

# QuantiTect® Whole Transcriptome Handbook

For preparation of cDNA from total RNA by whole transcriptome amplification



# QIAGEN Sample and Assay Technologies

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- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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# Contents

<b>Kit Contents</b>	<b>4</b>
<b>Shipping and Storage</b>	<b>4</b>
<b>Product Use Limitations</b>	<b>4</b>
<b>Product Warranty and Satisfaction Guarantee</b>	<b>5</b>
<b>Quality Control</b>	<b>5</b>
<b>Technical Assistance</b>	<b>5</b>
<b>Safety Information</b>	<b>6</b>
<b>Introduction</b>	<b>7</b>
Principle and procedure	7
<b>Equipment and Reagents to Be Supplied by User</b>	<b>10</b>
<b>Important Notes</b>	<b>11</b>
Quality of RNA template	11
Size of RNA transcripts	11
Amount of RNA template	11
<b>Protocol</b>	
■ <b>Whole Transcriptome Amplification</b>	<b>12</b>
<b>Troubleshooting Guide</b>	<b>16</b>
<b>Appendix A: Limitations of RNA Amplification</b>	<b>19</b>
<b>Appendix B: Determining cDNA Concentration and Yield</b>	<b>21</b>
<b>Appendix C: PicoGreen Quantification of Amplified cDNA</b>	<b>21</b>
<b>Ordering Information</b>	<b>24</b>

## Kit Contents

<b>QuantiTect Whole Transcriptome Kit</b>	<b>(25)</b>	<b>(100)</b>
<b>Catalog no.</b>	<b>207043</b>	<b>207045</b>
<b>Number of 50 <math>\mu</math>l reactions</b>	<b>25</b>	<b>100</b>
T-Script® Buffer (yellow lid)	400 $\mu$ l	400 $\mu$ l
T-Script Enzyme (blue lid)	25 $\mu$ l	100 $\mu$ l
Ligation Buffer (yellow lid)	600 $\mu$ l	600 $\mu$ l
Ligation Enzyme 1 (blue lid)	25 $\mu$ l	100 $\mu$ l
Ligation Enzyme 2 (blue lid)	25 $\mu$ l	100 $\mu$ l
Ligation Reagent (yellow lid)	200 $\mu$ l	200 $\mu$ l
REPLI-g® Midi DNA Polymerase (blue lid)	25 $\mu$ l	100 $\mu$ l
REPLI-g Midi Reaction Buffer (yellow lid)	730 $\mu$ l	2 x 1.45 ml
Handbook	1	1

## Shipping and Storage

The QuantiTect Whole Transcriptome Kit is shipped on dry ice. The kit, including all reagents and buffers, should be stored immediately upon receipt at  $-20^{\circ}\text{C}$  in a constant-temperature freezer. When stored under these conditions and handled correctly, this kit can be kept for at least 6 months after shipping without showing any reduction in performance. For longer storage, the kit should be stored at  $-70^{\circ}\text{C}$ .

## Product Use Limitations

The QuantiTect Whole Transcriptome Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of 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](http://www.qiagen.com)).

## Quality Control

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

## 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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QuantiTect Whole Transcriptome 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 see our Technical Support Center at [www.qiagen.com/Support](http://www.qiagen.com/Support) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://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/Support/MSDS.aspx](http://www.qiagen.com/Support/MSDS.aspx) 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

# Introduction

Gene expression profiling can be limited by the small amount of biological sample available. By using the QuantiTect Whole Transcriptome Kit, unlimited real-time PCR analyses of small and precious samples are possible. The kit allows uniform amplification of all transcripts within RNA samples, providing cDNA that can be archived for subsequent gene expression analysis by real-time PCR (the reverse-transcription step prior to PCR is not required). DNA yields achieved with the kit depend on the reaction conditions (see Table 1).

**Table 1. Typical cDNA yields**

Reaction conditions	Amplification time	Typical cDNA yield (per 50 µl reaction)	No. real-time PCR analyses
Standard reaction	2 hours	Up to 10 µg	Approx. 1000
High-yield reaction	8 hours	Up to 40 µg	Approx. 4000

The QuantiTect Whole Transcriptome Kit contains reverse transcriptase, DNA polymerase, and optimized buffers and reagents for the amplification of all transcripts within an RNA sample. The kit integrates cDNA synthesis with the proven quality of REPLI-g technology for nonbiased sequence amplification.

**Note:** The QuantiTect Whole Transcriptome Kit is not suitable for the amplification of small RNA molecules, such as tRNAs or miRNAs, or degraded RNA. RNA samples with a small degree of RNA damage may be usable, but each sample needs to be tested individually. Also, the QuantiTect Whole Transcriptome Kit amplifies cDNA derived from all regions of RNA transcripts, including 5' ends, but does not provide amplified cDNA corresponding to full-length RNA transcripts.

## Principle and procedure

The QuantiTect Whole Transcriptome Kit contains reagents for 3 sequential reactions:

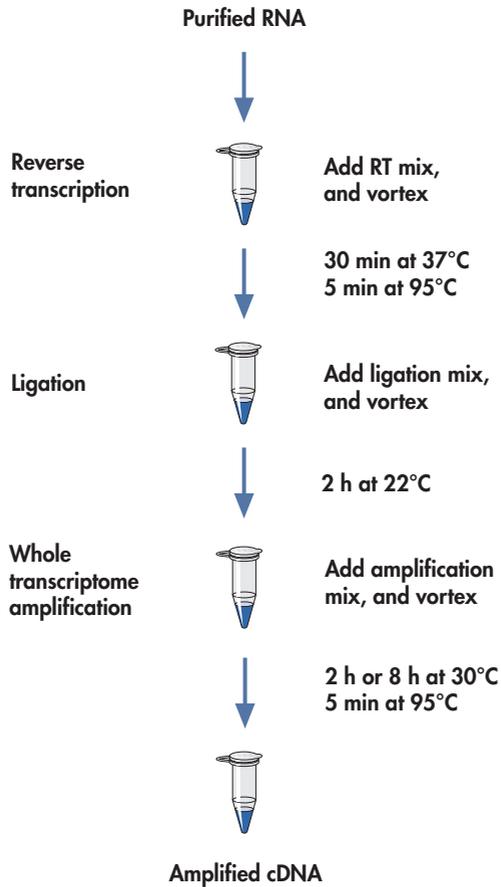
- **Reverse transcription:** cDNA is synthesized using an RT mix (T-Script Enzyme, which is a reverse transcriptase, and T-Script Buffer, which contains random and oligo-dT primers).
- **Ligation:** The synthesized cDNA is ligated using a high-efficiency ligation mix (Ligation Enzyme 1, Ligation Enzyme 2, Ligation Reagent, and Ligation Buffer).
- **Whole transcriptome amplification:** The ligated cDNA is amplified in an isothermal reaction lasting 2 or 8 hours using an amplification mix (REPLI-g Midi DNA Polymerase and REPLI-g Midi Reaction Buffer).

The QuantiTect Whole Transcriptome Kit provides highly uniform amplification across entire sequences, with negligible sequence bias. The amplified cDNA contains concatemers of transcript sequences, and is therefore of high molecular weight. Amplification is based on Multiple Displacement Amplification (MDA) technology (Figure 1), which carries out isothermal sequence amplification using a uniquely processive DNA polymerase. The DNA polymerase has a 3'→5' exonuclease proofreading activity to maintain high fidelity during replication and is used in the presence of exonuclease-resistant primers to achieve high yields of cDNA product. For more information about this technology, visit [www.qiagen.com/goto/WGA](http://www.qiagen.com/goto/WGA).



**Figure 1. QuantiTect whole transcriptome amplification.** cDNA is first synthesized from template RNA and then ligated (not shown). cDNA is then amplified by REPLI-g DNA polymerase, which moves along the cDNA template strand displacing the complementary strand. The displaced strand becomes a template for replication, allowing high yields of high-molecular-weight cDNA to be generated.

## QuantiTect Whole Transcriptome Procedure



## 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.

- Microcentrifuge tubes
- Thermal cycler, water bath, or heating block
- Microcentrifuge
- Vortexer
- Pipets and pipet tips
- Ice
- Nuclease-free water or TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)

## Important Notes

### Quality of RNA template

The efficiency of the amplification reaction with the QuantiTect Whole Transcriptome Kit depends on the quality and quantity of the starting RNA template. It is therefore important to use highly pure, intact RNA. Carrier RNA, which may be used in procedures for purification of very low amounts of target RNA, may affect the specific amplification of transcript sequences.

To ensure reproducible and efficient whole transcriptome amplification, we recommend purifying RNA template using silica-membrane technology. RNeasy® Kits, the PAXgene® Blood RNA Kit, and the QIAamp® RNA Blood Mini Kit (see ordering information, page 24) are based on this technology. They enable RNA purification from a variety of starting materials and provide high-quality RNA highly suited for use with the QuantiTect Whole Transcriptome Kit. For purification of low amounts of RNA in the presence of carrier RNA, we recommend the RNeasy Plus Micro Kit. If using other purification procedures, use of carrier RNA should be avoided.

### Size of RNA transcripts

The QuantiTect Whole Transcriptome Kit is not suitable for the amplification of small RNA molecules, such as tRNAs or miRNAs, or degraded RNA. Ideally, RNA transcripts should be longer than 500 nucleotides.

### Amount of RNA template

To ensure high reproducibility in whole transcriptome amplification, we recommend using a starting amount of 10 ng RNA template. Depending on the copy number of the transcripts, it may be possible to use smaller amounts of RNA template (see Appendix A, page 19). The minimum amount of starting RNA template should not be less than 100 pg.

## Protocol: Whole Transcriptome Amplification

### Important points before starting

- RNA should be purified prior to this procedure. QIAGEN kits provide purification of highly pure RNA from a range of sample types.
- This protocol is optimized for whole transcriptome amplification from  $\geq 10$  ng purified RNA. The RNA should be dissolved in nuclease-free water or TE buffer. **Smaller amounts ( $< 10$  ng RNA) can be used if the RNA is of high quality and your transcript of interest is of sufficient abundance** (see Appendix A, page 19).
- The QuantiTect Whole Transcriptome Kit is not suitable for the amplification of small RNA molecules, such as tRNAs or miRNAs, or degraded RNA. RNA samples with a small degree of RNA damage may be usable, but each sample needs to be tested individually.
- The QuantiTect Whole Transcriptome Kit amplifies cDNA derived from all regions of RNA transcripts, including 5' ends, but does not provide amplified cDNA corresponding to full-length RNA transcripts.
- All enzymes (i.e., T-Script Enzyme, Ligation Enzyme 1, Ligation Enzyme 2, and REPL-g Midi DNA Polymerase) should be thawed on ice just before reaction setup. All other kit components should be thawed at room temperature (15–25°C).
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- The RT mix, ligation mix, and amplification mix described in the protocol must always be prepared fresh. They cannot be stored for later use.

## Thermal cycling parameters

For increased speed and convenience, all incubation steps of the protocol can be preprogrammed on a thermal cycler (see Table 2).

**Table 2. Thermal cycling parameters**

Step	Time	Temperature	Additional comments
<b>Set the heating lid to 50°C for all steps</b>			
Reverse transcription	30 min	37°C	Add RT mix prior to incubation (step 2)
	5 min	95°C	Stops reverse transcription
Ligation	2 h	22°C	Add ligation mix prior to incubation (step 4)
Whole transcriptome amplification	2 h or 8 h*	30°C	Add amplification mix prior to incubation (step 6)
	5 min	95°C	Inactivates all enzymes
	∞	4°C	Cools amplified cDNA down

\* 2 h for standard cDNA yields, and 8 h for high cDNA yields.

## Procedure

1. Add  $\geq 10$  ng RNA in 1–5  $\mu$ l nuclease-free water or TE buffer to a microcentrifuge tube. Adjust the volume to 5  $\mu$ l using nuclease-free water.
2. Prepare the RT mix (see Table 3). Add 5  $\mu$ l RT mix to the RNA sample, mix by vortexing, and centrifuge briefly.

**Note:** The RT mix must be prepared fresh.

**Table 3. Preparing RT mix**

Component	Volume/reaction
T-Script Buffer	4 $\mu$ l
T-Script Enzyme	1 $\mu$ l
<b>Total volume*</b>	<b>5 <math>\mu</math>l</b>

\* Mix by vortexing and centrifuge briefly.

3. Incubate at 37°C for 30 min. Stop the reaction by incubating at 95°C for 5 min and then cooling to 22°C.

4. **Prepare the ligation mix (see Table 4). Add 10  $\mu$ l to the RT reaction, mix by vortexing, and centrifuge briefly.**

**Important:** When preparing the ligation mix, add the components in the order shown in Table 4.

**Note:** The ligation mix must be prepared fresh.

**Table 4. Preparing ligation mix**

Component	Volume/reaction
Ligation Buffer	6 $\mu$ l
Ligation Reagent	2 $\mu$ l
Ligation Enzyme 1	1 $\mu$ l
Ligation Enzyme 2	1 $\mu$ l
<b>Total volume*</b>	<b>10 <math>\mu</math>l</b>

\* Mix by vortexing and centrifuge briefly.

5. **Incubate at 22°C for 2 h.**
6. **Prepare the amplification mix (see Table 5). Add 30  $\mu$ l amplification mix to the ligation reaction, mix by vortexing, and centrifuge briefly.**

**Note:** The amplification mix must be prepared fresh.

**Table 5. Preparing amplification mix**

Component	Volume/reaction
REPLI-g Midi Reaction Buffer	29 $\mu$ l
REPLI-g Midi DNA Polymerase	1 $\mu$ l
<b>Total volume*</b>	<b>30 <math>\mu</math>l</b>

\* Mix by vortexing and centrifuge briefly.

7. **Incubate at 30°C for either 2 h (standard reaction) or 8 h (high-yield reaction).**  
For typical cDNA yields with these incubation times, see Table 1 (page 7).

**8. Stop the reaction by incubating at 95°C for 5 min.**

**Note:** If the amplified cDNA will be quantified using PicoGreen® reagent (see Appendices B and C, page 21), be aware that the reagent binds double-stranded DNA only. Therefore, quantify the cDNA before proceeding with the 95°C incubation, or remove an aliquot for later quantification.

**9. Store the amplified cDNA undiluted at -20°C until required for downstream applications.**

The amplified cDNA should be treated as genomic DNA (i.e., minimize the number of freeze–thaw cycles). Storage of nucleic acids at low concentration over a long period of time may result in acid hydrolysis. We therefore recommend storage of the amplified cDNA at a concentration of at least 100 ng/μl.

**10. If performing real-time PCR, dilute the amplified cDNA with water or TE buffer (see Table 6), and use 2 μl of the diluted cDNA for real-time PCR.**

It is not necessary to perform reverse transcription prior to real-time PCR.

**Table 6. Dilution of amplified cDNA**

Reaction conditions	Dilution
Standard reaction (2-h amplification)	1/50 (e.g., add 2 μl cDNA to 100 μl water)
High-yield reaction (8-h amplification)	1/250 (e.g., add 2 μl cDNA to 500 μl water)

## Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). 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 sample and assay technologies (for contact information, see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

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### Comments and suggestions

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#### Little or no amplified cDNA

- |   |   |
|---|---|
| a) Incorrect reaction temperature                                   | Be sure to carry out the RT, ligation, and amplification reactions at the temperatures specified in the protocol. If necessary, check the temperature of your thermal cycler, heating block, or water bath. |
| b) Pipetting error or missing reaction component                    | Check your pipets. Be sure to mix all reagents well after thawing and to store them on ice.   |
| c) Incorrect incubation time  | For the RT, ligation, and amplification reactions, be sure to use the incubation times specified in the protocol.   |
| d) RT mix, ligation mix, and amplification mix not freshly prepared | Always prepare the RT mix, ligation mix, and amplification mix fresh before use. Storage of these mixes prior to use may affect whole transcriptome amplification.  |
| e) Amplified cDNA denatured prior to PicoGreen quantification       | PicoGreen reagent binds double-stranded DNA only. Before incubating the amplified cDNA at 95°C (step 8 of the protocol), quantify the cDNA or remove an aliquot for later quantification.                   |

#### DNA yields of approximately 10 µg or 40 µg in negative (no-template) controls, but no positive result in downstream assay (e.g., PCR)

DNA is generated during REPLI-g reaction by random extension of primer-dimers

High-molecular-weight product can be generated by random extension of primer-dimers. This DNA will not affect the quality of actual samples or specific downstream genetic assays.

## Comments and suggestions

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### **DNA yields of approximately 10 µg or 40 µg in negative (no-template) controls and positive result in downstream assay (e.g., PCR)**

DNA is generated during REPLI-g reaction by contaminating DNA templates

Decontaminate all laboratory equipment, and take all necessary precautions to avoid contamination of reagents and samples with extraneous DNA.

If possible, work in a laminar-flow hood. Use sterile equipment and barrier pipet tips only, and keep amplification chemistry and DNA templates in separate storage locations.

### **Little or no transcript detected in real-time PCR analysis, but DNA yield is approximately 10 µg or 40 µg**

a) RNA template degraded

Use nondegraded RNA or larger amounts of RNA, if possible. Only RNA transcripts longer than 500 nucleotides can be amplified.

b) Low-abundance transcript analyzed

Use larger amounts of RNA (see Appendix A, page 19). Up to 100 ng RNA template can be used as starting material.

c) Small transcripts analyzed

Small transcripts such as tRNA or miRNAs cannot be amplified by the QuantiTect Whole Transcriptome Kit. Only RNA transcripts longer than 500 nucleotides can be amplified.

d) Full-length transcripts analyzed

Due to random priming, amplification of full-length cDNA is not possible. We recommend analyzing smaller sequences from your target cDNA.

e) RNA template contains carrier RNA

Use RNA template that was purified without using carrier RNA. If carrier RNA needs to be used, purify RNA with the RNeasy Plus Micro Kit. For ordering information, see page 24.

f) Incorrect amount of RNA template

Do not use less than 100 pg RNA template. We recommend using at least 10 ng RNA template.

### Downstream application results not optimum

- |    |   |  |
|----|---|--|
| a) | Sensitive downstream applications may require DNA cleanup after REPL-g reaction | Contact QIAGEN Technical Services for DNA cleanup recommendations suitable for your application.   |
| b) | Microarray analysis carried out   | cDNA prepared using the QuantiTect Whole Transcriptome Kit is not intended for use with Affymetrix® GeneChip® arrays.  |
| c) | Little or no cDNA amplified   | Always prepare the RT mix, ligation mix, and amplification mix fresh before use. Storage of these mixes prior to use may affect whole transcriptome amplification. |

# Appendix A: Limitations of RNA Amplification

When carrying out transcriptome amplification using the QuantiTect Whole Transcriptome Kit, it is important to consider both the amount of starting material (i.e., the number of cells or the amount of RNA) and the copy number of the transcripts of interest.

Table 7 and Figure 2 show the relationship between the amount of starting material and transcript representation (note that this is only a guide: the number of transcripts per given amount of starting material can vary). In starting material where the copy number of a transcript is 10 or less (highlighted in bold in Table 7), stochastic problems will occur (i.e., the unequal distribution of a very low number of transcripts in a highly dilute solution). This may result in underrepresentation of the low-copy transcript at the start of transcriptome amplification. Special consideration should be given to mosaic transcripts, which are derived from genes that are expressed only in a subset of cells in tissues. Since these transcripts are not present in every cell, they will not be accurately represented in low amounts of starting material (i.e., 1–10<sup>2</sup> cells).

Reliable amplification depends on the copy number of the transcripts. If the recommended amount of starting material (i.e., 10 ng intact RNA) is amplified, all transcripts will be accurately represented after amplification. 10 ng of RNA corresponds to about 500 cells, and even low-copy transcripts are well represented in this RNA amount. Using lower amounts of RNA or a very limited number of cells means that the starting material could have a partial representation or an absence of low-copy transcripts (Figure 3 provides an example).

**Table 7. Transcript representation in different cell amounts**

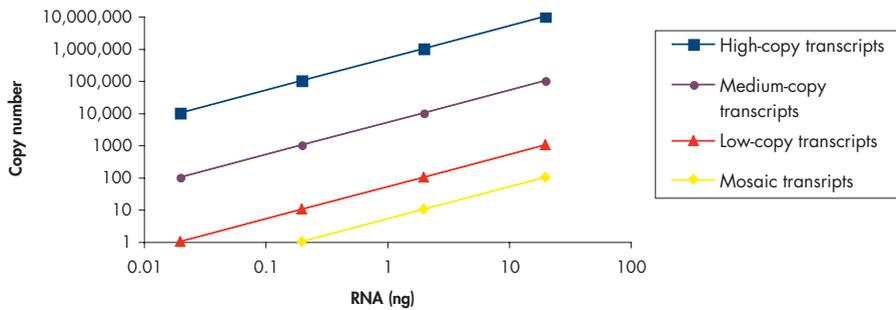
	10 <sup>3</sup> cells*	10 <sup>2</sup> cells <sup>†</sup>	10 cells <sup>‡</sup>	1 cell <sup>§</sup>
Amount of RNA (ng)	20	2	0.2	0.02
No. of high-copy transcripts	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>
No. of medium-copy transcripts	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>
No. of low-copy transcripts	10 <sup>3</sup>	10 <sup>2</sup>	<b>10</b>	<b>1</b>
No. of mosaic transcripts	10 <sup>2</sup>	<b>10</b>	<b>1</b>	<b>0</b>

\* Complete representation of all transcripts.

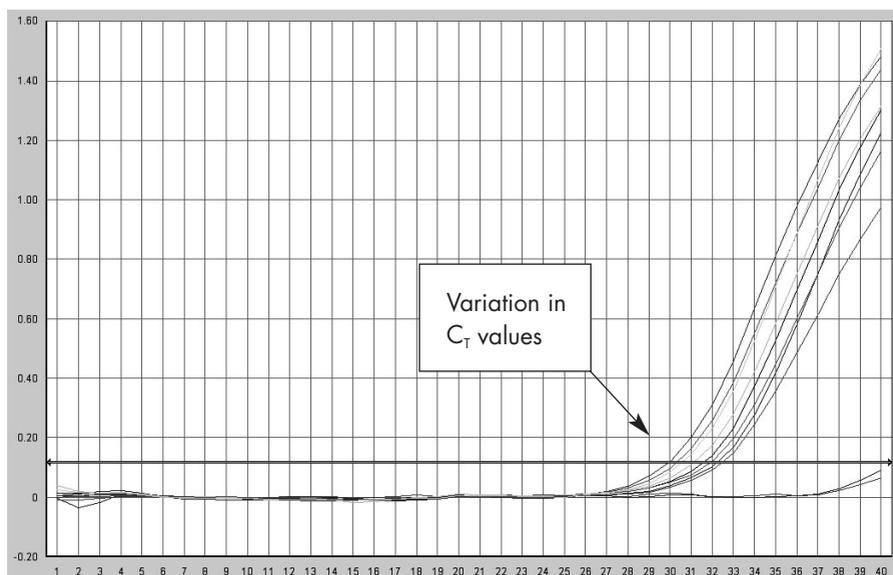
<sup>†</sup> Stochastic problems for mosaic transcripts.

<sup>‡</sup> Stochastic problems for low-copy and mosaic transcripts.

<sup>§</sup> Stochastic problems for low-copy transcripts and loss of mosaic transcripts.



**Figure 2. Transcript representation in different RNA amounts.** Relationship between amount of starting RNA and copy number of high-copy, medium-copy, low-copy, and mosaic transcripts.



**Figure 3. Stochastic problems when analyzing a low-copy transcript.** Eight replicate RNA samples (1 ng each) were amplified using the QuantiTect Whole Transcriptome Kit. Real-time PCR analysis of NFκB transcript was then performed using 10 ng amplified cDNA. The resulting  $C_T$  values were in the range of 30–32.5. This significant variation occurred because the stochastic variation in low-copy NFκB transcript in the replicate RNA samples is amplified, resulting in widely differing amounts of NFκB cDNA.

## Appendix B: Determining cDNA Concentration and Yield

Using the QuantiTect Whole Transcriptome Kit, a 50 µl reaction typically yields up to 10 µg (2 h incubation) or 40 µg (8 h incubation) of cDNA regardless of the amount of template RNA, allowing direct use of the amplified cDNA in most downstream applications. However, if accurate quantification of the cDNA is required, it is important to use a DNA quantification method that is specific for double-stranded DNA, since the cDNA sample contains unused reaction primers. PicoGreen reagent displays enhanced binding to double-stranded DNA and may be used, in conjunction with a fluorometer, to quantify the amount of amplified cDNA. A protocol for the quantification of amplified cDNA is provided in Appendix C.

## Appendix C: PicoGreen Quantification of Amplified cDNA

This protocol is designed for quantification of double-stranded amplified cDNA using PicoGreen reagent.

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

### Equipment and reagents to be supplied by user

- Quanti-iT™ PicoGreen dsDNA reagent (Invitrogen, cat. no. P7581)
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)
- Human genomic DNA (e.g., Promega, cat. no. G3041)
- 2 ml microcentrifuge tube
- 96-well plates (suitable for use in a fluorescence microplate reader)
- Fluorescence microplate reader (e.g., TECAN® Ultra)

### Procedure

#### Setup and reading of microplate

1. **In a 2 ml microcentrifuge tube, make a 1/150 dilution of PicoGreen stock solution in TE buffer. 20 µl is required for each quantification reaction. Cover the microcentrifuge tube in aluminum foil or place it in the dark to avoid photodegradation of the PicoGreen reagent.**

For example, to prepare enough PicoGreen working solution for 100 samples, add 13.3 µl PicoGreen to 1986.7 µl TE buffer.

**Important:** Prepare the PicoGreen/TE solution in a plastic container as the PicoGreen reagent may adsorb to glass surfaces.

**2. Prepare a 16 µg/ml stock solution of genomic DNA in TE buffer.**

DNA standards will be prepared from this stock solution.

**3. Make 200 µl of 1.6, 0.8, 0.4, 0.2, and 0.1 µg/ml DNA standards by further diluting the 16 µg/ml genomic DNA with TE buffer.**

**4. Transfer 20 µl of each DNA standard in duplicate into a 96-well plate labeled A (see Figure 4).**

**Note:** The 96-well plate must be suitable for use in a fluorescent microplate reader.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H			1.6	0.8	0.4	0.2	0.1	1.6	0.8	0.4	0.2	0.1

**Figure 4. 96-well plate.** Gray squares: genomic DNA standard (µg/ml).

**5. Place 2 µl of each amplified cDNA sample for quantification into a new 96-well plate and add 98 µl TE buffer to make a 1/50 dilution. Store the remaining amplified cDNA at -20°C.**

**6. Place 2 µl diluted cDNA (from step 5) into an unused well of 96-well plate A and add 18 µl TE to make a 1/500 dilution.**

The 1/50 dilutions can be stored at -20°C and used for future downstream sample analysis.

7. Add 20  $\mu$ l PicoGreen working solution (from step 1) to each sample (amplified cDNA and DNA standards) in 96-well plate A. Gently shake the plate on the bench top to mix the samples and reagent.
8. Centrifuge the 96-well plate briefly to collect residual liquid from the walls of the wells.
9. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescence filters (excitation approximately 480 nm; emission approximately 520 nm).

To ensure that the sample readings remain in the detection range of the microplate reader, adjust the instrument's gain so that the sample with the highest DNA concentration yields fluorescence intensity near the fluorimeter's maximum.

### Calculation of DNA concentration and yield

10. Generate a standard curve by plotting the concentration of DNA standards ( $\mu$ g/ml) (X-axis) against the fluorescence reading generated by the microplate reader (Y-axis). Plot an average of the fluorescence recorded for each DNA standard of the same concentration.
11. Use the standard curve to determine the concentration ( $\mu$ g/ml) of the diluted amplified cDNA sample. This is achieved by plotting the fluorescence reading of the sample against the standard curve and reading the DNA concentration on the X-axis.

**Note:** The calculation of DNA concentration depends on the standard curve and the determination of the slope. For accurate results, the standard curve should be a straight line. Any deviation from this may cause inaccuracies in the measurement of amplified cDNA concentrations.

12. Multiply the value determined in step 11 by 500 to show the concentration of undiluted sample cDNA (as the sample cDNA measured by PicoGreen fluorescence had been diluted 1 in 500).
13. To determine the total amount of cDNA in your sample, multiply the concentration of undiluted sample cDNA ( $\mu$ g/ml) (step 12) by the reaction volume in milliliters (i.e., for a 50  $\mu$ l reaction, multiply by 0.05).

## Ordering Information

Product	Contents	Cat. no.
QuantiTect Whole Transcriptome Kit (25)	For 25 x 50 µl reactions: T-Script Enzyme and Buffer, Ligation Enzymes, Reagent, and Buffer, and REPLI-g DNA Polymerase and Buffer	207043
QuantiTect Whole Transcriptome Kit (100)	For 100 x 50 µl reactions: T-Script Enzyme and Buffer, Ligation Enzymes, Reagent, and Buffer, and REPLI-g DNA Polymerase and Buffer	207045
<b>Accessories</b>		
<b>RNeasy Plus Micro Kit — for purification of concentrated total RNA from small amounts of tissue or small numbers of cells</b>		
RNeasy Plus Micro Kit (50)	50 RNeasy MinElute® Spin Columns, 50 gDNA Eliminator Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free DNase I, Carrier RNA, RNase-Free Reagents and Buffers	74034
<b>PAXgene Blood RNA Kit — for isolation and purification of intracellular RNA from whole blood stabilized in PAXgene Blood RNA Tubes</b>		
PAXgene Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes*	762164† 762174‡
<b>QIAamp RNA Blood Mini Kit — for purification of cellular RNA from fresh whole blood</b>		
QIAamp RNA Blood Mini Kit (50)	For 50 RNA preps: 50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304

\* Available from BD ([www.bd.com](http://www.bd.com)) and BD authorized distributors, cat. no. 762165.

† Canada and USA.

‡ All other countries.

# Ordering Information

Product	Contents	Cat. no.
<b>QuantiFast® SYBR® Green PCR Kit — for fast, quantitative, real-time PCR and two-step RT-PCR using SYBR Green I</b>		
QuantiFast SYBR Green PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (with ROX dye), 2 x 2 ml RNase-Free Water	204054
<b>QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at <a href="http://www.qiagen.com/GeneGlobe">www.qiagen.com/GeneGlobe</a>)</b>		
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions or 400 x 25 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies
<b>QuantiFast Probe PCR Kits — for fast, quantitative, real-time PCR and two-step RT-PCR using sequence-specific probes</b>		
For all instruments from Applied Biosystems except the Applied Biosystems® 7500:		
QuantiFast Probe PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (with ROX™ dye), 2 x 2 ml RNase-Free Water	204254
For the Applied Biosystems 7500 and instruments from Bio-Rad, Cepheid, Eppendorf, Roche, and Agilent:		
QuantiFast Probe PCR +ROX Vial Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 210 µl ROX Dye Solution, 2 x 2 ml RNase-Free Water	204354
<b>QuantiFast Multiplex PCR Kits — for fast, quantitative, multiplex, real-time PCR and two-step RT-PCR using sequence-specific probes</b>		
For instruments from Applied Biosystems except the Applied Biosystems 7500:		
QuantiFast Multiplex PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (with ROX dye), 2 x 2 ml RNase-Free Water	204654

\* Other kit sizes available; please inquire. For the Rotor-Gene® Q and other Rotor-Gene cyclers, Rotor-Gene Kits should be used instead; for details, visit [www.qiagen.com/goto/Rotor-GeneKits](http://www.qiagen.com/goto/Rotor-GeneKits).

## Ordering Information

Product	Contents	Cat. no.
For the Applied Biosystems 7500 and instruments from Bio-Rad, Cepheid, Eppendorf, Roche, and Agilent:		
QuantiFast Multiplex PCR +R Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (without ROX dye), 210 µl ROX Dye Solution, 2 x 2 ml RNase-Free Water	204754
<b>Related products</b>		
<b>REPLI-g Mini and Midi Kits — for highly uniform whole genome amplification from small or precious samples</b>		
REPLI-g Mini Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions (typical yield 10 µg per reaction)	150023
REPLI-g Midi Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions (typical yield 40 µg per reaction)	150043
<b>REPLI-g UltraFast Mini Kit — for the fastest reliable whole genome amplification</b>		
REPLI-g UltraFast Mini Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 20 µl ultrafast whole genome amplification reactions	150033

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