October 2012

QIAGEN® OneStep RT-PCR Handbook

For fast and highly sensitive one-step RT-PCR

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

QIAGEN OneStep RT-PCR Kit Catalog no. Number of reactions	(25) 210210 25	(100) 210212 100	(1000) 210215 1000
QIAGEN OneStep RT-PCR Enzyme Mix (contains the QIAGEN products Omniscript Reverse Transcriptase, Sensiscript Reverse Transcriptase, and HotStarTaq® DNA Polymerase)	50 µl	200 µl	2 x 1.0 ml
QIAGEN OneStep RT-PCR Buffer,* 5x	350 µl	1.15 ml	11.5 ml
Q-Solution®, 5x	2 ml	2.0 ml	10.0 ml
dNTP Mix, 10 mM each	50 µl	200 µl	2 x 1.0 ml
RNase-free water	1.9 ml	2 x 1.9 ml	2 x 20.0 ml
Quick-Start Protocol	1	1	1

* Contains 12.5 mM $MgCl_2$

Shipping and Storage Conditions

The QIAGEN OneStep RT-PCR Kit is shipped on dry ice and should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the product can be kept at least until the expiration date (see the inside of the kit lid) without showing any reduction in performance.

Intended Use

The QIAGEN OneStep RT-PCR 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.

Safety Information

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

24-hour emergency information

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

QIAGEN OneStep RT-PCR Enzyme Mix:	(see quality-control label inside kit lid for lot-specific values)
RT-PCR specificity and reproducibility assay:	RT-PCR specificity and reproducibility are tested in parallel 50-µl reactions containing 100 pg or 10 pg of human total RNA and 0.6 µM primers (specific for β-actin). After 35 cycles, a 0.3 kb product is detectable as a single, specific band.
Exonuclease activity assay:	Reactions are prepared using QIAGEN OneStep RT-PCR Enzyme Mix components and linear DNA. Exonuclease activity is indicated under "Exo.".
Endonuclease activity assay:	Reactions are prepared using QIAGEN OneStep RT-PCR Enzyme Mix components and DNA. Endonuclease activity is indicated under "Endo.".
RNase activity assay:	Reactions are prepared using QIAGEN OneStep RT-PCR Enzyme Mix components and RNA. RNase activity is indicated under "RNase".
Protease activity assay:	QIAGEN OneStep Enzyme Mix components are incubated in storage buffer. Protease activity is indicated under "Protease".
Buffers and reagents:	
QIAGEN OneStep RT-PCR Buffer, 5x:	Conductivity, performance in RT-PCR, and pH are tested.
Q-Solution, 5x:	Conductivity, pH, total aerobic microbial count, and performance in PCR are tested.
dNTP Mix:	Concentration and purity are verified by UV spectroscopy and HPLC. Performance in PCR is tested.
RNase-free water:	Conductivity, pH, and RNase activities are tested.

Product Specifications

Enzymes:

The QIAGEN OneStep RT-PCR Enzyme Mix contains a specially formulated enzyme blend for both reverse transcription and PCR. The unique combination of Omniscript® and Sensiscript® Reverse Transcriptases, with their high affinity for RNA templates, ensures highly efficient and sensitive transcription of RNA amounts from as little as 1 pg up to 2 µg.

Omniscript and Sensiscript Reverse Transcriptases are unique enzymes, and are different from the reverse transcriptases of Moloney murine leukemia virus (MMLV) or avian myeloblastosis virus (AMV). Omniscript and Sensiscript Reverse Transcriptases are recombinant heterodimeric enzymes expressed in *E. coli*.

HotStarTaq DNA Polymerase is a chemically modified form of a recombinant 94-kDa DNA polymerase (deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7), originally isolated from *Thermus aquaticus*, expressed in *E. coli*.

Buffers and reagents:

Storage buffer containing QIAGEN OneStep RT-PCR Enzyme Mix:	20 mM Tris·Cl, 100 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.5% (v/v) Nonidet P-40, 0.5% (v/v) Tween® 20, 50% glycerol (v/v), stabilizer; pH 9.0 (20°C)
QIAGEN OneStep RT-PCR Buffer:	5x concentrated. Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, 12.5 mM $MgCl_2$, DTT; pH 8.7 (20°C)
Q-Solution:	5x concentrated
dNTP Mix:	10 mM each of dATP, dCTP, dGTP, and dTTP; PCR-grade
RNase-free water:	Ultrapure quality, PCR-grade

Introduction

The QIAGEN OneStep RT-PCR Kit provides a convenient format for highly sensitive and specific RT-PCR using any RNA. The kit contains optimized components that allow both reverse transcription and PCR amplification to take place in what is commonly referred to as a "one-step" reaction.

QIAGEN OneStep RT-PCR Enzyme Mix

The QIAGEN OneStep RT-PCR Enzyme Mix contains a specially formulated enzyme blend specific for both reverse transcription and PCR amplification (see Table 1).

- **Omniscript and Sensiscript Reverse Transcriptases** are included in the QIAGEN OneStep RT-PCR Enzyme Mix and provide highly efficient and specific reverse transcription. Both reverse transcriptases exhibit a higher affinity for RNA, facilitating transcription through secondary structures that inhibit other reverse transcriptases. Omniscript Reverse Transcriptase is specially designed for reverse transcription of RNA amounts greater than 50 ng, and Sensiscript Reverse Transcriptase is optimized for use with very small amounts of RNA (<50 ng). This special enzyme combination in the QIAGEN OneStep RT-PCR Enzyme Mix provides highly efficient and sensitive reverse transcription of any RNA quantity from 1 pg to 2 µg.
- **HotStarTaq DNA Polymerase** included in the QIAGEN OneStep RT-PCR Enzyme Mix provides hot-start PCR for highly specific amplification. During reverse transcription, chemically modified HotStarTaq DNA Polymerase is completely inactive and does not interfere with the reverse-transcriptase reaction. After reverse transcription by Omniscript and Sensiscript Reverse Transcriptases, reactions are heated to 95°C for 15 min to activate HotStarTaq DNA Polymerase and to simultaneously inactivate the reverse transcriptases. This hot-start procedure using HotStarTaq DNA Polymerase eliminates extension from nonspecifically annealed primers and primer–dimers in the first cycle ensuring highly specific and reproducible PCR.

Although all of the enzymes are present in the reaction mix, the use of HotStarTaq DNA Polymerase ensures the temporal separation of reverse transcription and PCR allowing both processes to be performed sequentially in a single tube. Only one reaction mix needs to be set up: no additional reagents are added after the reaction starts.

QIAGEN OneStep RT-PCR Buffer

QIAGEN OneStep RT-PCR Buffer is designed to enable both efficient reverse transcription and specific amplification.

- The unique buffer composition allows reverse transcription to be performed at high temperatures (50°C). This high reaction temperature improves the efficiency of the reverse-transcriptase reaction by disrupting secondary structures and is particularly important for one-step RT-PCR performed with limiting template RNA amounts.
- It has been reported that one-step RT-PCR may exhibit reduced PCR efficiency compared to two-step RT-PCR. The combination of QIAGEN enzymes and the unique formulation of the QIAGEN OneStep RT-PCR Buffer ensures high PCR efficiency in one-step RT-PCR.
- The buffer contains the same balanced combination of KCl and $(NH_4)_2SO_4$ included in QIAGEN PCR Buffer. This formulation enables specific primer annealing over a wider range of annealing temperatures and Mg^{2+} concentrations than conventional PCR buffers.[†] The need for optimization of RT-PCR by varying the annealing temperature or the Mg^{2+} concentration is therefore minimized.

Q-Solution

The QIAGEN OneStep RT-PCR Kit is provided with Q-Solution, an innovative additive that facilitates amplification of difficult templates by modifying the melting behavior of nucleic acids.

- Q-Solution often enables or improves suboptimal RT-PCR caused by RNA and DNA templates that have a high degree of secondary structure or that are GC-rich.
- Unlike other commonly used additives such as DMSO, Q-Solution is used at just one working concentration. For further information, please read the protocol "QIAGEN OneStep RT-PCR Kit and Q-Solution", page 14.

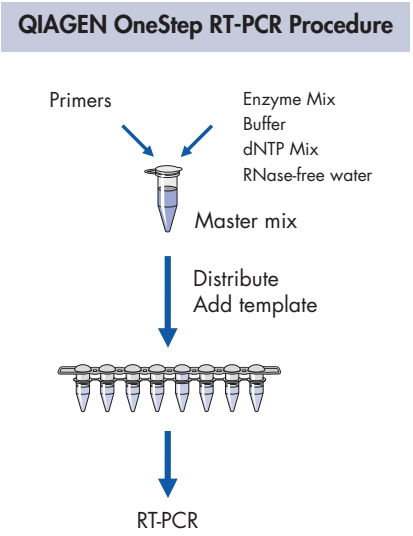
Table 1. All you need for reliable OneStep RT-PCR results

OneStep RT-PCR Kit contents	Features
HotStarTaq® DNA Polymerase	Highly specific products
Omniscript® and Sensiscript® RT	Wide range of RNA amounts (1 pg – 2 µg) High sensitivity
OneStep RT-PCR Buffer	Minimal optimization needed No inhibition of PCR by reverse transcriptases
Q-Solution	Facilitates amplification of GC-rich templates

[†] For further information see our comprehensive brochure *"Critical success factors and new technologies for PCR and RT-PCR"*. To obtain a copy, visit the QIAGEN web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributor (see back cover or visit www.qiagen.com).

QIAGEN OneStep RT-PCR Procedure

The QIAGEN OneStep RT-PCR Kit allows fast and easy RT-PCR setup. Whatever your application — virus detection, molecular diagnostics research, or gene expression — just mix all components together in one tube and start your thermal cycler program (see flowchart).



Equipment and Reagents to be Supplied by user

Primers: The QIAGEN OneStep RT-PCR Kit is designed to be used with **gene-specific primers**. The use of random oligomers or oligo-dT primers is not recommended.

RNase inhibitor*: (optional) RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, and C, as well as human placental RNases. It helps to minimize the risk of RNA degradation during experimental setup.

* The use of RNAse inhibitor is optional because the buffer composition has an inhibitory effect on RNAses.

Protocol: QIAGEN OneStep RT-PCR Kit

This protocol serves as a guideline for one-step RT-PCR. Reverse transcription and PCR are carried out sequentially in the same tube. All components required for both reactions are added during setup, and there is no need to add additional components once the reaction has been started. The protocol has been optimized for 1 pg – 2 µg of total RNA. Optimal reaction conditions, such as incubation times and temperatures during PCR amplification, will vary and need to be determined individually.

Important notes before starting

- HotStarTaq DNA Polymerase, contained in the QIAGEN OneStep RT-PCR Enzyme Mix, requires **initial activation by incubation at 95°C for 15 min** before amplification can take place (see step 6 of this protocol). This incubation also inactivates the reverse transcriptases. Do not heat activate the HotStarTaq DNA Polymerase until the reverse-transcriptase reaction is finished.
- The QIAGEN OneStep RT-PCR Kit is designed to be used with **gene-specific primers** at a final concentration of **0.6 µM**. The use of random oligomers or oligo-dT primers is not recommended since it will result in the amplification of nonspecific products.
- Set up all reactions on ice.
- Make sure the thermal cycler is preheated to 50°C before placing samples in it.
- The 5x QIAGEN OneStep RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which will produce satisfactory results in most cases.
- An RNase-free environment should be maintained during RNA isolation and reaction setup.
- Set up the reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Thaw template RNA, primer solutions, dNTP Mix, 5x QIAGEN OneStep RT-PCR Buffer, and RNase-free water, and place them on ice.**

   It is important to mix the solutions completely before use to avoid localized differences in salt concentration.

2. **Prepare a master mix according to Table 2.**

   The master mix typically contains all the components required for RT-PCR except the template RNA. Prepare a volume of master mix 10% greater than that required for the total number of reactions to be performed. A negative control (without template RNA) should be included in every experiment (see Appendix J, page 33).

Table 2. Reaction components for one-step RT-PCR

Component	Volume/reaction*	Final concentration
Master mix		
RNase-free water (provided)	Variable	–
5x QIAGEN OneStep RT-PCR Buffer†	10.0 µl	1x
dNTP Mix (containing 10 mM of each dNTP)	2.0 µl	400 µM of each dNTP
Primer A	Variable	**0.6 µM‡**
Primer B	Variable	**0.6 µM‡**
QIAGEN OneStep RT-PCR Enzyme Mix	2.0 µl	–
RNase inhibitor (optional)§	Variable	5–10 units/reaction
Template RNA		
Template RNA, added at step 4	Variable	1 pg – 2 µg/reaction
Total volume	**50.0 µl**	**–**

* When reducing reaction volumes, adjust the volumes accordingly. More information is provided in supplementary protocols, which are available from www.qiagen.com/literature/default.aspx.

† Contains 12.5 mM $MgCl_2$

‡ A final primer concentration of 0.6 µM is optimal for most primer–template systems. However, in some cases using other primer concentrations (i.e., 0.5–1.0 µM) may improve amplification performance.

§ The use of RNase inhibitor is optional; because the buffer composition has an inhibitory effect on RNAses.

3. **Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.**

   Mix gently, for example, by pipetting the master mix up and down a few times.

4. **Add template RNA (≤ 2 µg/reaction) to the individual PCR tubes.**

   The QIAGEN OneStep RT-PCR Kit can be used with total RNA, messenger RNA, or viral RNA.

5. **Program the thermal cycler according to the program outlined in Table 2.**

   Table 2 describes a typical thermal cycler program. The program includes steps for both reverse transcription and PCR. The PCR amplification segment must start with an initial heating step at 95°C for 15 min to activate HotStarTaq DNA Polymerase. For maximum yield and specificity, temperatures and cycling times can be further optimized for each new target and primer pair. However, the protocol gives satisfactory results in most cases.

6. **Start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 50°C. Then place the PCR tubes in the thermal cycler.**

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer-term storage.

Table 3. Thermal cycler conditions

			Additional comments
Reverse transcription:	30 min	50°C	A reverse-transcription reaction temperature of 50°C is recommended. However, if satisfactory results are not obtained using 50°C, the reaction temperature may be increased up to 60°C.
Initial PCR activation step:	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step. Omniscript and Sensiscript Reverse Transcriptases are inactivated and the cDNA template is denatured.
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers.
Extension:	1 min	72°C	For RT-PCR products of 1–2 kb, increase the extension time by 30–60 s. For RT-PCR products over 2 kb, see appendix, page 30.
Number of cycles:	25–40		The cycle number is dependent on the amount of template RNA and the abundance of the target transcript. See Appendix C, page 29.
Final extension:	10 min	72°C	

Protocol: QIAGEN OneStep RT-PCR Kit and Q-Solution

This protocol is designed for using Q-Solution in one-step RT-PCR. Q-Solution changes the melting behavior of nucleic acids and can be used for RT-PCR systems that do not work well under standard conditions. When using Q-Solution the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution. This recommendation should also be followed if another RT-PCR additive (such as DMSO) was previously used for a particular primer–template system.

When using Q-Solution, the following effects may be observed depending on the individual RT-PCR assay:

Case A: Q-Solution enables RT-PCR that previously failed.

Case B: Q-Solution increases RT-PCR specificity in certain primer–template systems.

Case C: Q-Solution has no effect on RT-PCR performance.

Case D: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction. In this case, addition of Q-Solution disturbs the previously optimal primer–template annealing. Therefore, when using Q-Solution for the first time for a particular primer–template system, always perform reactions in parallel with and without Q-Solution.

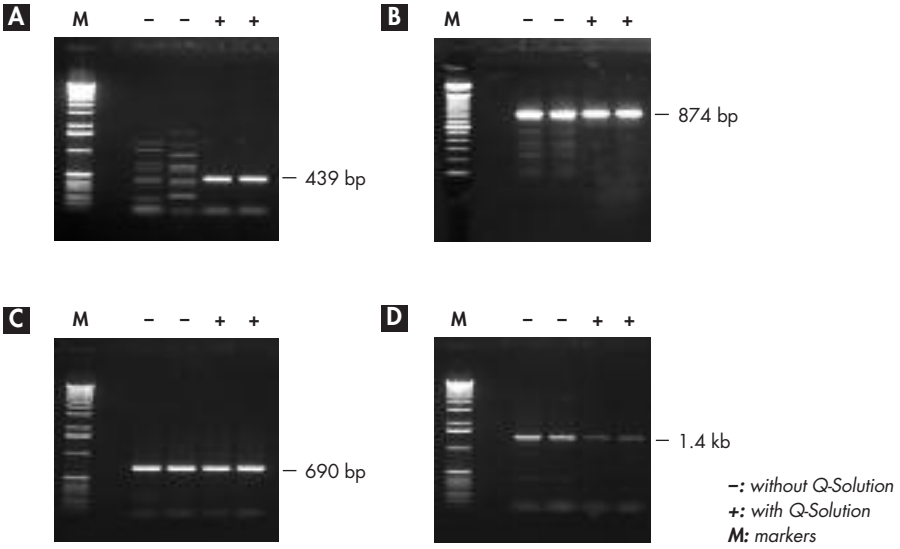


Figure 1. RT-PCR effects using Q-Solution.

Important notes before starting

- HotStarTaq DNA Polymerase, contained in the QIAGEN OneStep RT-PCR Enzyme Mix, requires **initial activation by incubation at 95°C for 15 min** before amplification can take place (see step 6 of this protocol). This incubation also inactivates the reverse transcriptases. Do not heat activate the HotStarTaq DNA Polymerase until the reverse-transcriptase reaction is finished.
- Q-Solution modifies the melting behavior of nucleic acids and can be used for primer–template systems that do not perform well using standard conditions. When using Q-Solution the first time for a particular primer–template system, always perform parallel reactions with and without Q-Solution.
- The QIAGEN OneStep RT-PCR Kit is designed for use with **gene-specific primers** at a final concentration of **0.6 µM**. The use of random oligomers or oligo-dT primers is not recommended since it will result in the amplification of nonspecific products.
- Set up all reactions on ice.
- Make sure the thermal cycler is preheated to 50°C before placing samples in it.
- The 5x QIAGEN OneStep RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which will produce satisfactory results in most cases.
- An RNase-free environment should be maintained during RNA isolation and reaction setup.
- Set up reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Thaw template RNA, primer solutions, dNTP Mix, 5x QIAGEN OneStep RT-PCR Buffer, Q-Solution, and RNase-free water, and place them on ice.**

   It is important to mix the solutions completely before use to avoid localized differences in salt concentration.

2. **Prepare a master mix according to Table 4.**

   The master mix typically contains all the components required for RT-PCR except the template RNA. Prepare a volume of master mix 10% greater than that required for the total number of reactions to be performed. A negative control (without template RNA) should be included in every experiment (see Appendix J, page 33).

   When using Q-Solution for the first time for a particular primer–template system, always perform parallel reactions with and without Q-Solution.

3. **Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.**

   Mix gently, for example, by pipetting the master mix up and down a few times.

Table 4. Reaction components for one-step RT-PCR using Q-Solution

Component	Volume/reaction*	Final concentration
Master mix		
RNase-free water (provided)	Variable	–
5x QIAGEN OneStep RT-PCR Buffer†	10.0 µl	1x
dNTP Mix (containing 10 mM of each dNTP)	2.0 µl	400 µM of each dNTP
5x Q-Solution	10.0 µl	1x
Primer A	Variable	**0.6 µM‡**
Primer B	Variable	**0.6 µM‡**
QIAGEN OneStep RT-PCR Enzyme Mix	2.0 µl	–
RNase inhibitor (optional)§	Variable	5–10 units/reaction
Template RNA		
Template RNA, added at step 4	Variable	1 pg – 2 µg/reaction
Total volume	**50.0 µl**	**–**

* When reducing reaction volumes, adjust the volumes accordingly. More information is provided in supplementary protocols, which are available from www.qiagen.com/literature/default.aspx.

† Contains 12.5 mM $MgCl_2$

‡ A final primer concentration of 0.6 µM is optimal for most primer–template systems. However, in some cases using other primer concentrations (i.e., 0.5–1.0 µM) may improve amplification performance.

§ The use of RNase inhibitor is optional because the buffer composition has an inhibitory effect on RNAses.

4. **Add template RNA (≤ 2 µg/reaction) to the individual PCR tubes.**

   The QIAGEN OneStep RT-PCR Kit can be used with total RNA, messenger RNA, or viral RNA.

5. **Program the thermal cycler according to the program outlined in Table 5, page 17.**

   Table 4 describes a typical thermal cycler program. The program includes steps for both reverse transcription and PCR. The PCR amplification segment must start with an initial heating step at 95°C for 15 min to activate HotStarTaq DNA Polymerase. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target and primer pair.

6. **Start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 50°C. Then place the PCR tubes in the thermal cycler.**

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer-term storage.

Table 5. Thermal cycler conditions

			Additional comments
Reverse transcription:	30 min	50°C	A reverse-transcription reaction temperature of 50°C is recommended. However, if satisfactory results are not obtained using 50°C, the reaction temperature may be increased up to 60°C.
Initial PCR activation step:	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step. Omniscript and Sensiscript Reverse Transcriptases are inactivated and the cDNA template is denatured.
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers.
Extension:	1 min	72°C	For RT-PCR products of 1–2 kb, increase the extension time by 30–60 s. For RT-PCR products over 2 kb, see Appendix B, page 24.
Number of cycles:	25–40		The cycle number is dependent on the amount of template RNA and the abundance of the target transcript. See Appendix C, page 29.
Final extension:	10 min	72°C	

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. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Little or no product		
a)	Pipetting error or missing reagent	Check the concentrations and storage conditions of reagents, including primers and dNTP Mix. Repeat the RT-PCR.
b)	HotStarTaq DNA Polymerase not activated	Ensure that the cycling program included the HotStarTaq DNA Polymerase activation step (15 min at 95°C) as described in step 6 of the protocols (pages 11 and 14).
c)	HotStarTaq DNA Polymerase activated too early	Check the cycling program. Ensure that the reverse-transcription reaction is complete (30 min at 50°C) before activating the HotStarTaq DNA Polymerase (15 min at 95°C).
d)	Reverse-transcription reaction temperature incorrect	A reverse-transcription reaction temperature of 50°C is recommended. However, if desired results are not obtained using 50°C, reaction temperatures of 45–60°C may be used.
e)	Primer concentration not optimal or primers degraded	A primer concentration of 0.6 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the RT-PCR with different primer concentrations from 0.5–1.0 µM in 0.1 µM increments. In particular, when performing highly sensitive RT-PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel*.

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

		Comments and suggestions
f)	RT-PCR conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Q-Solution. Follow the protocol on page 14.
g)	Incorrect nucleotide concentration	Use 0.4 mM of each dNTP. Different nucleotide concentrations can reduce the amount of RT-PCR product.
h)	Problems with starting template	Check the concentration, integrity, purity, and storage conditions of the starting RNA template (see Appendix A, page 23). If necessary, make new serial dilutions of template RNA from stock solutions. Repeat the RT-PCR using the new dilutions.
i)	Enzyme concentration too low	Ensure that 2 µl of QIAGEN OneStep RT-PCR Enzyme Mix per reaction was used.
j)	Insufficient number of cycles	Increase the number of cycles in increments of 5 cycles (see Appendix C, page 29).
k)	Incorrect PCR annealing temperature or time	Decrease annealing temperature in 2°C steps. Annealing time should be between 30 and 60 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix E, page 30).
l)	Incorrect denaturation temperature or time	Denaturation should be at 94°C for 30 to 60 seconds. Ensure that the cycling program included the HotStarTaq DNA Polymerase activation step (15 min at 95°C) as described in step 6 of the protocols (pages 11 and 14).
m)	Insufficient starting template	Increase the template amount. If this is not possible, perform a second round of PCR using a nested-PCR approach (see Appendix D, page 30).
n)	Primer design not optimal	Review primer design (see Appendix B, page 24). Only use gene-specific primers. Do not use random oligomers or oligo-dT primers.
o)	RT-PCR of long fragments	Increase the concentration of template RNA. When amplifying products longer than 2 kb, use the modified reaction conditions described in Appendix F, page 30.

		Comments and suggestions
p)	Reactions overlaid with mineral oil when using a thermal cycler with a heated lid	When using a thermal cycler with a heated lid that is switched on, do not overlay the reactions with mineral oil as this may decrease the yield of RT-PCR product.
q)	Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.
Product is multibanded		
a)	Reactions set up at room temperature (15–25°C)	Be sure to set up the RT-PCR on ice to avoid premature cDNA synthesis.
b)	Starting conditions for reverse-transcriptase reaction incorrect	Make sure that thermal cycler is preheated to 50°C before placing samples in it.
c)	Reverse-transcription reaction temperature too low	A reverse-transcription reaction temperature of 50°C is recommended. However, if the desired results are not obtained using 50°C, the reaction temperature may be increased in increments of 2°C up to 60°C.
d)	RT-PCR cycling conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Q-Solution. Follow the protocol on page 14.
e)	PCR annealing temperature too low	Increase annealing temperature in increments of 2°C. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix E, page 30).
f)	Primer concentration not optimal or primers degraded	A primer concentration of 0.6 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the RT-PCR with different primer concentrations from 0.5–1.0 µM in 0.1 µM increments. In particular, when performing highly sensitive RT-PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel*.

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

		Comments and suggestions
g)	Primer design not optimal	Review primer design (see Appendix B, page 24). Use only gene-specific primers. Do not use random oligomers or oligo-dT primers.
h)	Contamination with genomic DNA	Pretreat starting RNA template with DNase I. Alternatively, use primers located at splice junctions of the target mRNA to avoid amplification from genomic DNA (see Appendix J, page 33).
Product is smeared		
a)	Too much starting template	Check the concentration of the starting RNA template (see Appendix A, page 23). If necessary, make new serial dilutions of template RNA from stock solutions. Repeat the RT-PCR using the new dilutions.
b)	Carry-over contamination	If the negative control (without template RNA) shows a RT-PCR product or a smear, exchange all reagents. Use disposable pipet tips containing hydrophobic filters to minimize cross-contamination. Set up all reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
c)	Reactions set up at room temperature (15–25°C)	Be sure to set up the RT-PCR on ice to avoid premature cDNA synthesis.
d)	Starting conditions for reverse-transcriptase reaction incorrect	Make sure that thermal cycler is preheated to 50°C before placing samples in it.
e)	RT-PCR cycling conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Q-Solution. Follow the protocol on page 14.
f)	Enzyme concentration too high	Ensure that 2 µl QIAGEN OneStep RT-PCR Enzyme Mix per reaction was used.
g)	Too many cycles	Reduce the number of cycles in steps of 3 cycles.

		Comments and suggestions
h)	Primer concentration not optimal or primers degraded	A primer concentration of 0.6 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the RT-PCR with different primer concentrations from 0.5–1.0 µM in 0.1 µM increments. In particular, when performing highly sensitive RT-PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel*.
i)	Primer design not optimal	Review primer design (see Appendix B, page 24). Use only gene-specific primers. Do not use random oligomers or oligo-dT primers.

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

Appendix A: Starting template

The efficiencies of reverse transcription and PCR are highly dependent on the quality and quantity of the starting RNA template.

It is important to have intact RNA as starting template. Even trace amounts of contaminating RNases in the RNA sample can cause RNA cleavage, resulting in shortened cDNA products. Chemical impurities, such as protein, poly-anions (e.g., heparin), salts, EDTA, ethanol, phenol, and other solvents, can affect the activity and processivity of the reverse transcriptases and the *Taq* DNA polymerase. To ensure reproducible and efficient RT-PCR, it is important to determine the quality and quantity of the starting RNA.

For best results, we recommend starting with RNA purified using silica-membrane technology. For example, RNeasy® Kits, QIAamp® Viral RNA Kits, and the QIAamp RNA Blood Mini Kit can be used to isolate RNA from a variety of starting materials and provide high-quality RNA ideal for use in reverse-transcription and RT-PCR applications. Alternatively RNA can be isolated from whole blood using the PAXgene® Blood RNA Kit.

Storage of RNA

Purified RNA may be stored at –20°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable for at least 1 year.

Determining concentration and purity of RNA

- The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Note that absorbance measurements cannot discriminate between DNA and RNA.
- To determine RNA concentration, we recommend dilution of the sample in water, since the relationship between absorbance and concentration (A_{260} reading of 1 = 40 µg/ml RNA) is based on an extinction coefficient calculated for RNA in water. To ensure significance, readings should be between 0.1 and 1.0.
- The ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. To determine RNA purity, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1* in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer using the same solution.

* Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

Integrity of RNA

The integrity and size distribution of total RNA can be checked by denaturing agarose-gel electrophoresis and ethidium bromide* staining. The respective ribosomal bands (Table 6) should appear as sharp bands on the stained gel. The intensity of the 28S ribosomal RNA band should be approximately twice that of the 18S rRNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear of lower molecular weight species, it is likely that the RNA sample suffered major degradation during preparation.

Table 6. Sizes of ribosomal RNAs from various sources

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

Appendix B: Primer design, concentration, and storage

Standard RT-PCR primers

Prerequisites for successful one-step RT-PCR include the design of optimal primer pairs, the use of appropriate primer concentrations, and the correct storage of primer solutions. Some general guidelines are given in Table 7, page 25. The QIAGEN OneStep RT-PCR Kit is designed to be used with **gene-specific primers only**. The use of random oligomers or oligo-dT primers is not recommended since this will result in the amplification of nonspecific products.

* Ethidium bromide is toxic and/or mutagenic. Take appropriate safety measures.

Table 7. General guidelines for standard RT-PCR primers

Length:	18–30 nucleotides
G/C content:	40–60%
T_m:	Simplified formula for estimating melting temperature (T_m): T_m = 2°C x (A+T) + 4°C x (G+C) Whenever possible, design primer pairs with similar T_m values. Optimal PCR annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m. Primer T_m values should not be lower than the reverse-transcription reaction temperature (e.g., 50°C).
Location:	■ Design primers so that one half of the primer hybridizes to the 3' end of one exon and the other half to the 5' end of the adjacent exon (see Figure 2A, page 27). Primers will anneal to cDNA synthesized from spliced mRNAs, but not to genomic DNA. Thus, amplification of contaminating DNA is eliminated. ■ Alternatively, RT-PCR primers should be designed to flank a region that contains at least one intron (see Figure 2B, page 27). Products amplified from cDNA (no introns) will be smaller than those amplified from genomic DNA (containing introns). Size difference in products is used to detect the presence of contaminating DNA. ■ If only the mRNA sequence is known, choose primer annealing sites that are at least 300–400 bp apart. It is likely that fragments of this size from eukaryotic DNA contain splice junctions. As explained in the previous point and Figure 2B, such primers may be used to detect DNA contamination.

Table continues on next page.

Table 7 (continued)

Sequence:

- Avoid complementarity of two or more bases at the 3' ends of primer pairs to reduce primer–dimer formation.
- Avoid mismatches between the 3' end of the primer and the target-template sequence.
- Avoid runs of 3 or more G or C nucleotides at the 3' end.
- Avoid a 3'- end T. Primers with a T at the 3' end have a greater tolerance of mismatch.
- Avoid complementary sequences within a primer sequence and between the primers of a primer pair.
- Commercially available computer software can be used for primer design.

Concentration:

- Spectrophotometric conversion for primers:

  1 A_{260} unit ≡ 20–30 µg/ml

- Molar conversions:

Primer length	pmol/µg	30 pmol (0.6 µM in 50 µl)
18mer	168	178 ng
20mer	152	198 ng
25mer	121	248 ng
30mer	101	297 ng

- Use 0.5–1.0 µM of each primer in one-step RT-PCR. For most applications, a primer concentration of 0.6 µM will be optimal.

Storage:

Lyophilized primers should be dissolved in a small volume of distilled water or TE to make a concentrated stock solution. Prepare small aliquots of working solutions containing 10 pmol/µl to avoid repeated thawing and freezing. Store all primer solutions at –20°C. Primer quality can be checked on a denaturing polyacrylamide gel*; a single band should be seen.

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

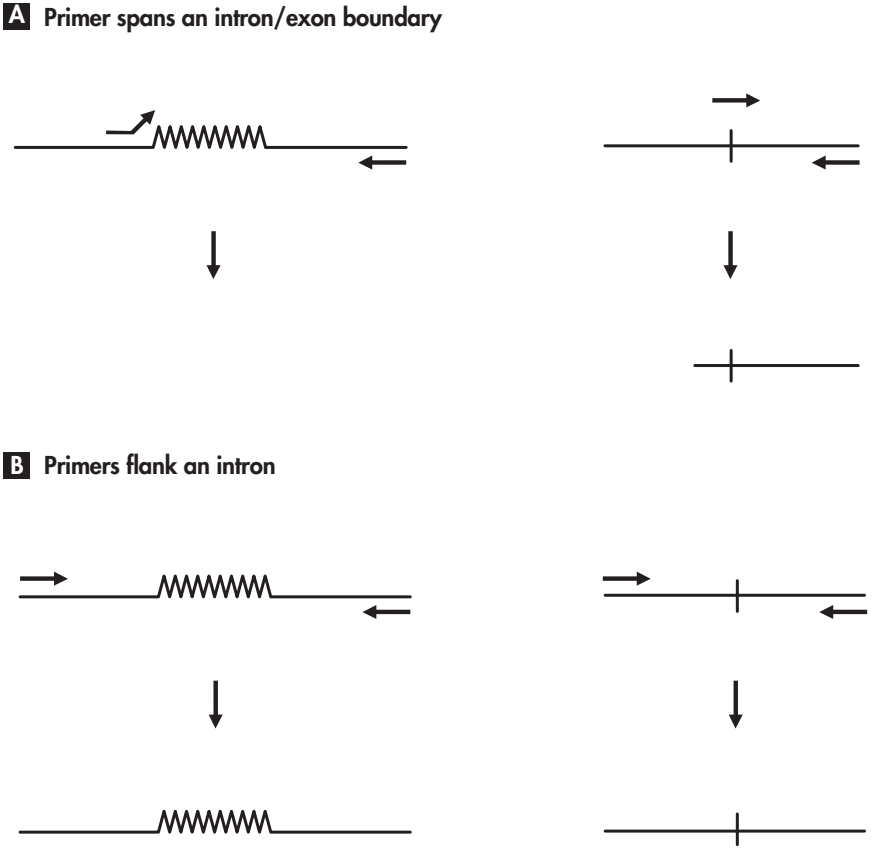


Figure 2. Primer design for RT-PCR. Primer design to A eliminate or B detect amplification from contaminating genomic DNA

Degenerate RT-PCR primers

Occasionally, the exact nucleotide sequence of the target RNA will not be known, for instance when it has been deduced from an amino acid sequence or when a family of closely related sequences is to be amplified. To enable such templates to be amplified by one-step RT-PCR, degenerate primers can be used. These are actually mixtures of several primers whose sequences differ at the positions that correspond to the uncertainties in the template sequence.

Hot-start PCR using HotStarTaq DNA Polymerase, which is a component of the QIAGEN OneStep RT-PCR Enzyme Mix, often improves amplification specificity in RT-PCR using degenerate primers by reducing the formation of nonspecific RT-PCR products and primer–dimers. Table 8 gives recommendations for further optimizing one-step RT-PCR using degenerate primers. To help determine the best location for degenerate primers, Table 8 lists the codon redundancy of each amino acid.

Table 8. Guidelines for design and use of degenerate primers

Sequence:	■ Avoid degeneracy in the 3 nucleotides at the 3' end. ■ If possible, use Met- or Trp-encoding triplets at the 3' end. ■ To increase primer–template binding efficiency, reduce degeneracy by allowing mismatches between the primer and template, especially towards the 5' end (but not at the 3' end). ■ Try to design primers with less than 4-fold degeneracy at any given position.
RT-PCR conditions	When optimizing one-step RT-PCR using degenerate primers, modify RT-PCR conditions in the following order:
Primer concentration:	First try a primer concentration of 0.6 µM. If this primer concentration results in poor RT-PCR amplification, increase the primer concentration in increments of 0.2 µM until satisfactory results are obtained.
Template concentration:	Increase starting RNA amount (up to 2 µg).
PCR annealing temperature:	Reduce annealing temperature in steps of 2°C.

Table 9. Codon redundancy

Amino acid	Number of codons
Met, Trp	1
Cys, Asp, Glu, Phe, His, Lys, Asn, Gln, Tyr	2
Ile	3
Ala, Gly, Pro, Thr, Val	4
Leu, Arg, Ser	6

Appendix C: Number of cycles

A cycling program usually consists of 25–40 cycles, depending on the number of copies of the starting template. Increasing the number of cycles does not necessarily lead to a higher yield of RT-PCR product; instead it may increase nonspecific background and decrease the yield of specific RT-PCR product. Table 10 gives the approximate number of target copies contained in 100 ng total human RNA according to the abundance class of the respective target. Table 11 provides general guidelines for choosing the number of cycles.

Table 10. mRNA classification based on abundance

Class	Copies/cell	Example	Approximate number of copies in 100 ng total human RNA
Abundant	1000–10,000	GAPDH, β-Actin	$7 \times 10^6 - 7 \times 10^7$
Intermediate	100–1000	Translation factors	$7 \times 10^5 - 7 \times 10^6$
Rare	<10	TNF, M-CSF	7×10^4

Table 11. General guidelines for choosing the number of PCR cycles

Number of copies of starting template	Number of cycles*
10–100	45–50
100–1000	40–45
1000–10,000	30–35
>10,000	25–35

* When performing quantitative RT-PCR, the range of cycle numbers resulting in a linear relationship between target quantity and product yield must be determined emperically.

Appendix D: Sensitive RT-PCR assays

RT-PCR can be performed to detect even a single RNA molecule. However, amplification of such a low number of target sequences is often limited by the generation of nonspecific RT-PCR products and primer–dimers. The combination of HotStarTaq DNA Polymerase and QIAGEN OneStep RT-PCR Buffer increases specificity in the first cycle and throughout PCR. Thus HotStarTaq DNA Polymerase is well suited to such highly sensitive RT-PCR assays.

Nested PCR

If PCR sensitivity is too low, a nested PCR method can increase RT-PCR product yield. Nested RT-PCR involves reverse transcription followed by two rounds of amplification reactions. The first-round PCR is performed according to the QIAGEN OneStep RT-PCR Protocol (page 11 or 14). Subsequently, an aliquot of the first-round PCR product, for example 1 µl of a $1/10^3$ – $1/10^4$ dilution, is subjected to a second round of PCR. The second-round PCR is performed using two new primers that hybridize to sequences nternal to the first-round primer target sequences. In this way, only specific first-round RT-PCR products (and not nonspecific products) will be amplified in the second round. Alternatively, it is possible to use one internal and one first-round primer in the second PCR; this method is referred to as semi-nested PCR.

Appendix E: Touchdown PCR

Touchdown PCR uses a cycling program with varying annealing temperatures. It is a useful method to increase the specificity of RT-PCR. The annealing temperature in the initial PCR cycle should be 5–10°C above the T_m of the primers. In subsequent cycles, the annealing temperature is decreased in steps of 1–2°C per cycle until a temperature is reached that is equal to, or 2–5°C below, the T_m of the primers. Touchdown PCR enhances the specificity of the initial primer–template duplex formation and hence the specificity of the final RT-PCR product.

To program your thermal cycler for touchdown PCR, you should refer to the manufacturer's instructions.

Appendix F: Amplification of long RT-PCR products

The QIAGEN OneStep RT-PCR Protocol is optimized for amplification of products of up to 2 kb. When amplifying RT-PCR products larger than 2 kb, it is recommended to use the modified cycling conditions described in Table 12, page 31.

For amplification of very long RT-PCR products of up to 12.5 kb, we recommend the QIAGEN LongRange 2 Step RT-PCR Kit.

Table 12. Modified thermal-cycler conditions for long RT-PCR products

			Additional comments
Reverse transcription:	30 min	**45°C**	For RT-PCR products longer than 2 kb, a reverse-transcription reaction temperature of 45°C is recommended.
Initial PCR activation step:	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step. Omniscript and Sensiscript Reverse Transcriptases are inactivated and template cDNA is denatured.
3-Step cycling			
Denaturation:	**10 s**	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below T_m of primers.
Extension:	**1 min x EPL**	**68°C**	EPL is the expected product length in kb; e.g., for a 3 kb product, the extension step would last 1 min x 3 = 3 min
Number of cycles:	25–40		The cycle number is dependent on the amount of template RNA and the abundance of the respective target.
Final extension:	10 min	**68°C**	

Appendix G: Multiplex RT-PCR

Multiplex RT-PCR is a demanding amplification technique that allows the simultaneous detection of several RNA targets in a single reaction. Multiplex RT-PCR is used in applications such as analysis of chromosome translocations, detection of RNA virus serotypes, study of cytokine gene expression, and quantitative/semiquantitative RT-PCR (see also page 32).

Primers should be designed according to the guidelines given in Table 7, page 25. Table 13 provides further guidelines for optimization of multiplex RT-PCR conditions.

Table 13. Guidelines for multiplex RT-PCR

Step 1	**Optimize cycling conditions for individual primer pairs**
	■ Set up one-step RT-PCR samples according to the protocol "QIAGEN OneStep RT-PCR Kit" (see page 11) using a primer concentration of 0.6 µM. Each reaction should contain only one primer pair.
	■ Determine one set of RT-PCR conditions (template amount, number of cycles, annealing temperature, and extension time) that produces satisfactory yield from each individual primer pair.
Step 2	**Perform multiplex RT-PCR**
	Use 0.6 µM of each primer and the optimized cycling conditions from step 1.
Step 3	**Optimize multiplex RT-PCR**
	■ If the RT-PCR from step 2 results in different product yields, reduce the concentration of the primers yielding the most prominent RT-PCR product(s) in steps of 0.1 µM until all products are produced in similar quantities. Concentrations as low as 0.05 µM may be sufficient to amplify abundant transcripts.
	■ If altering the primer concentrations fails to improve the yield of long RT-PCR products, increase the extension time in increments of 30 s.

Appendix H: Co-amplification of an internal control

The relative abundance of a transcript in different samples can be estimated by semiquantitative or relative RT-PCR. Typically, the signal from the RT-PCR product is normalized to the signal from an internal control included in all samples and amplified at the same time as the target. The normalized data from different samples can then be compared. Transcripts of housekeeping genes such as GAPDH or β-actin are frequently chosen as internal controls because they are abundantly expressed at relatively constant rates in most cells. However, the internal control transcript is usually more abundant than the transcript under study. This difference in abundance can lead to preferential amplification of the internal control and, in some cases, prevent amplification of the target RT-PCR product. Often, such problems can be overcome by reducing the internal-control primer concentration. The guidelines in Table 14, page 33, may be helpful in developing co-amplification conditions.

Table 14. Guidelines for co-amplification of an internal control

- Choose similar amplicon sizes for the target and the internal control but be sure that the products can be easily distinguished on an agarose gel.
- Determine RT-PCR conditions that are suitable for both amplicons by varying template amount, number of cycles, annealing temperature, and extension time.
- Initially, try primer concentrations of 0.6 µM for the target transcript and 0.3 µM for the internal control transcript.
- If the yield of internal standard greatly exceeds that of the specific target using the concentrations given above, reduce the internal-control primer concentration in steps of 0.05–0.1 µM. The optimal primer concentration for the internal control depends on the relative abundance and efficiency of amplification of the control and target transcripts. Control transcripts may be much more highly expressed than the target transcript. If the difference in abundance is too great, then RT-PCR of the internal control may interfere with the amplification of the target transcript.

Appendix I: Purification of RT-PCR products

After amplification, the RT-PCR sample contains a complex mixture of specific RT-PCR product and residual reaction components such as primers, unincorporated nucleotides, enzymes, salts, mineral oil, and possibly nonspecific amplification products. Before the specific RT-PCR product can be used in subsequent experiments, it is often necessary to remove these contaminants. The QIAquick® and MinElute® systems offer a quick and easy method for purifying the final RT-PCR product (see ordering information, page 38). For more information about QIAquick or MinElute products, please call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Appendix J: Control of contamination

General control of nucleic acid contamination

It is extremely important to include at least one negative control in every round of RT-PCR. This control lacks the template RNA to detect possible contamination of the reaction components.

Controls for DNA contamination

Contamination of RT-PCR by genomic DNA can often be detected or eliminated by the choice of primer location (see Table 7, page 25). If it is not possible to use such primers, DNA contamination can be detected using control reactions in which reverse-transcriptase activity is inhibited. Because no cDNA synthesis takes place in the control reaction, the only template available for amplification is contaminating DNA.

Reverse-transcriptase activity is inhibited by keeping control reactions on ice and placing them in the thermal cycler only after it has reached 95°C for the HotStarTaq DNA Polymerase activation step (before cycling). Alternatively, control reactions are set up using all components except RNA. The RNA is then added during the HotStarTaq DNA Polymerase activation step. The high-temperature step inactivates reverse transcriptase, and in the absence of reverse transcription, the only DNA template for PCR is contaminating DNA. Therefore, formation of PCR products in these reactions indicates the presence of contamination.

General physical precautions

- Separate the working areas for setting up one-step RT-PCR amplifications and RNA and DNA handling, including the addition of starting template, PCR product analysis, or plasmid preparation. Ideally, use separate rooms.
- Use a separate set of pipets for the RT-PCR master mix. Use of pipet tips with hydrophobic filters is strongly recommended.
- Prepare and freeze small aliquots of primer solutions and dNTP Mix. Use of fresh distilled water is strongly recommended.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with 10% (v/v) commercial bleach solution.* Afterwards, the benches and pipets should be rinsed with distilled water.

General chemical precautions

RT-PCR stock solutions can also be decontaminated using UV light. This method is laborious, however, and its efficiency is difficult to control and cannot be guaranteed. We recommend storing solutions in small aliquots and using fresh aliquots for each RT-PCR.

* Commercial bleach solutions typically comprise 5.25% sodium hypochlorite. Sodium hypochlorite is an irritant and should be handled with caution.

Appendix K: General remarks for 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, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

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.

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. Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

* DEPC is a suspected carcinogen and should be handled with great care. Wear gloves and use a fume hood when using this chemical.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% (v/v) to inactivate RNases on glass or plasticware, or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Ordering Information

Product	Contents	Cat. no.
QIAGEN OneStep RT-PCR Kit (25)	For 25 one-step RT-PCRs: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,* dNTP Mix,† 5x Q-Solution, RNase-free water	210210
QIAGEN OneStep RT-PCR Kit (100)	For 100 one-step RT-PCRs: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,* dNTP Mix,† 5x Q-Solution, RNase-free water	210212
QIAGEN OneStep RT-PCR Kit (1000)	For 1000 one-step RT-PCRs: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer,* dNTP Mix,† 5x Q-Solution, RNase-free water	210215
Related Products		
Omniscript Reverse Transcriptase Kit — for reverse transcription using ≥ 50 ng RNA		
Omniscript Reverse Transcriptase Kit (10)‡	For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,‡ RNase-free water	205110
Sensiscript Reverse Transcriptase Kit — for reverse transcription using < 50 ng RNA		
Sensiscript Reverse Transcriptase Kit (50)	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,‡ RNase-free water	205211

* Contains 12.5 mM $MgCl_2$

† Contains 10 mM of each dNTP

‡ Contains 5 mM of each dNTP

‡ Larger kit sizes available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
HotStarTaq DNA Polymerase — for highly specific hot-start PCR		
HotStarTaq DNA Polymerase (250)†‡	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	203203
HotStarTaq *Plus* DNA Polymerase — for highly specific hot-start PCR without optimization		
HotStarTaq *Plus* DNA Polymerase (250)†	250 units HotStarTaq Plus DNA Polymerase, 10x PCR Buffer, 10x CoralLoad® PCR Buffer, 5x Q-Solution, 25 mM $MgCl_2$	203603
QIAGEN LongRange PCR Kits — for reliable and accurate long-range PCR		
QIAGEN LongRange PCR Kit (20)†	For 20 x 50 µl reactions: LongRange PCR Enzyme Mix (40 U), LongRange PCR Buffer, 5x Q-Solution, RNase-Free Water, 10 mM dNTPs	206401
QIAGEN LongRange 2Step RT-PCR Kit (20)†	For 20 x 50 µl PCRs: Reverse transcription step (10 x 20 µl reactions) - LongRange Reverse Transcription Enzyme, Buffer, dNTPs, Oligo-dT, RNase Inhibitor, RNase-Free Water; PCR step - QIAGEN LongRange PCR Kit	205920
QIAquick PCR Purification Kit — for direct purification of PCR fragments		
QIAquick PCR Purification Kit (50)†	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
MinElute PCR Purification Kit — for purification of PCR products (70 bp to 4 kb) in low elution volumes		
MinElute PCR† Purification Kit (50)	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004

* Contains 15 mM $MgCl_2$

† Larger kit sizes available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
PAXgene Blood rNA Kit — For isolation and purification of intracellular RNA from whole blood stabilized in PAXgene Blood RNA Tubes		
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	762174
RNeasy Kits — for total RNA isolation from animal cells or tissues, yeast, or bacteria		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Midi Kit (10)*	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Maxi Kit (12)	12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75162

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.

* Larger kit sizes available; see www.qiagen.com.

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