 first-strand cDNA synthesis from cultured cells

- RNase-free plastic tubes (up to 1.5 ml for storing FastLane lysates, and greater than 20 µl for genomic DNA elimination and reverse transcription)
- Ice
- Heating block or water bath (capable of reaching 95°C)
- Vortexer
- Microcentrifuge
- Optional: gene-specific primers

For quantitative, real-time PCR

- Optimized kit for quantitative, real-time PCR, which includes *Taq* polymerase, quantitative, real-time PCR buffer, primers, probe or SYBR® Green I dye, and nucleotides (for details, see Appendix E, page 22).
- QIAGEN offers the following ready-to-run solutions for quantitative, real-time PCR using SYBR Green I dye:
  - QuantiTect® SYBR Green PCR Kit — preoptimized master mix
  - QuantiTect Primer Assays — functionally validated primer sets

  QIAGEN offers the following ready-to-run solutions for quantitative, real-time PCR using sequence-specific probes:
  - QuantiTect Probe PCR Kit — preoptimized master mix for single PCR
  - QuantiTect Multiplex PCR Kits — preoptimized master mix for multiplex PCR
  - QuantiTect Gene Expression Assays — functionally validated primer–probe sets
  - QuantiTect Custom Assays — custom-designed primer–probe sets
  - QuantiTect Endogenous Control Assays — functionally validated primer–probe sets for housekeeping genes

  For more details, visit www.qiagen.com/geneXpression . For ordering information, see page 24.

Protocol: High-Speed Preparation of First-Strand cDNA from Cultured Cells for Real-Time PCR

Important points before starting

- This protocol has been developed for use with adherent cultured cells grown in 24-well plates. When using a 24-well plate, ≤4 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 plate, see Appendix C, page 20. If growing cells in suspension, refer to Appendix D, page 21.
- If working with RNA for the first time, read Appendix A, page 18.
- Set up reverse-transcription reactions on ice for optimal results.
- RNase inhibitor and dNTPs do not need to be added to the reverse-transcription reaction, as they are already present in Quantiscript Reverse Transcriptase and Quantiscript RT Buffer, respectively.
- RT Primer Mix (supplied) or gene-specific primers (not supplied) should be used. RT Primer Mix is optimized to provide high cDNA yields for all regions of RNA transcripts.
- Separate denaturation and annealing steps are not necessary before starting the reverse-transcription reaction.
- After reverse transcription, the reaction must be inactivated by incubation at 95°C for 3 minutes.
- For details on performing real-time PCR after reverse transcription, see Appendix E, page 22. 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.
- For details on appropriate controls for real-time RT-PCR, see Appendix F, page 22.

Procedure

1. **Seed an appropriate number of cells (e.g., 1 x 10^4 cells) per well of a 24-well plate.**

   Note: If using another type of plate, see Appendix C, page 20.
2. **Incubate the cells according to your experimental procedure or until confluent.**
3. **Aspirate the cell-culture medium using a pipet, and discard.**

4. **Add 500 µl Buffer FCW per well.**

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

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

6. **Add 200 µl Buffer FCP per well. Incubate for 5 min at room temperature (15–25°C).**

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

7. **Transfer the FastLane lysates (containing stabilized RNA) into appropriately sized tubes. Proceed immediately to step 8.**

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

8. **Thaw the following solutions at room temperature (15–25°C): FastLane lysates from step 7 (if necessary), gDNA Wipeout Buffer, RNase-free water, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, and RT Primer Mix.**

   Mix each solution by flicking the tubes. Centrifuge briefly to collect residual liquid from the sides of the tubes.

   Note: Store Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, and RT Primer Mix on ice immediately after thawing. Store gDNA Wipeout Buffer and RNase-free water at room temperature.

9. **Prepare the genomic DNA elimination reaction according to Table 1.**

   Mix thoroughly by inverting the tube several times. Centrifuge briefly to collect residual liquid from the side of the tube, and then store at room temperature (15–25°C).

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than that required for the total number of reactions to be performed. Then distribute the appropriate volume of master mix into individual reaction tubes followed by the individual FastLane lysates. Keep the tubes at room temperature.

Table 1. Genomic DNA Elimination Reaction Components

Component	Volume/reaction	Final concentration
gDNA Wipeout Buffer, 7x	2 µl	1x
FastLane lysate (from step 7)	1–4 µl	
RNase-free water	Variable	
Total volume	**14 µl**	**–**

10. **Incubate for 5 min at 42°C. Then place immediately on ice.**

11. **Prepare the reverse-transcription master mix on ice according to Table 2.**

    Mix thoroughly by flicking the tube for no more than 5 s. Centrifuge briefly to collect residual liquid from the side of the tube, and then store on ice. The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA. RT Primer Mix is optimized to provide high cDNA yields for all regions of RNA transcripts.

    Note: Template RNA (i.e., FastLane lysate from step 10) is added later at step 12.

    Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than that required for the total number of reactions to be performed. Then distribute the appropriate volume of master mix into individual reaction tubes. Keep the tubes on ice.

Table 2. Reverse-Transcription Reaction Components*

Component	Volume/reaction	Final concentration
Reverse-transcription master mix		
Quantiscript Reverse Transcriptase†	1 µl	
Quantiscript RT Buffer, 5x‡§	4 µl	1x
RT Primer Mix§	1 µl	
Template RNA		
FastLane lysate (from step 10)	14 µl (added at step 12)	
Total volume	**20 µl**	**–**

* If you prefer not to set up a master mix, then first mix Quantiscript RT Buffer, RT Primer Mix, and template RNA together, and add Quantiscript Reverse Transcriptase last. Proceed immediately to step 13.

† Also contains RNase inhibitor.

‡ Includes Mg^{2+} and dNTPs.

§ For convenience, premix RT Primer Mix and 5x Quantiscript RT Buffer in a 1:4 ratio if RT Primer Mix will be used routinely for reverse transcription. This premix is stable when stored at –20°C.

12. **Add template RNA (i.e., FastLane lysate from step 10) to each tube containing reverse-transcription master mix.**

    Mix thoroughly by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the sides of the tubes.

13. **Incubate for 30 min at 42°C.**

    Note: The incubation time can be reduced, but this should be tested individually for each template.

14. **Incubate for 3 min at 95°C to inactivate Quantiscript Reverse Transcriptase.**

15. **Add an aliquot of each finished reverse-transcription reaction to real-time PCR mix (see Appendix E, page 22).**

    Note: The aliquot should not form more than 10% of the total PCR volume.

    Store reverse-transcription reactions on ice and proceed directly with real-time PCR, or for long-term storage, store reverse-transcription reactions at –20°C. For real-time PCR, we recommend using QuantiTect Kits and Assays (see page 11).

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 (see back cover for contact information).

		Comments and suggestions
No product, or product detected late in real-time 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 cDNA procedure followed by real-time PCR. Determine which cell number gives optimal 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 Buffer FCP	Refer to Appendix C, page 20, to find the appropriate volume of Buffer FCP to add per well of your multiwell plate.
d)	Incorrect setup of reverse-transcription reaction	Be sure to set up the reaction on ice.
e)	Volume of reverse-transcription reaction added to the real-time PCR too high	Adding a high volume of reverse-transcription reaction to the PCR mix may reduce amplification efficiency and the linearity of the reaction. Generally, the volume of reverse-transcription reaction added should not exceed 10% of the final PCR volume.
f)	Temperature of reverse-transcription reaction	Reverse transcription should be carried out at 42°C. Check the temperature of your heating block or water bath. In rare cases, when analyzing RNAs with a very high degree of secondary structure, it may be advantageous to increase the temperature up to 50°C. However, temperatures >42°C will reduce the activity of Quantiscript Reverse Transcriptase and therefore affect the cDNA yield.

		Comments and suggestions
g)	Pipetting error or missing reagent when setting up reverse-transcription reaction	Check the pipets used for experimental setup. Mix all reagents well after thawing, and repeat the reverse-transcription reaction.
h)	RNA denatured	Denaturation of the template RNA is not necessary. If denaturation was performed, the integrity of the RNA may be affected.
i)	Short incubation time	The standard reverse-transcription reaction requires a 30-min incubation.
j)	Incorrect concentration or degradation of primers for reverse-transcription reaction	If using a gene-specific primer for reverse transcription, check the concentration and integrity of the primer. If necessary, perform reverse transcription with different primer concentrations or use the supplied RT Primer Mix. If using RT Primer Mix, be sure to use 1 µl of RT Primer Mix in a 20 µl reaction.
k)	Incubation temperature too high	Reverse transcription should be carried out at 42°C. Higher temperatures may reduce the length of cDNA products or the activity of Quantiscript Reverse Transcriptase. Check the temperature of your heating block or water bath.
No product, or product detected late in real-time PCR, or only primer–dimers detected		
Problems occurring during real-time PCR		Refer to the instructions for the real-time PCR kit you are using.

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 cDNA 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: Determination of Quality of RNA

The integrity and size distribution of total RNA can be checked as follows. Stabilizing agents are first removed from FastLane lysates by using the RNeasy® Mini Kit (see ordering information, page 24) and the RNA cleanup protocol supplied with the kit. The cleaned-up RNA samples are then analyzed by denaturing agarose gel electrophoresis and ethidium bromide* staining. The respective ribosomal bands (Table 3) should appear as sharp bands on the stained gel. 28S ribosomal RNA bands should be present at approximately twice the amounts of the 18S RNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

Table 3. Size of Ribosomal RNAs (rRNA) from Various Sources

Source	rRNA	Size (kb)
Mouse	18S	1.9
	28S	4.7
Human	18S	1.9
	28S	5.0

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

Appendix C: Seeding and Processing Cells for Different Plate Formats

The protocol on page 12 is for use with 24-well plates seeded with ≤4 x 10^4 cells per well. If using another type of plate, refer to Table 4 below for the number of cells to seed per well and the volumes of Buffer FCW and Buffer FCP to add per well. Grow the cells according to your experimental procedure or until confluent.

Table 4. 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	Volume of Buffer FCP to add per well
384-well plate†	5 x 10^3	25 µl	12.5 µl
96-well plate†	1 x 10^4	100 µl	50 µl
48-well plate	2 x 10^4	250 µl	100 µl
24-well plate	4 x 10^4	500 µl	200 µl
12-well plate	8 x 10^4	1000 µl	400 µl
6-well plate	1 x 10^5	2000 µl	1000 µl

* 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.

† If working with 384- or 96-well plates, the QuantiTect Reverse Transcription Kit is also required, since additional reverse-transcription reactions will be performed (see ordering information, page 24).

Appendix D: Processing Suspended Cells

The protocol on page 12 has been developed for use with adherent cultured cells grown in 24-well plates. If growing cells in suspension in a 24-well plate, follow steps D1 to D7 below, and then continue from step 8 of the protocol on page 12.

Important points before starting

- When using a 24-well plate, ≤4 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 C for the number of cells to seed per well and the volumes of Buffer FCW and Buffer FCP to add per well.
- If working with frozen cell pellets, start the procedure at step D3. If cells are already washed with Buffer FCW, start the procedure at step D5.

Procedure

D1. Pellet the cells by centrifugation in an appropriate vessel according to your experimental procedure.

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

D2. Aspirate the cell-culture medium using a pipet, and discard.

D3. Add 500 µl Buffer FCW per sample.

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

D4. Pellet cells by centrifugation in an appropriate vessel according to your experimental procedure.

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

D5. Aspirate Buffer FCW using a pipet, and discard.

If desired, the cell pellet can be stored frozen after removal of Buffer FCW.

D6. Add 200 µl Buffer FCP per sample. Incubate for 5 min at room temperature (15–25°C).

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

D7. Transfer FastLane lysates (containing stabilized RNA) into appropriately sized tubes. Proceed immediately to step 8 on page 13.

Note: If a pause in the procedure is required, store the tubes containing FastLane lysates at –20°C.

Appendix E: Quantitative, Real-Time, Two-Step RT-PCR

In quantitative, real-time, two-step RT-PCR, cDNA is first synthesized by reverse transcription. An aliquot of the finished reverse-transcription reaction is then used for PCR. Reverse transcription and PCR are performed sequentially in 2 separate reaction tubes.

E1. Carry out reverse transcription according to the protocol on page 12.

E2. Add an aliquot of each finished reverse-transcription reaction to real-time PCR mix.

Note: No more than 1/10 of the final PCR volume should derive from the finished reverse-transcription reaction. For example, for a 50 µl PCR assay, use ≤5 µl of the finished reverse-transcription reaction.

E3. Carry out real-time PCR as recommended by the supplier.

We recommend using QuantiTect Kits and Assays (see page 11).

Appendix F: Recommended Controls for Quantitative, Real-Time RT-PCR

RT control

With the FastLane Cell cDNA Kit, genomic DNA is efficiently removed in a single step. However, all reverse-transcription experiments should include a negative control to test for contaminating genomic DNA. Genomic DNA contamination can be detected by performing a control reaction in which no reverse transcription is possible. This control contains all components including template RNA, except for Quantiscript Reverse Transcriptase. Reverse transcription therefore cannot take place and the only template available is contaminating genomic DNA. In rare cases in which genomic DNA is still amplified, detection of contaminating DNA can be eliminated with specially designed primers or probes (Figure 2).

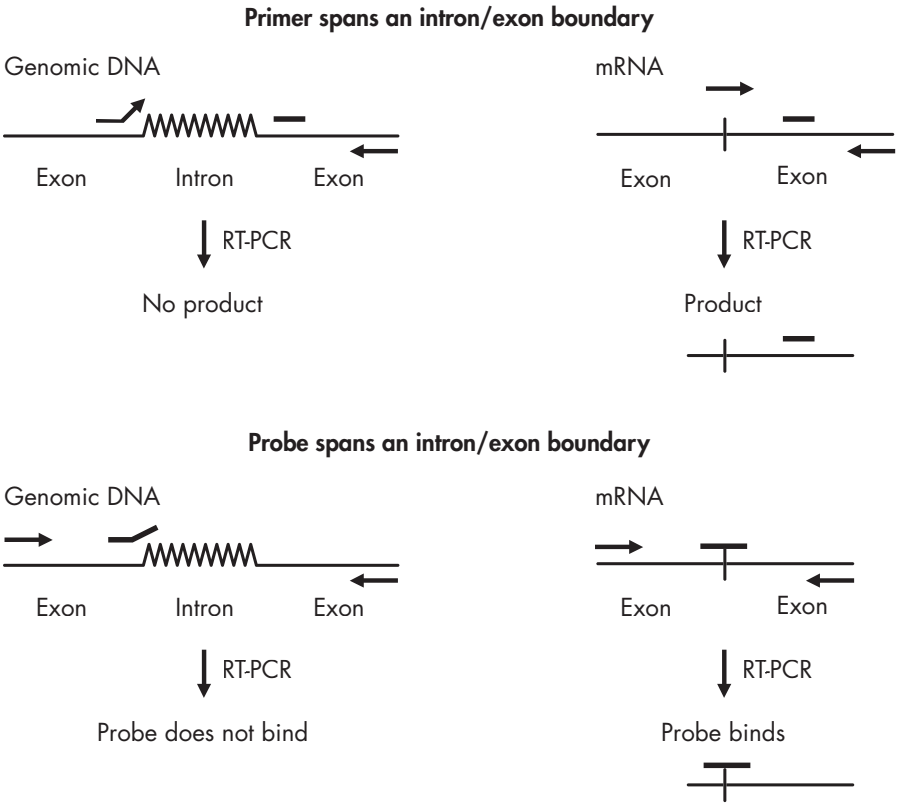


Figure 2 Primer/probe design to eliminate signals from contaminating genomic DNA.

Positive control

In some cases, it may be necessary to include a positive control containing a known concentration of template. This is usually a substitute for absolute standards and is used only to test for presence or absence of the target, but does not yield detailed quantitative information. Ensure that the positive control contains at least the minimum amount of RNA required for accurate detection.

No template control (NTC)

All real-time PCR quantification experiments should include an NTC containing all the components of the reaction except for the template. This enables detection of carryover contamination from previous experiments.

Ordering Information

Product	Contents	Cat. no.
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
Related product		
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311
QuantiTect Reverse Transcription Kit (200)	For 200 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205313
Accessories		
QuantiTect SYBR Green PCR Kit — for quantitative, real-time PCR and two-step RT-PCR using SYBR Green I		
QuantiTect SYBR Green PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix (providing a final concentration of 2.5 mM $MgCl_2$), 2 x 2 ml RNase-Free Water	204143
QuantiTect Primer Assays — for use in quantitative, real-time RT-PCR with SYBR Green detection		
QuantiTect Primer Assay (200)†	For 200 x 50 µl reactions (for use in a 96-well plate or single tubes) or 500 x 20 µl reactions (for use in a 384-well plate or single capillaries): 10x QuantiTect Primer Assay (lyophilized)	Varies

* Larger kit size available; for details, visit www.qiagen.com/PCR .

† Visit www.qiagen.com/GeneGlobe to search for and order QuantiTect Primer Assays.

Ordering Information

Product	Contents	Cat. no.
QuantiTect Probe PCR Kit — for quantitative, real-time PCR and two-step RT-PCR using sequence-specific probes		
QuantiTect Probe PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix (providing a final concentration of 4 mM $MgCl_2$), 2 x 2 ml RNase-Free Water	204343
QuantiTect Multiplex PCR Kits — for quantitative, multiplex, real-time PCR and two-step RT-PCR using sequence-specific probes		
QuantiTect Multiplex PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Multiplex PCR Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water	204543
QuantiTect Multiplex PCR NoROX Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Multiplex PCR NoROX Master Mix (contains no ROX dye), 2 x 2 ml RNase-Free Water	204743
QuantiTect Gene Expression Assays — for gene expression analysis using functionally validated, quantitative, real-time RT-PCR assays		
QuantiTect Gene Expression Assay (100)†	For 100 x 50 µl reactions (for use in a 96-well plate or single tubes) or 250 x 20 µl reactions (for use in a 384-well plate or single capillaries): 0.5 ml 10x QuantiTect Assay Mix (dyes available: FAM)	Varies

* Larger kit size available; for details, visit www.qiagen.com/PCR .

† Visit www.qiagen.com/GeneGlobe to search for and order QuantiTect Gene Expression Assays.

Ordering Information

Product	Contents	Cat. no.
QuantiTect Custom Assays — for gene expression analysis using custom-designed, quantitative, real-time RT-PCR assays		
QuantiTect Custom Assay (100)*	For 100 x 50 µl reactions (for use in a 96-well plate or single tubes) or 250 x 20 µl reactions (for use in a 384-well plate or single capillaries): 0.25 ml 20x Primer Mix, 0.25 ml 20x QuantiProbe™ (dyes available: FAM, Yakima Yellow™, TET)	–
QuantiTect Endogenous Assays — for gene expression analysis of endogenous control genes using functionally validated, quantitative, real-time RT-PCR assays		
QuantiTect Endogenous Control Assay (100)†	For 100 x 50 µl reactions (for use in a 96-well plate or single tubes) or 250 x 20 µl reactions (for use in a 384-well plate or single capillaries): 0.5 ml 10x QuantiTect Assay Mix (dyes available: FAM, Yakima Yellow)	Varies
RNeasy Mini Kit — for purification of up to 100 µg total RNA		
RNeasy Mini Kit (50)‡	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	74104

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* Visit www.qiagen.com/goto/assays to design your own QuantiTect Custom Assays. QuantiTect Custom Assays are available in larger sizes. Yakima Yellow dye can substitute for VIC® or HEX dye.

† Visit www.qiagen.com/GeneGlobe to view the full range of QuantiTect Endogenous Control Assays. Yakima Yellow dye can substitute for VIC or HEX dye.

‡ Larger kit size available; for details, visit www.qiagen.com/RNA .

Notes

www.qiagen.com

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