October 2010

Sensiscript® Reverse Transcription Handbook

Sensiscript Reverse Transcriptase for

First-strand cDNA synthesis using <50 ng RNA
Two-tube RT-PCR
One-tube RT-PCR

QIAGEN

WWW.QIAGEN.COM

Contents

Kit Contents

Sensiscript® RT Kit **Catalog no.** **Number of reactions**	**(50)** **205211** **50**	**(200)** **205213** **200**
Sensiscript Reverse Transcriptase	50 reactions	200 reactions
Buffer RT, 10x	150 µl	4 x 150 µl
dNTP Mix, 5 mM each	100 µl	4 x 100 µl
RNase-free water	1.1 ml	4 x 1.1 ml
Handbook	1	1

Shipping and Storage

Sensiscript RT Kits are shipped on dry ice. The kits, including all reagents and buffers, should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the products can be kept at least until the expiration date (see kit, inside lid) without showing any reduction in performance.

Technical Assistance

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

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

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on the back page.

Product Use Limitations

The Sensiscript RT Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Product Warranty and Satisfaction Guarantee

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

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

Safety Information

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

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Product Specifications

Sensiscript Reverse Transcriptase is a new, unique enzyme, different from the reverse transcriptases of Moloney murine leukemia virus (MMLV) or avian myeloblastosis virus (AMV). Sensiscript Reverse Transcriptase is a recombinant heterodimeric enzyme expressed in *E.coli*.

RNA-directed DNA-polymerase activity (reverse transcriptase):	Yes
DNA-directed DNA-polymerase activity:	Yes
RNase H activity:	Yes
Functional absence of other RNase activity:	Yes
Functional absence of endonuclease activity:	Yes
Functional absence of exonuclease activity:	Yes
Functional absence of protease activity:	Yes

Quality Control

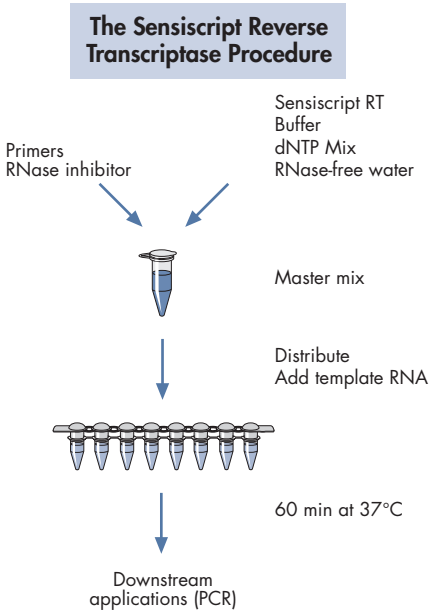
QIAGEN Quality Control carefully checks each lot of Sensiscript Reverse Transcriptase for cDNA-synthesis efficiency and functional absence of RNases, exonucleases, endonucleases, and proteases. All buffers and reagents are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination. For more information, please call for a certificate of analysis, or contact one of the QIAGEN Technical Service Departments or local distributors listed on the back page.

Introduction

QIAGEN offers two unique reverse transcriptases for a wide range of applications.

- **Sensiscript Reverse Transcriptase** is specially designed for highly sensitive reverse transcription with small amounts of RNA (<50 ng).
- **Omniscript® Reverse Transcriptase** is designed for efficient and sensitive reverse transcription with 50 ng – 2 µg RNA. Please see the *Omniscript Reverse Transcription Handbook* for protocols.

Sensiscript Reverse Transcriptase has a high affinity for RNA, which enables efficient and sensitive reverse transcription of any template, leading to high yields of cDNA. Sensiscript Reverse Transcriptase is provided ready to use with dNTPs and with an optimized reaction buffer which, together with the high affinity for RNA of the Sensiscript Reverse Transcriptase, enables read-through of templates with high GC content or complex secondary structures. Due to this pre-optimization, tedious pipetting and pre-incubation steps are eliminated, and no additional RNase H digestion step is needed (see flowchart).



Starting template

Reverse transcriptases are used in vitro for first-strand cDNA synthesis with RNA as the starting template. The efficiency of the reaction is 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 transcriptase. To ensure reproducible and efficient reverse transcription, it is important to determine the quality and quantity of the starting RNA. See "Appendix B: Storage, Quantification, and Determination of Quality of RNA", page 20.

For best results, we recommend starting with RNA purified using silica-gel–membrane technology. For example, RNeasy® Kits, PAXgene™ Blood RNA 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, high-quality mRNA can be used, purified, for example, with Oligotex® mRNA and Oligotex Direct mRNA Kits. See pages 27–29 for ordering information.

Reverse transcription of RNA is required when quantifying RNA (e.g., by RT-PCR or real-time RT-PCR) or cloning a sequence of RNA. For RNA quantification, RNA is transcribed into single-stranded cDNA using random primers, gene-specific primers, or oligo-dT primers that specifically hybridize to the poly-A–tail of mRNAs. For RNA cloning, reverse transcription is performed using gene-specific primers, and is followed by second-strand cDNA synthesis, catalyzed by DNA polymerases such as *Taq* DNA polymerase or DNA polymerases from *E. coli* or phages.

For the quantification of RNA transcripts, real-time RT-PCR is the most sensitive and reliable method. Real-time RT-PCR begins with the reverse transcription of RNA into cDNA, and is followed by PCR amplification of the cDNA. The quantity of cDNA is determined during the exponential phase of PCR by the detection of fluorescence signals that exceed a certain threshold. Fluorescence signals are generated by fluorophores incorporated into the PCR product (e.g., in SYBR® Green assays) or by fluorophores which are coupled to short oligonucleotide probes (i.e., in probe-based assays). In real-time RT-PCR, the level of RNA transcripts is calculated from the number of the PCR cycle at which the threshold is exceeded. This cycle is called the threshold cycle or the crossing point.

Sensiscript Reverse Transcriptase is suitable for use in the following applications:

- Standard RT and RT-PCR
- Real-time RT-PCR
- Synthesis of double-stranded cDNA for cloning

- RACE (Rapid Amplification of cDNA Ends)
- Differential display RT-PCR
- Linear RNA amplification (e.g., according to Eberwine*)
- Exponential RNA amplification (NASBA®, TMA)
- SAGE (Serial Analysis of Gene Expression)
- Labeling for microarrays
- Analysis of transcription start site by primer extension
- Analysis of LMD samples

Enzymatic activities of reverse transcriptase

Reverse transcriptase enzymes are generally derived from RNA-containing retroviruses such as avian myeloblastosis virus (AMV), Moloney murine leukemia virus (MMLV), or human immunodeficiency virus (HIV). QIAGEN now offers the first commercially available reverse transcriptases from a new source.

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

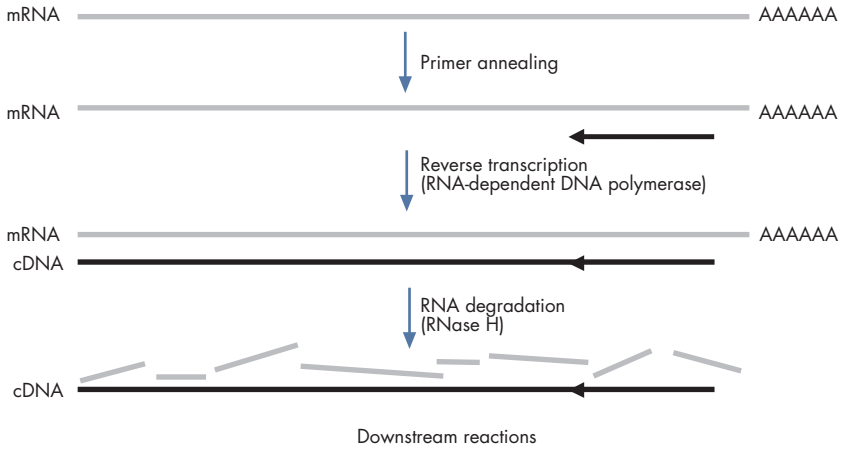


Figure 1 Sensiscript Reverse Transcriptase in first-strand cDNA synthesis.

* Phillips, J. and Eberwine, J.H. (1996) Antisense RNA Amplification: A Linear Amplification Method for Analyzing the mRNA Population from Single Living Cells. Methods **10**, 283.

RNA-dependent DNA polymerase (reverse transcriptase)

The RNA-dependent DNA-polymerase activity (reverse transcription) transcribes complementary DNA (cDNA) from an RNA template. This activity allows synthesis of cDNA for cloning, PCR, RNA sequencing, and primer extension.

RNase H activity of Sensiscript Reverse Transcriptase

A ribonuclease activity (RNase H) of Sensiscript Reverse Transcriptase specifically degrades only the RNA in RNA:DNA hybrids. This Sensiscript RNase H activity affects RNA that is hybridized to cDNA. It has no effect on pure RNA. Furthermore, the Sensiscript RNase H activity, acting during reverse transcription, may improve the sensitivity of subsequent PCR.

Reagents to Be Supplied by User

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

- **RNase inhibitor**

  RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, and C, as well as human placental RNases. For best results, we highly recommend using RNase inhibitor to minimize the risk of RNA degradation during experimental setup. RNase inhibitor is commonly supplied at a concentration of 40 units/µl.

- **Primers**

  Oligo-dT primers and specific primers are commonly used at a final concentration of 0.1–1.0 µM each, and random nonamers are commonly used at a final concentration of 10 µM in the reverse-transcription reaction.

For RT-PCR:

- ***Taq* DNA polymerase, PCR buffer, primers, reagents, and additional nucleotides for PCR.**

  See "Guidelines: Two-Tube RT-PCR" and "Guidelines: One-Tube RT-PCR", page 14 and 15.

Sensiscript Protocol for Reverse Transcription of <50 ng RNA

This is the standard protocol for first-strand cDNA synthesis using <50 ng RNA and Sensiscript Reverse Transcriptase from QIAGEN. Sensiscript Reverse Transcriptase is especially designed for optimal performance with low amounts of RNA. For best results with >50 ng RNA, we recommend using Omniscript Reverse Transcriptase from QIAGEN. The amount of RNA corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present, and regardless of the primers used or cDNA analyzed.

Important points before starting

- If working with RNA for the first time, please read the recommendations in "Appendix A: General Remarks for Handling RNA" (page 18).
- The protocol is optimized for use with <50 ng RNA. This amount corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present, and regardless of the primers used or cDNA analyzed. For best results with >50 ng RNA, use Omniscript Reverse Transcriptase from QIAGEN.
- Set up all reactions on ice to avoid premature cDNA synthesis and minimize the risk of RNA degradation.
- Separate denaturation and annealing steps are generally not necessary. However, for some RNAs with a high degree of secondary structure, a denaturation step may be desired. If so, denature the RNA in RNase-free water before reaction setup: incubate the RNA for 5 min at 65°C, then place immediately on ice. Do not denature the RNA in the reaction mix.
- When using oligo-dT primers, a primer length of at least 12 nucleotides and a final concentration of 1 µM is recommended. When using random primers, a primer length of 9 nucleotides and final concentration of 10 µM is recommended. Concentration and length of other primers should be individually optimized.
- For some transcripts, more sensitive detection in the subsequent PCR or real-time PCR is possible if a mixture of oligo-dT primers and random primers are used. If using random primers for reverse transcription, we strongly recommend the use of random nonamers at a final concentration of 10 µM.
- If PCR is to be performed following reverse transcription (two-tube RT-PCR), see "Guidelines: Two-Tube RT-PCR", page 14. Always be sure to:
  - Set up all reaction mixtures in an area separate from that used for DNA preparation or RT-PCR product analysis.
  - Use reagents and pipets set aside only for the setup of reverse transcription and PCR.
  - Use disposable pipet tips containing hydrophobic filters to minimize the risk of cross contamination.

Procedure

1. **Thaw template RNA solution on ice. Thaw the primer solutions (not supplied), 10x Buffer RT, dNTP Mix, and RNase-free water at room temperature. Store on ice immediately after thawing. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.**
2. **Dilute RNase inhibitor (not supplied) to a final concentration of 10 units/µl in ice-cold 1x Buffer RT (dilute an aliquot of the 10x Buffer RT accordingly using the RNase-free water supplied). Mix carefully by vortexing for no more than 5 s, and centrifuge briefly to collect residual liquid from the sides of the tube.**

   Commercially available RNase inhibitor is commonly supplied at 40 units/µl. Dilute to make it easier to pipet small amounts when preparing the master mix in step 3.

   Note: Prepare a fresh dilution of RNase inhibitor. To minimize the amount of RNase inhibitor and Buffer RT used, dilute no more than needed for your current series of reactions.
3. **Prepare a fresh master mix on ice according to the table on page 13. Mix thoroughly and carefully by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tube, and store on ice.**

   The master mix contains all components required for first-strand synthesis except the template RNA. If setting up more than one reaction, prepare a volume of master mix 10% greater than that required for the total number of reverse-transcription reactions to be performed.

   Note: If performing RT-PCR, see "Guidelines: Two-Tube RT-PCR" and "Guidelines: One-Tube RT-PCR", page 14 and 15.
4. **If setting up more than one reverse-transcription reaction, distribute the appropriate volume of master mix into individual reaction tubes. Keep tubes on ice.**
5. **Add template RNA to the individual tubes containing the master mix. Mix thoroughly and carefully by vortexing for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tube.**

   Note: The protocol is optimized for use with <50 ng RNA. This amount corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present, and regardless of the primers used or cDNA analyzed.
6. **Incubate for 60 min at 37°C.**
7. **Add an aliquot of the finished reverse-transcription reaction to the PCR mix.**

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

Store reverse-transcription reactions on ice and proceed directly with PCR,* or for long-term storage, store reverse-transcription reactions at –20°C.

Component	Volume/reaction	Final concentration
Master mix		
10x Buffer RT	2.0 µl	1x
dNTP Mix (5 mM each dNTP)	2.0 µl	0.5 mM each dNTP
Oligo-dT primer (10 µM)*	2.0 µl	1 µM*
RNase inhibitor (10 units/µl)†	1.0 µl	10 units (per reaction)
Sensiscript Reverse Transcriptase	1.0 µl	
RNase-free water	Variable	–
Template RNA		
Template RNA, added at step 5	Variable	<50 ng‡ (per reaction)
Total volume	**20 µl**	**–**

* Not provided. If using specific primers, concentration should be 0.1–1.0 µM. If using random primers, a primer length of 9 nucleotides and final concentration of 10 µM is recommended. See "Important notes before starting".

† Not provided. If supplied at >10 units/µl, dilute in 1x Buffer RT (dilute an aliquot of the 10x Buffer RT accordingly).

‡ This amount corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present, and regardless of the primers used or cDNA analyzed.

Notes

* We recommend the QuantiTect Probe PCR Kit (cat. no. 204343) for real-time PCR using sequence-specific probes, or the QuantiTect SYBR Green PCR Kit (cat. no. 204143) for real-time PCR using SYBR Green I.

Guidelines: Two-Tube RT-PCR

In two-tube RT-PCR, cDNA is first synthesized by reverse transcription. An aliquot of the finished reverse-transcription reaction is then used for PCR. In two-tube RT-PCR, reverse transcription and PCR are performed sequentially in two separate reaction tubes. Random primers, oligo-dT primers, or gene-specific primers can be used in two-step RT-PCR. Use of oligo-dT or random primers allows quantification of a cDNA pool from one reverse-transcription reaction. In addition, cDNAs can be stored for later analyses.

1. **Carry out the reverse-transcription reaction following the protocol on pages 11–13, using Sensiscript Reverse Transcriptase and <50 ng RNA.**

   Note: The protocol is optimized for use with <50 ng RNA. This amount corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present, and regardless of the primers used or cDNA analyzed. For best results with >50 ng RNA, we recommend using Omniscript Reverse Transcriptase from QIAGEN.

2. **Add an aliquot of the finished reverse-transcription reaction to the PCR mix.**

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

3. **Carry out PCR with *Taq* DNA polymerase as recommended by the supplier.**

   We have consistently obtained excellent results using *Taq* DNA Polymerase and HotStarTaq® DNA Polymerase from QIAGEN. See page 24 and 25 for ordering information.

Guidelines: One-Tube RT-PCR

In one-tube RT-PCR, both reverse transcription and PCR are performed in the same tube. *Taq* DNA polymerase and all necessary primers and reagents are added during reaction setup. cDNA is synthesized by reverse transcription at 37°C while the *Taq* DNA polymerase in the reaction mix has little activity. After allowing sufficient time for reverse transcription, the temperature is raised, inactivating the reverse transcriptase and allowing the *Taq* DNA polymerase to amplify the cDNA.

Since both the reverse-transcription reaction and PCR are performed in the same buffer, it is not possible to separately optimize the two reactions. The reverse transcription step can have poor efficiency with some PCR buffers. For one-tube RT-PCR without optimization, we recommend using the QIAGEN OneStep RT-PCR Kit.

Troubleshooting Guide

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

		Comments and suggestions
Little or no cDNA product		
a)	Incorrect setup	Be sure to set up the reaction on ice.
b)	Volume of reverse-transcription reaction added to the PCR or real-time PCR was too high	Adding a high volume of reverse transcription reaction to the PCR mix may reduce amplification efficiency and the linearity of the reaction. Generally, the volume of reverse-transcription reaction added should not exceed 1/5 of the final PCR volume.
c)	Temperature of reaction	Reverse transcription should be carried out at 37°C. Check the temperature of your heating block or water bath. In rare cases, when analyzing RNAs with a very high degree of secondary structure, it may be advantageous to increase the temperature to 42°C or even 50°C. However, temperatures >42°C will reduce the activity of Sensiscript Reverse Transcriptase and therefore affect the cDNA yield and length when using standard templates.
d)	Pipetting error or missing reagent	Check the pipettes used for experimental set up. Mix all reagents well after thawing, store on ice immediately after thawing, and repeat reverse-transcription reaction.
e)	Poor quality or wrong quantity of starting template	Check the concentration, integrity, and purity of starting RNA-template (see "Appendix B: Storage, Quantification, and Determination of Quality of RNA", page 20). Mix well after thawing the RNA template, and use RNase inhibitor at a final concentration of 0.5 U/µl in the assay. Even minute amounts of RNases can affect the length of cDNA-synthesis products and sensitivity in RT-PCR, especially with small amounts of RNA.
f)	RNA concentration too high	Sensiscript Reverse Transcriptase is designed for use with <50 ng RNA. This amount corresponds to the entire amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present, and regardless of the primers used or cDNA analyzed. For best results with >50 ng RNA, we recommend using

		QIAGEN Omniscript Reverse Transcriptase. See page 24 for ordering information.
g)	Incorrect nucleotide concentration or nucleotide degradation	Use the dNTP Mix provided in the kit. Different nucleotide concentrations can reduce the amount of cDNA product. Storage of nucleotides at room temperature will cause degradation of the nucleotides.
h)	Incorrect denaturation conditions	Usually, denaturation of the RNA–primer mix is not necessary, but, in some cases denaturation of the starting template allows more efficient priming. If so, denature the RNA in RNase-free water (provided in the kit). High denaturation temperatures (>65°C) or prolonged denaturation time (>5 min) can affect the integrity of RNA, causing shortened cDNA products.
i)	Incorrect primer concentration or primer degradation	Check the concentration and integrity of primer used for reverse transcription. If necessary, perform reverse transcription with different primer concentrations or primers. If using random primers, be sure to perform the RT reaction with random nonamers at a final concentration of 10µM.
j)	Short incubation time	The standard reverse-transcription reaction requires a 60-minute incubation. In rare cases, when analyzing RNAs with a very high degree of secondary structure, it may be advantageous to increase the incubation time to 2 hours.

Short cDNA products

a)	Various reasons	See points (c)–(j) under "Little or no cDNA product" above.
b)	Incubation temperature too high	Reverse transcription should be carried out at 37°C. Higher temperatures may reduce the length of cDNA products. Check the temperature of your heating block or water bath.
c)	Reverse transcriptase inactivated following reaction	Heat inactivation of Sensiscript Reverse Transcriptase is not recommended prior to analysis of long cDNAs, which are more susceptible to DNA cleavage. Perform first-strand cDNA synthesis without final heat inactivation of reverse transcriptase.

Appendix A: 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 non-disposable 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.

Disposable plasticware

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

Non-disposable plasticware

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* (see "Solutions", page 19). 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 0.1% 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.

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

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water,* and then rinsed with ethanol† and allowed to dry.

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% 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. Trace amounts of DEPC will modify purine residues in RNA by carboxymethylation. Carboxymethylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: QIAGEN reverse-transcriptase buffers and RNase-free water are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

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

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage of RNA

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

Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. To ensure significance, readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml (A_{260}=1 ⇒ 40 µg/ml). This relation is valid only for measurements in water. Therefore, if it is necessary to dilute the RNA sample, this should be done in water. As discussed on the next page (see "Purity of RNA", page 21), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH,* 1 mM EDTA* followed by washing with RNase-free water (see "Solutions", page 19). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 20 µl of RNA sample + 180 µl distilled water (1/10 dilution).

Measure absorbance of diluted sample in a 0.2-ml cuvette (RNase-free).

A_{260} = 0.2

Concentration of RNA sample	= 40 µg/ml x A_{260} x dilution factor
	= 40 µg/ml x 0.2 x 10
	= 80 µg/ml
Total amount	= concentration x volume of sample in ml
	= 80 µg/ml x 0.1 ml
	= 8 µg of RNA

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

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, 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 with the same solution.

For determination of RNA concentration, however, we still 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 (see "Quantification of RNA", page 20).

Integrity of RNA

The integrity and size distribution of total RNA can be checked by denaturing agarose gel electrophoresis‡ and ethidium bromide‡ staining (see "Appendix C: Protocol for RNA Formaldehyde Agarose Gel Electrophoresis", page 22). The respective ribosomal bands (Table 1) should appear as sharp bands on the stained gel. 28S ribosomal RNA bands should be present at approximately twice the amounts of the 18S RNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

Table 1. Size of ribosomal RNAs from various sources

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

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

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

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

Appendix C: Protocol for RNA Formaldehyde Agarose Gel Electrophoresis

The following protocol for formaldehyde agarose (FA) gel electrophoresis is routinely used at QIAGEN and gives enhanced sensitivity for gel and subsequent analysis (e.g., northern blotting). A key feature is the concentrated RNA loading buffer that allows a larger volume of RNA sample to be loaded onto the gel than conventional protocols (e.g., Sambrook et al., eds. (1989) Molecular cloning — a laboratory manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

FA gel preparation

To prepare FA gel (1.2% agarose) of size 10 x 14 x 0.7 cm, mix:

1.2 g	agarose*
10 ml	10x FA gel buffer (see composition below)

add RNase-free water to 100 ml

If smaller or larger gels are needed, adjust the quantities of components proportionately.

Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde* and 1 µl of a 10 mg/ml ethidium bromide* stock solution. Mix thoroughly and pour onto gel support. Prior to running the gel, equilibrate in 1x FA gel running buffer (see composition below) for at least 30 min.

RNA sample preparation for FA gel electrophoresis

Add 1 volume of 5x RNA loading buffer (see composition below) to 4 volumes of RNA sample (for example 10 µl of loading buffer and 40 µl of RNA) and mix.

Incubate for 3–5 min at 65°C, chill on ice, and load onto the equilibrated FA gel.

Gel running conditions

Run gel at 5–7 V/cm in 1x FA gel running buffer.

Composition of FA gel buffers

10x FA gel buffer

200 mM	3-[N-morpholino]propanesulfonic acid (MOPS) (free acid)*
50 mM	sodium acetate*
10 mM	EDTA*

pH to 7.0 with NaOH*

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

1x FA gel running buffer

100 ml	10x FA gel buffer
20 ml	37% (12.3 M) formaldehyde*
880 ml	RNase-free water

5x RNA loading buffer

16 µl	saturated aqueous bromophenol blue solution*†
80 µl	500 mM EDTA, pH 8.0*
720 µl	37% (12.3 M) formaldehyde*
2 ml	100% glycerol*
3.084 ml	formamide*
4 ml	10 x FA gel buffer

RNase-free water to 10 ml

Stability: approximately 3 months at 4°C

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

† To make a saturated solution, add solid bromophenol blue to distilled water. Mix and continue to add more bromophenol blue until no more will dissolve. Centrifuge to pellet the undissolved powder, and carefully pipet the saturated supernatant.

Ordering Information

Product	Contents	Cat. no.
Sensiscript RT Kits — for reverse transcription using <50 ng RNA		
Sensiscript RT Kit (50)	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water	205211
Sensiscript RT Kit (200)	For 200 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water	205213
Omniscript RT Kits — for reverse transcription using ≥50 ng RNA		
Omniscript RT Kit (50)*	For 50 reverse-transcription reactions: 200 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water	205111
Omniscript RT Kit (200)*	For 200 reverse-transcription reactions: 800 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix (containing 5 mM each dNTP), RNase-free water	205113
Related Products		
HotStarTaq DNA Polymerase — for hot-start PCR		
HotStarTaq DNA Polymerase (250 U)	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203203
HotStarTaq DNA Polymerase (1000 U)	4 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	203205

* Other kit sizes and/or formats available; please inquire.

Ordering Information

Product	Contents	Cat. no.
***Taq* DNA Polymerase — for standard PCR**		
Taq DNA Polymerase (250 U)*	250 units *Taq* DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$	201203
Taq PCR Core Kit (250 U)*	250 units *Taq* DNA Polymerase, 10x PCR Buffer (containing 15 mM $MgCl_2$), 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix (containing 10 mM each dNTP)	201223
Taq PCR Master Mix Kit (250 U)*	3 x 1.7 ml *Taq* PCR Master Mix containing 250 units *Taq* DNA Polymerase total and providing a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP; 3 x 1.7 ml distilled water	201443
QuantiTect® Probe Kits — for quantitative, real-time PCR and RT-PCR using sequence-specific probes		
QuantiTect Probe PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix (providing a final concentration of 4 mM $MgCl_2$), 2 x 1.9 ml RNase-free water	204343
QuantiTect Probe RT-PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe RT-PCR Master Mix (providing a final concentration of 4 mM $MgCl_2$), 100 µl QuantiTect RT Mix, 2 x 1.9 ml RNase-free water	204443

* Other kit sizes and/or formats available; please inquire.

Ordering Information

Product	Contents	Cat. no.
QuantiTect SYBR® Green Kits — for quantitative, real-time PCR and RT-PCR using SYBR Green		
QuantiTect SYBR Green PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix (providing a final concentration of 2.5 mM $MgCl_2$), 2 x 1.9 ml RNase-free water	204143
QuantiTect SYBR Green RT-PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green RT-PCR Master Mix (providing a final concentration of 2.5 mM $MgCl_2$), 100 µl QuantiTect RT Mix, 2 x 1.9 ml RNase-free water	204243
QIAGEN Oligonucleotide Synthesis Service — high-quality oligos, modified oligos, and longmers		
Oligonucleotide Synthesis Service	Custom-made oligonucleotides and a wide range of modified oligos, including Molecular Beacons, dual-labeled probes, and many more	Inquire

* Larger kit sizes available; please inquire.

Ordering Information

Product	Contents	Cat. no.
QIAquick® PCR Purification Kits — 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 Kits — 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 (2ml)	28004
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
RNeasy Plant Kits — for total RNA isolation from plants and fungi		
RNeasy Plant Mini Kit (20)*	20 RNeasy Mini Spin Columns, 20 QIAshredder™ Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74903

* Other kit sizes and/or formats available; please inquire.

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 Kits — for high-throughput RNA minipreparation from cells		
RNeasy 96 Kit (4)*†	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Collection Microtubes (1.2 ml), Caps, RNase-free Reagents and Buffers	74181
QIAamp RNA Blood Kits — for total RNA isolation from whole human blood		
QIAamp RNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304
QIAamp Viral RNA Kits — for isolation of viral RNA from cell-free body fluids		
QIAamp Viral RNA Mini Kit (50)†	For 50 microspin viral RNA preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Buffers, Collection Tubes (2 ml)	52904
Oligotex mRNA Kits — for isolation of poly A$^+$ mRNA from total RNA		
Oligotex mRNA Mini Kit†‡	For 12 mRNA minipreps: 200 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	70022

* Requires use of either QIAvac 96 or the Plate Rotor 2 x 96 and a special centrifuge.

† Larger kit sizes and/or formats available; please inquire.

‡ Not available in Japan.

Ordering Information

Product	Contents	Cat. no.
Oligotex Direct mRNA Kits — for isolation of poly A+ mRNA directly from animal cells or tissues		
Oligotex Direct mRNA Micro Kit*†	For 12 mRNA micropreps: 250 µl Oligotex Suspension, Small Spin Columns, Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	72012
Oligotex Direct mRNA Mini Kit*†	For 12 mRNA minipreps: 420 µl Oligotex Suspension, Small Spin Columns,Collection Tubes (1.5 ml), RNase-free Reagents and Buffers	72022
PAXgene Blood RNA Kits — for isolation of cellular RNA from whole blood		
PAXgene Blood RNA Kit (50)	For 50 RNA preps: 50 PAXgene RNA Spin Columns, buffers, proteinase K, and processing tubes; to be used with PAXgene Blood RNA Tubes (cat. no. 762115 US and Canada; 762125 all other countries)	762134
PAXgene Blood RNA Validation Kit (10)	For 10 RNA preps: 10 PAXgene Blood RNA Tubes, 10 PAXgene RNA Spin Columns, buffers, proteinase K, and processing tubes	762132
PAXgene 96 Blood RNA Kit (4)	For 4 x 96 RNA preps: 4 PAXgene 96 RNA Plates, 4 PAXgene 96 Filter Plates, buffers, proteinase K, RNase-Free DNase Sets, AirPore Tape Sheets, collection vessels; to be used with PAXgene Blood RNA Tubes (cat. no. 762115 US and Canada; 762125 all other countries	762331

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* Larger kit sizes and/or formats available; please inquire.

† Not available in Japan.

Notes

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