December 2015

OneStep *Ahead* RT-PCR Kit Handbook

For ultrafast and highly sensitive one-step RT-PCR

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAGEN OneStep *Ahead* RT-PCR Kit	**(100)**	**(200)**	**(2000)**
Catalog No.	**220211**	**220213**	**220216**
Number of reactions (25 µl)	**50**	**200**	**2000**
QIAGEN OneStep *Ahead* RT-Mix (25x), containing the following QIAGEN products: Omniscript® Reverse Transcriptase, Sensiscript® Reverse Transcriptase and an RNase Inhibitor	50 µl	200 µl	10 x 200 µl
QIAGEN OneStep *Ahead* RT-PCR Master Mix (2.5x), containing HotStarTaq DNA Polymerase, QuantiNova DNA Polymerase, a hot-start proofreading enzyme, OneStep *Ahead* RT-PCR buffer and dNTP mix (dATP, dCTP, dGTP and dTTP)	500 µl	2 x 1 ml	20 x 1 ml
Q-Solution®, 5x	400 µl	2 ml	5 x 2 ml
OneStep *Ahead* Template Tracer, 25x	200 µl	200 µl	10 x 200 µl
OneStep *Ahead* Master Mix Tracer, 125x	50 µl	50 µl	10 x 50 µl
RNase-Free Water	1.9 ml	2 x 1.9 ml	20 x 1.9 ml
Quick-Start Protocol OneStep *Ahead* RT-PCR	1	1	1

Storage

The QIAGEN OneStep *Ahead* RT-PCR Kit is shipped on dry ice and should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer. When the kits are stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality control label inside the kit box). The OneStep *Ahead* Template Tracer and Master Mix Tracer can also be stored at 2–8°C until the expiration date. The OneStep *Ahead* RT-PCR Master Mix can also be stored at 2–8°C for up to 6 months, depending on the expiration date.

If desired, the OneStep *Ahead* Master Mix Tracer can be added to 2.5x OneStep *Ahead* RT-PCR Master Mix for long-term storage. For details, see "Adding Master Mix Tracer to the RT-PCR Master Mix", page 12.

Intended Use

The QIAGEN OneStep *Ahead* 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.

Quality Control

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

Product Information

The QIAGEN OneStep *Ahead* RT-PCR Kit contains:

25x OneStep *Ahead* RT-Mix

Component	Description
Omniscript Reverse Transcriptase	Omniscript Reverse Transcriptase is a recombinant, heterodimeric enzyme expressed in *E. coli*. It is provided in an inactive state and has minimal enzymatic activity at ambient temperature. The enzyme is activated during the reverse-transcription step at 50°C.
Sensiscript Reverse Transcriptase	Sensiscript Reverse Transcriptase is a recombinant, heterodimeric enzyme expressed in *E. coli*. It is provided in an inactive state and has minimal enzymatic activity at ambient temperature. The enzyme is activated during the reverse-transcription step at 50°C.
RNase Inhibitor	The RNase inhibitor is a recombinant mammalian protein that inhibits eukaryotic RNases such as RNase A and B.
RT blocker	Enables heat-mediated activation of the reverse transcriptases.

2.5x OneStep *Ahead* RT-PCR Master Mix

Component	Description
QuantiNova DNA Polymerase	QuantiNova DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 5 minute, 95°C incubation step.
HotStarTaq DNA polymerase	HotStarTaq DNA Polymerase is a chemically modified form of the *Taq* polymerase. QuantiNova DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. Its activation occurs in a gradual fashion.
Hot-start proofreading enzyme	The high-fidelity proofreading DNA polymerase with 3'→5' exonuclease activity is also heat-activated by the initial 5 minute incubation step at 95°C. Its addition ensures increased amplification accuracy and processivity.
OneStep *Ahead* RT-PCR Buffer	Contains Tris·HCl , KCl, NH_4SO_4, $MgCl_2$ and additives enabling fast cycling and direct loading of the reactions onto agarose gels.
dNTP mix	Contains dATP, dCTP, dGTP and dTTP of ultrapure quality.

Other components

Component	Description
OneStep *Ahead* Master Mix Tracer	Orange dye allows tracking of master mix addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 50 bp on a 1% agarose gel.
OneStep *Ahead* Template Tracer	Blue dye allows tracking of template RNA addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 4000 bp on a1% agarose gel.
Q-Solution, 5x	5x concentrated.
RNase-Free Water	Ultrapure quality, PCR-grade.

Introduction

The QIAGEN OneStep *Ahead* 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.

The kit comprises the following components:

QIAGEN OneStep *Ahead* RT-Mix

- The QIAGEN OneStep *Ahead* RT-Mix contains a specially formulated enzyme blend of Omniscript and Sensiscript Reverse Transcriptase, as well as an RNase inhibitor. Both transcriptases provide highly efficient and specific reverse transcription and 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. Sensiscript Reverse Transcriptase is optimized for use with very small amounts of RNA (<50 ng). The enzyme combination in QIAGEN's OneStep *Ahead* RT-Mix provides highly efficient and sensitive reverse transcription of any RNA quantity – even down to 0.1 pg.
- The RT-blocker facilitates heat-mediated activation of the reverse transcriptases and thus prevents nonspecific enzyme activity at room temperature. This allows for reaction setup at room temperature, which adds convenience and facilitates use with automated (robotic) workflows.
- The 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.

QIAGEN OneStep *Ahead* RT-PCR Master Mix

The OneStep *Ahead* RT-PCR Master Mix contains a well-balanced blend of three different DNA Polymerases, QIAGEN OneStep *Ahead* RT-PCR Buffer and dNTPs.

DNA polymerase blend:

- QuantiNova DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. QuantiNova DNA Polymerase is provided in an antibody-mediated inactive state and has no enzymatic activity at ambient temperature. The enzyme is fully activated after the 5 minute incubation step at 95°C and starts amplifying with high specificity from the first cycle.
- HotStarTaq DNA Polymerase is a chemically modified form of a recombinant 94-kDa DNA polymerase, originally isolated from *Thermus aquaticus* and expressed in *E. coli*. Its activation occurs in a gradual fashion and eventually it boosts the amplification process to ensure high yields of PCR product.
- The high-fidelity proofreading DNA polymerase with 3'→5' exonuclease activity is also heat-activated by the initial 5 minute incubation step at 95°C. Its addition ensures increased amplification accuracy and processivity.
- The hot-start procedure 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 hot-start DNA polymerases 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 *Ahead* 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, which is particularly important for one-step RT-PCR performed with limited 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 *Ahead* RT-PCR Buffer ensure 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, minimizing the need for optimization of RT-PCR by varying the annealing temperature or the Mg^{2+} concentration.

Q-Solution

The QIAGEN OneStep *Ahead* RT-PCR Kit is provided with Q-Solution, an 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 templates that have a high degree of secondary structure or that are GC-rich.
- Unlike other commonly used additives, for example DMSO, Q-Solution is used at just one working concentration. For further information, please read "OneStep RT-PCR using Q-Solution", page 21.

OneStep *Ahead* Master Mix Tracer and Template Tracer

- The blue and orange dyes in the OneStep *Ahead* Template Tracer and in the Master Mix Tracer, respectively, allow tracking of pipetted samples during the RT-PCR setup. When the blue template is added to the orange OneStep *Ahead* RT-PCR Master Mix, the color changes to green. The use of these tracers is optional. The Template Tracer is provided as a 25x concentrate and should be diluted (using water) to obtain a 1x final concentration in the sample. To generate a template dilution series, dilute the 25x concentrate (using template and water) to obtain a final concentration of 1x OneStep *Ahead* Blue Template Tracer. The Master Mix Tracer is provided as a 125x concentrate and can be added directly to the master mix stock vial to obtain a 1x final concentration. These tracers do not reduce sample stability or RT-PCR performance.
- Reactions can be directly loaded onto agarose gels after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking of the subsequent electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

QIAGEN OneStep *Ahead* RT-PCR Procedure

The QIAGEN OneStep *Ahead* 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 Figure 1).

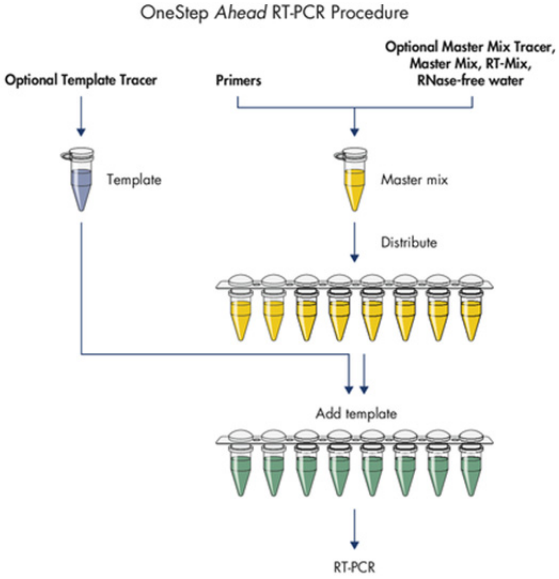

Figure 1. OneStep *Ahead* RT-PCR procedure with additional tracer dyes.

Adding Master Mix Tracer to the RT-PCR Master Mix

OneStep *Ahead* RT-PCR Master Mix

The OneStep *Ahead* Master Mix Tracer can be added directly to the OneStep *Ahead* RT-PCR Master Mix for long-term storage. Since the amount of tracer added is very small, the concentration of the master mix will not be changed and the master mix can be used as indicated in the protocols.

Table 1. Addition of OneStep *Ahead* Master Mix Tracer to the RT-PCR Master Mix

Volume of 2.5x OneStep *Ahead* RT-PCR Master Mix	Volume of 125x OneStep *Ahead* Master Mix Tracer
0.5 ml	10 µl
1.0 ml	20 µl

Equipment and Reagents to be Supplied by User

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

Protocol: Using QIAGEN OneStep *Ahead* RT-PCR Kit

Important points before starting

- 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 0.1 pg – 1 µg of total RNA.
- The blend of DNA polymerases, including a proofreading enzyme, contained in the QIAGEN OneStep *Ahead* RT-PCR Master Mix require a 5 minute heat-activation step at 95°C after the reverse-transcription step. This also inactivates the reverse transcriptases.
- The OneStep *Ahead* RT-Mix contains an RNase inhibitor and a blend of Omniscript and Sensiscript reverse transcriptases, as well as an RT-blocker for heat-mediated activation of the reverse-transcription step.
- Heat-mediated activation of the reverse transcriptases prevents nonspecific enzyme activity. This allows reaction setup at room temperature, facilitating the use of this kit in automated (robotic) workflows.
- The kit is designed for use with gene-specific primers at a final concentration of 0.5 µM. The use of random oligomers or oligo-dT primers is not recommended.
- QIAGEN OneStep *Ahead* RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which provides satisfactory results in most cases.
- The blue and orange dyes in the OneStep *Ahead* Template Tracer and in the OneStep *Ahead* Master Mix Tracer, respectively, allow tracking of pipetted samples during RT-PCR setup. When the blue template is added to the orange OneStep *Ahead* RT-PCR Master Mix, the color changes to green. The use of these tracers is optional. The Template Tracer is provided as a 25x concentrate and should be used in a 1x final concentration in the sample. To generate a template dilution series, dilute the 25x concentrate (using template and water) to obtain a final concentration of 1x OneStep *Ahead* Blue Template Tracer in

the diluent. The Master Mix Tracer is provided as a 125x concentrate and can be added directly to the master mix stock vial to obtain a 1x concentration in the final reaction mix. These tracers do not reduce sample stability or RT-PCR performance.

- Reactions can be directly loaded onto agarose gels after cycling. The tracer dyes allow monitoring of the loading process and tracking during the subsequent electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.
- The QIAGEN OneStep *Ahead* RT-PCR Kit contains Q-Solution, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.

Procedure

1. Thaw OneStep *Ahead* RT-PCR Master Mix, template RNA, primer solutions and RNase-Free Water. Mix thoroughly before use.
2. Prepare a reaction mix according to Table 2. The reaction mix contains all the components except the template RNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

   Due to the 2-phase hot-start of both the RT and the PCR reactions, it is not necessary to keep samples on ice during reaction setup or while programming the cycler. However, we recommend storing the RT-Mix at -20°C immediately after use.

   Note: A negative control (without template RNA) should be included in every experiment.

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

Component	Volume/reaction	Final concentration
OneStep *Ahead* RT-PCR Master Mix, 2.5x	10 µl	1x
OneStep *Ahead* RT-Mix, 25x	1 µl	1x
Primer A	Variable	0.5 µM
Primer B	Variable	0.5 µM
RNase-Free Water	Variable	–
Optional: OneStep *Ahead* Master Mix Tracer,125x	0.2 µl	1x
Template RNA (added at step 4)	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	**25 µl**	

3. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.
4. Add template RNA (1 µg – 100 fg per reaction, depending on target transcript abundance) to the individual PCR tubes. The QIAGEN OneStep *Ahead* RT-PCR Kit can be used with total RNA, messenger RNA, viral RNA or *in vitro* transcribed RNA.

   Optional: The Template Tracer can be added to the template RNA before pipetting into the reaction Mix. This allows the pipetting process to be monitored, thus minimizing pipetting errors.
5. Program the thermal cycler according to the manufacturer's instructions and using the conditions outlined in Table 3. The protocol includes steps for both reverse transcription and PCR and gives satisfactory results in most cases.

Table 3. One-step RT-PCR cycling conditions for amplicons < 1 kbp

Step	Time	Temperature	Additional comments
Reverse transcription	10 min	50°C	OmniScript and SensiScript RTs are activated and reverse transcription takes place. The reaction temperature may be increased up to 55°C if satisfactory results are not obtained at 50°C.
Initial PCR activation	5 min	95°C	This heating step activates the DNA polymerase blend, inactivates Omniscript and Sensiscript Reverse Transcriptases and denatures the cDNA template.
3-step cycling:	10 s	95°C	Do not exceed this temperature.
Denaturation	10 s	55°C	Approximately 5°C below T_m of primers.
Extension	10 s	72°C	For RT-PCR products up to 1 kb, an extension time of 10 s is sufficient.
Number of cycles	40		The optimal cycle number depends on the amount of template RNA and the abundance of the target transcript.
Final extension	2 min	72°C	

For amplification of RT-PCR products >1kbp, please refer to Appendix B (page 24).

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/or protocols in this handbook or sample and assay technologies (for contact information, 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. Repeat the RT-PCR.
b) DNA polymerases not activated	Ensure that the cycling program included the DNA polymerase activation step (5 min at 95°C) as described in the cycling protocols, step 2.
c) HotStarTaq DNA Polymerase activated too early	Check the cycling program. Ensure that the reverse-transcription reaction is complete (10 min at 50°C) before activating the DNA polymerases (5 min at 95°C).
d) Reverse-transcription reaction temperature incorrect	A reverse-transcription reaction temperature of 50°C is recommended. If desired results are not obtained using 50°C, use reaction temperatures of 45–60°C.
e) Primer concentration not optimal or primers degraded	A primer concentration of 0.5 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.
f) RT-PCR conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Q-Solution. Follow the protocol described in Appendix A on page 22.
g) Problems with starting template	Check the concentration, integrity, purity and storage conditions of the starting RNA template (see Appendix D, page 29). If necessary, make new serial dilutions of template RNA from stock solutions. Repeat the RT-PCR using the new dilutions.
h) Enzyme concentration too low	Ensure that 1 µl of RT-Mix per 25 µl reaction was used.
i) Insufficient number of cycles	Increase the number of cycles in increments of 5 cycles.

		Comments and suggestions
j)	Incorrect PCR annealing temperature or time	Decrease annealing temperature in 2°C steps. Annealing time should be between 10 and 20 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix F, page 38).
k)	Incorrect denaturation temperature or time	Denaturation should be at 95°C for 10 seconds. Ensure that the cycling program included the DNA polymerase activation step (5 min at 95°C) as described in the cycling protocols, step 3.
l)	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 F, page 38).
m)	Primer design not optimal	Review primer design (see Appendix E, page 31). Only use gene-specific primers. Do not use random oligomers or oligo-dT primers.
n)	RT-PCR of long fragments	Increase the concentration of template RNA. When amplifying products longer than 1 kbp, use the modified reaction conditions described in Appendix B, page 24.
o)	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.
p)	Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been programmed correctly.
Product is multibanded		
a)	Reverse-transcription reaction temperature too low	A reverse-transcription reaction temperature of 50°C is recommended. If the desired results are not obtained using 50°C, the reaction temperature may be increased in increments of 1°C up to 55°C.
b)	RT-PCR cycling conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Q-Solution. Follow the protocol on page 21.
c)	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 F, page 38).
d)	Primer concentration not optimal or primers degraded	A primer concentration of 0.5 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.
e)	Primer design not optimal	Review primer design (see Appendix E, page 31). Use only gene-specific primers. Do not use random oligomers or oligo-dT primers.

		Comments and suggestions
f)	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 E, page 31).
Product is smeared		
a)	Too much starting template	Check the concentration of the starting RNA template (see Appendix D, page 29). 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)	RT-PCR cycling conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Q-Solution. Follow the protocol on page 21.
d)	RT enzyme concentration too high	Ensure that 1 µl QIAGEN OneStep *Ahead* RT-Mix Enzyme Mix per 25- reaction was used.
e)	Too many cycles	Reduce the number of cycles in steps of 3 cycles.
f)	Primer concentration not optimal or primers degraded	A primer concentration of 0.5 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.
g)	Primer design not optimal	Review primer design (see Appendix E, page 31). Use only gene-specific primers. Do not use random oligomers or oligo-dT primers.

Appendix A: One-Step RT-PCR Using Q-Solution

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

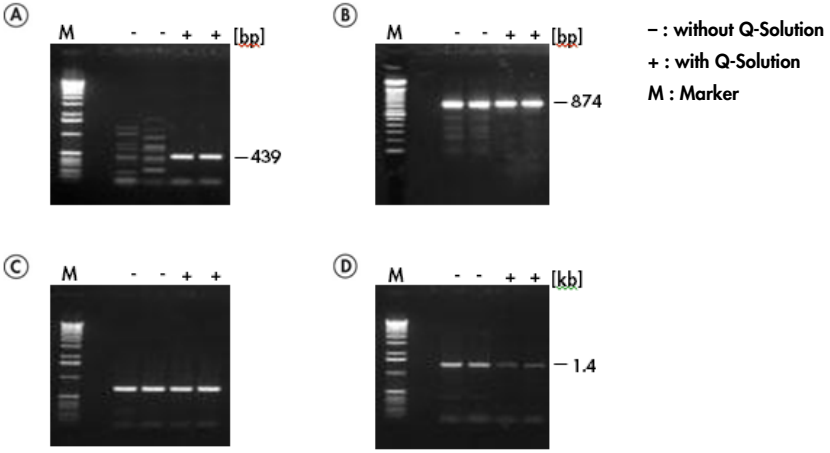

Figure 2. RT-PCR effects using Q-Solution.

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

Component	Volume/reaction	Final concentration
Reaction mix OneStep *Ahead* RT-PCR Master Mix, 2.5x	10 µl	1x
OneStep *Ahead* RT-Mix, 25x	1 µl	1x
Q-Solution	5 µl	1x
Primer A	Variable	0.5 µM
Primer B	Variable	0.5 µM
RNase-Free Water	Variable	–
Optional: OneStep *Ahead* Master Mix Tracer,125x	0.2 µl	1x
Template RNA	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	**25 µl**	

1. Mix the reaction thoroughly and dispense appropriate volumes into PCR tubes or wells of a PCR plate.
2. Add template RNA (1 µg – 100 fg per reaction, depending on target transcript abundance) to the individual PCR tubes. The QIAGEN OneStep *Ahead* RT-PCR Kit can be used with total RNA, messenger RNA, viral RNA or in vitro transcribed RNA.

3. Program the thermal cycler according to the manufacturer's instructions and using the conditions outlined in Table 3. The protocol includes steps for both reverse transcription and PCR and gives satisfactory results in most cases.

Appendix B: One-step RT-PCR for 1–4 kbp Amplicons

Notes before starting

- 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 0.1 pg – 1 µg of total RNA.
- The blend of DNA polymerases, including a proofreading enzyme, contained in the QIAGEN OneStep *Ahead* RT-PCR Master Mix require a 5 minute heat-activation step at 95°C after the reverse-transcription step. This also inactivates the reverse transcriptases.
- The OneStep *Ahead* RT-Mix contains an RNase inhibitor and a blend of Omniscript and Sensiscript reverse transcriptases, as well as an RT-blocker for heat-mediated activation of the reverse-transcription step.
- Heat-mediated activation of the reverse transcriptases prevents nonspecific enzyme activity. This allows reaction setup at room temperature, facilitating the use of this kit in automated (robotic) workflows.
- The kit is designed for use with gene-specific primers at a final concentration of 0.5 µM. The use of random oligomers or oligo-dT primers is not recommended.
- QIAGEN OneStep *Ahead* RT-PCR Buffer provides a final concentration of 2.5 mM $MgCl_2$ in the reaction mix, which provides satisfactory results in most cases.
- The blue and orange dyes in the OneStep *Ahead* Template Tracer and in the Master Mix Tracer, respectively, allow tracking of pipetted samples during RT-PCR setup. When the blue template is added to the orange OneStep *Ahead* RT-PCR Master Mix, the color changes to green. The use of these tracers is optional. The Template Tracer is provided as a 25x concentrate and should be used in a 1x final concentration in the sample. To generate a template dilution series, dilute the 25x concentrate (using template and water) to obtain a final concentration of 1x OneStep *Ahead* Blue Template Tracer in the diluent.

The Master Mix Tracer is provided as a 125x concentrate and can be added directly to the master mix stock vial to obtain a 1x concentration in the final reaction mix. These tracers do not reduce sample stability or RT-PCR performance.

- Reactions can be directly loaded onto agarose gels after cycling. The tracer dyes allow monitoring of the loading process and tracking during the subsequent electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

Procedure

1. Thaw OneStep *Ahead* RT-PCR Master Mix, template RNA, primer solutions and RNase-Free Water. Mix thoroughly before use.
2. Prepare a reaction mix according to Table 5. The reaction mix contains all the components except the template RNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

   Due to the 2-phase hot-start of both the RT and the PCR reactions, it is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template RNA) should be included in every experiment.

Table 5. Reaction setup for one-step RT-PCR

Component	Volume/reaction	Final concentration
Reaction mix OneStep *Ahead* RT-PCR Master Mix, 2.5x	10 µl	1x
OneStep *Ahead* RT-Mix, 25x	1 µl	1x
Primer A	Variable	0.5 µM
Primer B	Variable	0.5 µM
RNase-Free Water	Variable	–
Optional: OneStep *Ahead* Master Mix Tracer, 125x	0.2 µl	1x
Template RNA	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	**25 µl**	

Table 6. One-step RT-PCR cycling conditions for amplicons 1-4 kbp

Step	Time	Temperature	Comment
Reverse transcription	15 min	45°C	OmniScript and SensiScript RTs are activated and reverse transcription takes place.
Initial PCR activation	5 min	95°C	This heating step activates the DNA polymerase blend, inactivates Omniscript and Sensiscript Reverse Transcriptases and denatures the cDNA template.
3-step cycling:			
Denaturation	15 s	95°C	Do not exceed this temperature.
Annealing	15 s	55°C	Approximately 5°C below T_m of primers.
Extension	1–4 min	68°C	Allow 1min per kbp amplicon size.
Number of cycles	40		The optimal cycle number depends on the amount of template RNA and the abundance of the target transcript.
Final extension	5 min	68°C	

Appendix C: Duplex RT-PCR

Duplex RT-PCR allows the simultaneous detection of two different RNA targets in a single reaction. Duplex RT-PCR is used in applications such as analysis of chromosome translocations, detection of RNA virus serotypes, coamplification of an internal or external control and quantitative/semiquantitative RT-PCR. Primers should be designed according to the guidelines given below.

Step 1 Select optimal primer pairs

- Choose similar amplicon sizes for both targets, but ensure that the products can be easily distinguished by your read-out system, e.g., gel or capillary electrophoresis.
- Keep amplicon sizes ideally below 300 bp or clearly below 1 kb.

Step 2 Optimize cycling conditions for individual primer pairs

- Set up one-step RT-PCR samples according to the protocol "Using QIAGEN OneStep *Ahead* RT-PCR Kit" (see page 14) using a primer concentration of 0.5 µM. Each reaction should contain only one primer pair.
- Determine one set of RT-PCR conditions (template amount and annealing temperature) that produces satisfactory yield from each individual primer pair.

Step 3 Perform duplex RT-PCR according to Table 7. Use 0.5 µM of each primer and the optimized cycling conditions from step 1.

Step 4 Optimize duplex 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 10 s.

Table 7. One-step RT-PCR cycling conditions for amplicons < 1 kbp in duplex RT-PCR

Step	Time	Temperature	Comment
Reverse transcription	10 min	50°C	OmniScript and SensiScript RTs are activated and reverse transcription takes place. The reaction temperature may be increased up to 55°C if satisfactory results are not obtained at 50°C.
Initial PCR activation	5 min	95°C	This heating step activates the DNA polymerase blend, inactivates Omniscript and Sensiscript Reverse Transcriptases and denatures the cDNA template.
3-step cycling:			
Denaturation	15 s	95°C	Do not exceed this temperature.
Annealing	20 s	55°C	Approximately 5°C below T_m of primers.
Extension	20 s	72°C	
Number of cycles	40		The optimal cycle number depends on the amount of template RNA and the abundance of the target transcript.
Final extension	2 min	72°C	

Appendix D: 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-gel–membrane technology. For example, RNeasy® Kits, QIAamp® Viral RNA Kit and the QIAamp RNA Blood Mini Kit can be used to isolate RNA from a variety of starting materials and provide high-quality RNA 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 diluting the sample in water as 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.

RNA integrity

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 8) 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 8. 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

* Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.
† Ethidium bromide is toxic and/or mutagenic. Take appropriate safety measures.

Appendix E: 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 9. The QIAGEN OneStep *Ahead* 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.

Table 9. General guidelines for standard RT-PCR primers

	Description
Storage buffer	18–30 nucleotides
Storage	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 3A, page 34). 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 3B, page 34). 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 3B, such primers may be used to detect DNA contamination.

<table>
<tr><th></th><th>Description</th></tr>
<tr><td>Sequence</td><td>

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.

</td></tr>
<tr><td>Concentration</td><td>

Spectrophotometric conversion for primers:

1 A_{260} unit ≡ 20–30 µg/ml

Molar conversions:

<table>
<tr><th>Primer length</th><th>pmol/µg</th><th>25 pmol (0.5 µM in 25 µl)</th></tr>
<tr><td>18mer</td><td>168</td><td>74 ng</td></tr>
<tr><td>20mer</td><td>152</td><td>83 ng</td></tr>
<tr><td>25mer</td><td>121</td><td>103 ng</td></tr>
<tr><td>30mer</td><td>101</td><td>124 ng</td></tr>
</table>

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

</td></tr>
<tr><td>Storage:</td><td>

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.

</td></tr>
</table>

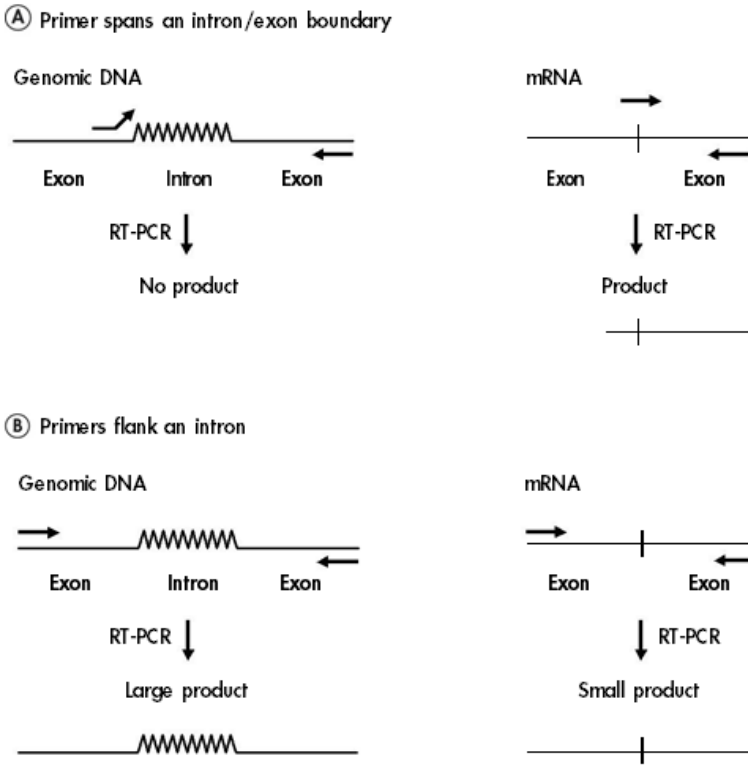

Figure 3. Primer design for RT-PCR. **A** Primer design to eliminate or **B** detect amplification from contaminating genomic DNA.

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, as enabled by QIAGEN OneStep *Ahead* RT-PCR Kit, often improves amplification specificity in RT-PCR using degenerate primers by reducing the formation of nonspecific RT-PCR products and primer–dimers. Table 10 gives recommendations to optimize one-step RT-PCR using degenerate primers. To help determine the best location for degenerate primers, Table 11 lists the codon redundancy of each amino acid.

Table 10. Guidelines for design and use of degenerate primers

	Description
Storage buffer	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 0.5 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 1 µg)
PCR annealing temperature	Reduce annealing temperature in steps of 2°C.

<table>
<tr><th></th><th>Description</th></tr>
<tr><td>Concentration</td><td>

Spectrophotometric conversion for primers:

1 A_{260} unit ≡ 20–30 µg/ml

Molar conversions:

<table>
<tr><th>Primer length</th><th>pmol/µg</th><th>25 pmol (0.5 µM in 25 µl)</th></tr>
<tr><td>18mer</td><td>168</td><td>74 ng</td></tr>
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<tr><td>30mer</td><td>101</td><td>124 ng</td></tr>
</table>

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.

</td></tr>
<tr><td>Storage:</td><td>

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.

</td></tr>
</table>

Table 11. Codon redundancy

Amino acid	rRNA size (kb)
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 F: 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 *Ahead* 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 *Ahead* RT-PCR Protocol. Subsequently, an aliquot of the first-round PCR product, for example 1 µl of a 1:1000 – 1:10000 dilution, is subjected to a second round of PCR. The second-round PCR is performed using two new primers that hybridize to sequences internal 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.

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, refer to the manufacturer's instructions.

Appendix G: 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 45). For more information about QIAquick or MinElute products, please visit **www.qiagen.com**.

Appendix H: 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 in order 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 Figure 3, page 34). If it is not possible to use such primers, DNA contamination can be detected using control reactions in which the addition of the RT-mix is omitted. Because no cDNA synthesis takes place in the control reaction, the only template available for amplification is contaminating DNA.

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. Use of freshly 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 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 I: 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. In order 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. Non-disposable 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.

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.

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

Ordering Information

Product	Contents	Cat. no.
QIAGEN OneStep *Ahead* RT-PCR Kit – for fast and efficient one-step RT-PCR		
QIAGEN OneStep *Ahead* RT-PCR (50)	For 50 one-step RT-PCRs: QIAGEN OneStep *Ahead* RT-Mix, 2.5x QIAGEN OneStep *Ahead* RT-PCR Master Mix*, Master Mix Tracer, Template Tracer, 5x Q-Solution, RNase-Free Water	220211
QIAGEN OneStep *Ahead* RT-PCR Kit (200)	For 200 one-step RT-PCRs: QIAGEN OneStep *Ahead* RT-Mix, 2.5x QIAGEN OneStep *Ahead* RT-PCR Master Mix*, Master Mix Tracer, Template Tracer, 5x Q-Solution, RNase-Free Water	220213
QIAGEN OneStep *Ahead* RT-PCR Kit (2000)	For 2000 one-step RT-PCRs: QIAGEN OneStep *Ahead* RT-Mix, 2.5x QIAGEN OneStep *Ahead* RT-PCR Master Mix*, Master mix Tracer, Template Tracer, 5x Q-Solution, RNase-Free Water	220216

* Contains 12.5 mM $MgCl_2$

Product	Contents	Cat. no.
Related Products		
Omniscript Reverse Transcriptase Kits – 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 Kits – 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
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

* Contains 5 mM of each dNTP

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

Product	Contents	Cat. no.
QIAGEN LongRange PCR Kit – 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
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 – 4 kb) in low elution volumes		
MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
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

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

Product	Contents	Cat. no.
RNeasy Maxi Kit (6)*	6 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-Free Reagents and Buffers	75161

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Visit **www.qiagen.com/geneXpression** to find out more about standardized solutions for gene expression analysis – from RNA preparation to real-time RT-PCR.

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

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Notes

Notes

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