Omniscript™ Reverse Transcriptase Handbook

Omniscript Reverse Transcriptase
for
First-strand cDNA synthesis
Two-tube RT-PCR

April 1999

QIAGEN

Trademarks

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http://www.qiagen.com

Contents

Kit Contents

Omniscript RT Kit	**(10)**	**(50)**	**(200)**
Catalog No.	**205110**	**205111**	**205113**
Number of reactions*	**10**	**50**	**200**
Omniscript Reverse Transcriptase	40 units	200 units	800 units
Buffer RT, 10x	30 µl	150 µl	4 x 150 µl
dNTP Mix, 5 mM each	20 µl	100 µl	4 x 100 µl
RNase-free water	1.1 ml	1.1 ml	4 x 1.1 ml

** Number of standard reactions (50 ng – 2 µg RNA each)*

Shipping and Storage

Omniscript™ 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.

Solutions and Reagents to be Supplied by User

- **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, random hexamers, or specific primers are commonly used at a final concentration of 0.1–1 µM each in the reverse-transcription reaction.

For RT-PCR:

- ***Taq* polymerase, PCR buffer, primers, reagents, and additional nucleotides for PCR**
  See "Guidelines: RT-PCR", page 13.

Technical Assistance Click Here for Contact Addresses

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 Omniscript 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 last page.

Product Use Limitations

Omniscript RT Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

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.

Introduction

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

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

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 (Fig. 1). A description of these activities is given below.

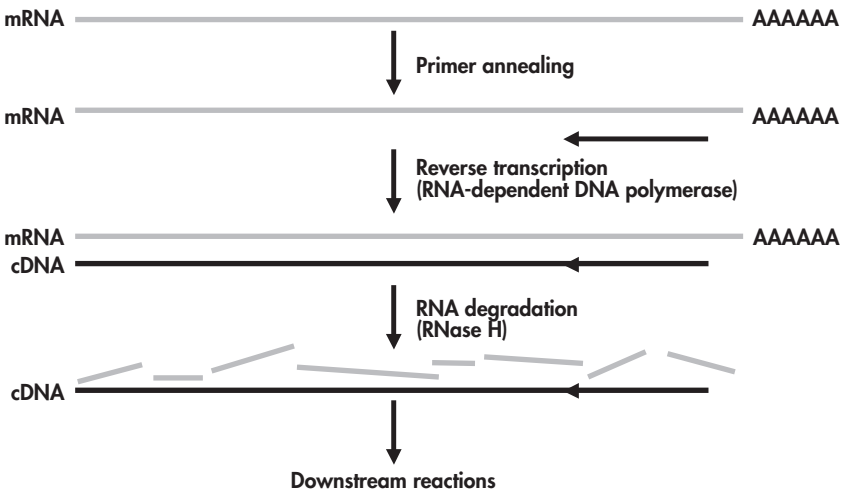

Figure 1. *Omniscript Reverse Transcriptase in first-strand cDNA synthesis*

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 Omniscript Reverse Transcriptase

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

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, Quantitation, and Determination of Quality of RNA", page 18.

For best results, we recommend starting with RNA purified using silica-gel–membrane technology. For example, RNeasy® Kits, QIAamp® Viral RNA Kits, and the QIAamp RNA Blood Mini Kit can be used to isolate RNA from a variety of starting materials and provide high-quality RNA ideal for use in reverse-transcription and RT-PCR applications. Alternatively, high-quality mRNA can be used, purified, for example, with Oligotex™ mRNA and Oligotex Direct mRNA Kits. See page 22 for ordering information.

Product Specifications

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

Unit definition:	Omniscript Reverse Transcriptase activity (deoxynucleoside-triphosphate:DNA deoxynucleotidyl-transferase, RNA-directed, E.C.2.7.7.49) is determined by an assay based on Houts, G.E. et al. (1979) J. Virol. **29**, 517, where one unit is defined as the enzyme activity which incorporates 1.0 nmol TTP into acid-insoluble products in 10 minutes at 37°C with poly-A template RNA and oligo-$dT_{12–18}$ primer.
RNA-directed DNA-polymerase activity (reverse transcriptase):	Yes; 4 units/µl
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 assays the units and carefully checks each lot of Omniscript 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 last page.

Omniscript Protocol for Reverse Transcription

This is the standard protocol for first-strand cDNA synthesis using 50 ng to 2 µg RNA and 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. With >2 µg RNA, scale up the reaction linearly to the appropriate volume. For best results with <50 ng RNA, we recommend using Sensiscript Reverse Transcriptase from QIAGEN, which is especially designed for optimal performance with low amounts of RNA.

Important notes before starting

- If working with RNA for the first time, please read the recommendations in "Appendix A: General Remarks for Handling RNA" (page 16).
- The protocol is optimized for use with 50 ng to 2 µg of 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. With >2 µg RNA, scale up the reaction linearly to the appropriate volume.
- 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. Concentration and length of other primers should be individually optimized. Final primer concentrations of 0.1–1.0 µM are generally recommended.
- If PCR is to be performed following reverse transcription (two-tube RT-PCR), see "Guidelines: RT-PCR", page 13. 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 your 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 sec, and centrifuge briefly to collect residual liquid from the sides of the tubes.**

   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 on ice a fresh master mix according to the table on page 11. Mix thoroughly and carefully by vortexing for no more than 5 sec. 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: The protocol is optimized for use with 50 ng to 2 µg RNA. With >2 µg RNA, scale up the reaction linearly to the appropriate volume. Calculate the scale-up factor from 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 example, with 4 µg RNA, double the volumes of all reaction components for a final 40-µl reaction volume.

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 sec. Centrifuge briefly to collect residual liquid from the walls of the tube.**

6. **Incubate for 60 min at 37°C.**

7. **For analysis of shorter cDNAs by PCR or other downstream enzymatic applications, Omniscript Reverse Transcriptase can be inactivated by heating the reaction mixture to 93°C for 5 min followed by rapid cooling on ice.**

   Note: Inactivation of Omniscript Reverse Transcriptase is not recommended prior to analysis of long cDNAs, which are more susceptible to DNA cleavage.

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 20-µl reaction)
Omniscript Reverse Transcriptase	1.0 µl	4 units (per 20-µl reaction)
RNase-free water	Variable	
Template RNA		
Template RNA, added at step 5	Variable	Up to 2 µg‡ (per 20-µl reaction)
Total volume	**20.0 µl**	–

* *Not provided. If using hexamer primers or specific primers, concentration should be individually optimized. 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

Guidelines: RT-PCR

RT-PCR can be performed as two-tube RT-PCR or one-tube RT-PCR. In two-tube RT-PCR, the reverse-transcription reaction and PCR are performed sequentially in two separate reaction tubes. In one-tube RT-PCR, both reactions are performed in the same tube: after completion of the reverse-transcription reaction, the temperature is raised and the thermal cycling (PCR) begins. Two-tube RT-PCR is generally recommended over one-tube RT-PCR so that the PCR step can be optimized without affecting the reverse-transcription step.

Recommendations for two-tube RT-PCR are given below. For one-tube RT-PCR, we recommend using Sensiscript Reverse Transcriptase from QIAGEN. Please see the *Sensiscript Reverse Transcriptase Handbook* for guidelines.

Guidelines for two-tube RT-PCR using Omniscript Reverse Transcriptase

1. **Carry out the reverse-transcription reaction following the protocol on pages 9–11, using Omniscript Reverse Transcriptase.**

   Note: The protocol is optimized for use with 50 ng to 2 µg RNA. With >2 µg RNA, scale up the reaction linearly to the appropriate volume. Calculate the scale-up factor from 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 Sensiscript Reverse Transcriptase from QIAGEN, which is especially designed for optimal performance with small amounts of RNA.

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 22 for ordering information.

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(s) in this handbook or molecular biology applications (see last page for contact information).

	Comments and Suggestions
Little or no cDNA product	
a) Incorrect setup	Be sure to set up the reaction on ice.
b) 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 Omniscript Reverse Transcriptase and therefore affect the cDNA yield and length when using standard templates.
c) 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.
d) Poor quality or wrong quantity of starting template	Check the concentration, integrity, and purity of starting RNA-template (see "Appendix B: Storage, Quantitation, and Determination of Quality of RNA", page 18). 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.
e) RNA concentration too high or too low	Omniscript Reverse Transcriptase is designed for use with 50 ng – 2 µg 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. With >2 µg RNA, scale up the reaction linearly to the appropriate volume. For best results with <50 ng,

	we recommend using QIAGEN Sensiscript Reverse Transcriptase. See page 22 for ordering information.
f) 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.
g) 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.
h) 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.
i) 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)–(i) 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 Omniscript 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 17). 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.

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

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.

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

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

Appendix B: Storage, Quantitation, 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.

Quantitation 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 below (see "Purity of RNA", page 19), 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 17). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

An example of the calculation involved in RNA quantitation 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

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 "Quantitation of RNA", page 18).

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 Formaldehyde Agarose Gel Electrophoresis", page 20). 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.*

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

** Toxic and/or mutagenic. Take appropriate safety measures.*

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

** Toxic and/or mutagenic. Take appropriate safety measures.*

† 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.
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,* RNase-free water	205111
Omniscript RT Kit (200)	For 200 reverse-transcription reactions: 800 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-free water	205113
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,* RNase-free water	205211
Sensiscript RT Kit (200)	For 200 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,* RNase-free water	205213
Related Products		
HotStarTaq DNA Polymerase† — for hot-start PCR		
HotStarTaq DNA Polymerase (250)	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,‡ 5x Q-Solution, 25 mM $MgCl_2$	203203
HotStarTaq DNA Polymerase (1000)	4 x 250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,‡ 5x Q-Solution, 25 mM $MgCl_2$	203205

* *Contains 5 mM each dNTP*

† *Purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e. an authorized thermal cycler.*

‡ *Contains 15 mM $MgCl_2$*

Ordering Information

Product	Contents	Cat. No.
***Taq* DNA Polymerase* — for standard PCR**		
Taq DNA Polymerase (250)†	250 units *Taq* DNA Polymerase, 10x PCR Buffer,‡ 5x Q-Solution, 25 mM $MgCl_2$	201203
Taq DNA Polymerase (1000)†	4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,‡ 5x Q-Solution, 25 mM $MgCl_2$	201205
Taq PCR Core Kit (250)	250 units *Taq* DNA Polymerase, 10x PCR Buffer,‡ 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix§	201223
Taq PCR Core Kit (1000)	4 x 250 units *Taq* DNA Polymerase, 10x PCR Buffer,‡ 5x Q-Solution, 25 mM $MgCl_2$, dNTP Mix§	201225
Taq PCR Master Mix Kit (250)	3 x 1.7 ml *Taq* PCR Master Mix¶ containing 250 units *Taq* DNA Polymerase total, 3 x 1.7 ml distilled H_2O	201443
Taq PCR Master Mix Kit (1000)	12 x 1.7 ml *Taq* PCR Master Mix¶ containing 1000 units *Taq* DNA Polymerase total, 12 x 1.7 ml distilled H_2O	201445
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

** Purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e. an authorized thermal cycler.*

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

‡ Contains 15 mM MgCl2

§ Contains 10 mM each dNTP

¶ Provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM each dNTP

Ordering Information

Product	Contents	Cat. No.
RNeasy Kits — for total RNA isolation from animal cells or tissues, yeast, or bacteria		
RNeasy Mini Kit (20)*	20 RNeasy Mini Spin Columns, Collection Tubes (1.5-ml and 2-ml), RNase-free Reagents and Buffers	74103
RNeasy Midi Kit (10)	10 RNeasy Midi Spin Columns, Collection Tubes (15-ml), RNase-free Reagents and Buffers	75142
RNeasy Midi Kit (50)	50 RNeasy Midi Spin Columns, Collection Tubes (15-ml), RNase-free Reagents and Buffers	75144
RNeasy Maxi Kit (6)	6 RNeasy Maxi Spin Columns, Collection Tubes (50-ml), RNase-free Reagents and Buffers	75161
RNeasy Maxi Kit (24)	24 RNeasy Maxi Spin Columns, Collection Tubes (50-ml), RNase-free Reagents and Buffers	75163
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
RNeasy Plant Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5-ml and 2-ml), RNase-free Reagents and Buffers	74904
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
RNeasy 96 Kit (24)†	For 24 x 96 total RNA preps: 24 RNeasy 96 Plates, Collection Microtubes (1.2-ml), Caps, RNase-free Reagents and Buffers	74183

** Larger kit sizes available; please inquire.*

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

Ordering Information

Product	Contents	Cat. No.
QIAamp RNA Blood Kits — for total RNA isolation from whole human blood		
QIAamp RNA Blood Mini Kit (20)*†	20 QIAamp Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5-ml and 2-ml), RNase-free Reagents and Buffers	52303
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)	29504
QIAamp Viral RNA Mini Kit (250)†	For 250 microspin viral RNA preps: 250 QIAamp Mini Spin Columns, Carrier RNA, Buffers, Collection Tubes (2-ml)	29506
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
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

* *Larger kit sizes available; please inquire.*

† *QIAamp Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of QIAamp Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. QIAamp Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.*

‡ *Not available in Japan*

Notes

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