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ipsogen® RT Handbook

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For reverse transcription of RNA

For research use only. Not for use in diagnostic procedures.

REF 679913

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QIAGEN Sample and Assay Technologies

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Contents

Intended Use

The *ipsogen* RT Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information 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.

Summary and Explanation

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. 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. For best results, we recommend starting with RNA purified using silica-gel–membrane technology. For example, the QIAGEN RNeasy® Mini Kit (cat. no. 74104), or RNeasy Midi Kit (cat. no. 75144) can be used to isolate RNA from a variety of starting materials and provide high-quality RNA highly suited for use in reverse-transcription and RT-PCR applications.

Principle of the Procedure

Reverse transcriptase is a multifunctional enzyme with 3 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 3 activities allows transcription of the single-stranded RNA genome into double-stranded DNA for retroviral infection. The RNA-dependent DNA-polymerase activity (reverse transcription) transcribes cDNA from an RNA template. This activity allows synthesis of cDNA for cloning, PCR, and RNA sequencing.

Materials Provided

Kit contents

***ipsogen* RT Kit**	**(33)**
Catalog no.	**679913**
Number of reactions	**33**
Reverse Transcriptase	36 µl
5x RT Buffer for reverse transcription	180 µl
dNTP Mix*	72 µl
Random Primer†	190 µl
RNase Inhibitor	18 µl
DTT‡	45 µl

* Deoxynucleotides 10 mM each.

† Random nonamer oligonucleotide.

‡ Dithiothreitol.

Materials Required but Not Provided

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

Consumables

- Nuclease-free, aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 0.5 ml or 0.2 ml RNase- and DNase-free PCR tubes
- Ice

Reagents

- Nuclease-free, PCR-grade water
- Reagents for 1.2% formaldehyde agarose gel electrophoresis

Equipment

- Microliter pipets* dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge* with rotor for 0.2 ml/0.5 ml reaction tubes (capable of attaining 10,000 rpm)
- Spectrophotometer,* or Agilent® BioAnalyzer®,* for RNA quantitation
- Equipment* for pulsed-field gel electrophoresis
- Thermomixer, heated orbital incubator, heating block, or water bath* (reverse transcription step)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

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.

Discard sample and assay waste according to your local safety regulations.

General precautions

qPCR tests require good laboratory practices, including equipment maintenance, that are dedicated to molecular biology and compliant with applicable regulations and relevant standards.

This kit is intended for research use. Reagents and instructions supplied in this kit have been tested for optimal performance. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data. All reagents are formulated specifically for use with this kit. For optimal performance of the procedure, no substitutions should be made.

Determining transcript levels using qPCR requires both the reverse transcription of the mRNA and the amplification of the generated cDNA by PCR. Therefore, the entire assay procedure must be performed under RNase-/DNase-free conditions.

Use extreme caution to prevent:

- RNase/DNase contamination, which might cause degradation of the template mRNA and the generated cDNA
- mRNA or PCR carryover contamination resulting in false positive signal

We therefore recommend the following.

- Use nuclease-free labware (e.g., pipets, pipet tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipet tips for all pipetting steps to avoid cross-contamination of the samples and reagents.
- Prepare pre-PCR master mix with dedicated material (pipets, tips, etc.) in a dedicated area where no DNA matrixes (cDNA, DNA, plasmid) are introduced. Add template in a separate zone (preferably in a separate room) with specific material (pipets, tips, etc.).

Reagent Storage and Handling

The kit is shipped on dry ice and must be stored at –30°C to –15°C upon receipt.

- Gently mix and centrifuge the tubes before opening.
- Store all kit components in original containers.

These storage conditions apply to both opened and unopened components. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance until the expiration date printed on the label.

Specimen Handling and Storage

Whole blood samples should be anti-coagulated with potassium EDTA and stored at 2–8°C for no more than 5 days before RNA extraction.

Procedure

Sample RNA preparation

RNA extraction must be performed with a recommended procedure (QIAGEN RNeasy Mini Kit, cat. no. 74104 or RNeasy Midi Kit, cat. no. 75144; or Life Technologies TRIzol®, cat. nos. 15596-026 and 15596-018).

The performance of an assay is dependent on the concentration and quality of input RNA. We therefore recommend qualifying the purified RNA, prior to downstream analysis, by agarose* gel electrophoresis, Agilent BioAnalyzer, or spectrophotometry.†

Protocol: Reverse transcription

Things to do before starting

- Thaw all necessary components and place them on ice.
- Mix contents of tubes well (do not vortex) and spin briefly (10 s at 10,000 rpm) to collect the liquid at the bottom of the tube.
- Adjust RNA samples to 0.1 µg/µl with nuclease-free water.

Note: A no template control (NTC) generated during reverse transcription using nuclease-free water as template can be used to check reverse transcription quality.

Procedure

1. **Incubate 1 µg of each RNA sample to be tested (10 µl) for 5 min at 65°C.**
2. **Immediately cool on ice for 5 min.**
3. **Centrifuge briefly (10 s at 10,000 rpm) to collect the liquid at the bottom of the tube. Keep on ice.**
4. **Prepare the reverse transcription premix on ice, and keep on ice (see Table 1).**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

† Optical density measured at 260 and 280 nm: OD of 1.0 at 260 nm is equivalent to approximately 40 µg/ml single-stranded RNA. An A_{260}/A_{280} ratio between 1.8 and 2.1 is indicative of highly purified RNA.

Table 1. Preparation of reverse transcription premix

Premix component	Volume per sample (µl)*	Final concentration
5x Reverse transcriptase buffer	5.0	1x
dNTP (10 mM each)	2.0	0.8 mM
Random nonamer (100 µM)	5.25	21 µM
RNase Inhibitor (40 U/µl)	0.5	0.8 U/µl
Reverse transcriptase (200 U/µl)	1.0	8 U/µl
DTT	1.25	–
RT premix volume per sample	**15**	

* Prepare n + 1, where n is the number of RNA samples

5. **Mix with care (do not vortex), centrifuge briefly, and add 15 µl of the premix to each RNA sample (for 40 ng/µl) and to the water control (NTC).**
6. **Mix each tube with care (do not vortex) and centrifuge briefly.**
7. **Run the reverse transcription program (see Table 2) using a thermomixer, heated orbital incubator, heating block, or water bath.**

Table 2. Settings for reverse transcription

Reverse transcription 1	25°C for 10 min
Reverse transcription 2	50°C for 60 min
Inactivation	85°C for 5 min
Cooling	4°C for 5 min

8. **Centrifuge briefly (10 s at 10,000 rpm) to collect the cDNA at the bottom of the tube.**
9. **Keep on ice, or store at –20°C, until qPCR is performed.**

Troubleshooting

For information on troubleshooting for this kit, see 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 protocol in this handbook or sample and assay technologies (for contact information, see "Contact Information", page 12).

Quality Control

This kit is manufactured according to ISO 13485:2003 standard. Certificates of Analysis are available upon request at www.qiagen.com/support/.

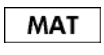
References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents sufficient for <N> reactions
	Use by
	Catalog number
	Lot number
	Material number
	Global Trade Item Number

Temperature limitation

Manufacturer

Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
ipsogen RT Kit (33)	For 33 reactions: Reverse transcriptase, 5x RT buffer, dNTP mix, Random primer, RNase Inhibitor, DTT	679913
Rotor-Gene Q® — for outstanding performance in real-time PCR		
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

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