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ipsogen® RT Kit Instructions for Use (Product Sheet)

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Version 2

IVD

For In Vitro Diagnostic Use

CE

REF 679823

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R2 MAT 1133762EN

Sample to Insight

Table of Contents

Intended Use

For *in vitro* diagnostic use.

The *ipsogen*® RT Kit contains all reagents, except template, necessary to perform a reverse transcription of reaction on RNA extracted from human samples. The *ipsogen* RT Kit is intended to be used in combination with QIAGEN in vitro diagnostics devices for which the *ipsogen* RT Kit is listed as an accessory kit.

The *ipsogen* RT Kit is not an automated device.

The *ipsogen* RT Kit is intended to be used only by professionals especially instructed, trained for molecular biology techniques, and familiar with the device technology. The device procedure is to be implemented in a molecular biology laboratory environment.

The *ipsogen* RT Kit is intended for *in vitro* diagnostic use.

Description

The *ipsogen* RT Kit allows reverse transcription of total RNA to be used in molecular diagnostic testing: the RNA-dependent DNA-polymerase activity (reverse transcription) transcribes cDNA from an RNA template.

Kit Contents

Contents	Volume (µL)
Reverse transcriptase	36
5x RT Buffer for reverse transcription	180
dNTP Mix*	72
Random Primer †	190
RNase Inhibitor	18
DTT ‡	45

* Deoxynucleotides 10 mM each

† Random nonamer oligonucleotide

‡ Dithiothreitol, 0.1M

Materials Required but Not Provided

Consumables

- Nuclease-free PCR grade water
- 0.5 mL or 0.2 mL nuclease-free tubes

Reagents

- Nuclease-free PCR grade water

  Note: Please consult the handbook of QIAGEN products for which the *ipsogen* RT Kit is listed as an accessory kit, to see whether a specific water reference shall be used.

Equipment

- Adjustable pipettes* dedicated for RT-PCR (1–10 µL; 10–100 µL; 20–200 µL; 100–1000 µL)

  Note: Two sets of pipets are recommended at minimum: one for the preparation and distribution of RT reaction mixes, one for RNA handling.

- Benchtop centrifuge* with rotor for 0.2 mL / 0.5 mL reaction tubes (capable of attaining 8000 x g or 10,000 rpm)
- Spectrophotometer*
- Thermal cycler* listed in the combination *in vitro* diagnostic QIAGEN product IFU for which the *ipsogen* RT Kit is listed as an accessory kit

*Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

- Heating block
- Ice or microtube and PCR plate cooler

Safety Information

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

For safety information relative to all other Materials Required but not Provided, please refer to the respective SDS - for reagents - and to the relevant instrument user manuals.

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

The following hazard and precautionary statements apply to components of the *ipsogen*RT Kit:

DTT

Contains: (R*, R*)-1,4-Dimercaptobutane-2,3-diol. Warning! Causes mild skin irritation. Wear protective gloves, protective clothing, eye protection/ face protection.

Emergency information

CHEMTREC

Outside USA & Canada +1 703-527-3887

Quality Control

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

Shipping and Storage

The *ipsogen* RT Kit is shipped on dry ice and must be stored at –30 to –15°C immediately upon receipt in a constant-temperature freezer.

When stored under the specified storage conditions, the *ipsogen* RT Kit is stable until the stated expiration date on box label.

Once opened, reagents can be stored in their original packaging at –30 to –15°C until the stated expiration date. Repeated freezing and thawing should be avoided. Do not exceed a maximum of 7 freeze-thaw cycles.

Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Specimen Storage and Handling

Following isolation, purified RNA may be stored at –30 to –15°C or lower (–90 to –65°C) if long-term storage is required.

Protocol: Reverse Transcription

Important points before starting

- Ensure reagents to be used are not expired and have been transported and stored according to the manufacturer's recommendations.
- The RT-negative control (RT-Neg) is generated during the reverse transciption step using nuclease-free PCR-grade water.

  Note: Please consult the handbook of QIAGEN products for which the *ipsogen* RT Kit is listed as an accessory kit, to see whether a specific water reference shall be used for the RT-Neg.
- The required output is 1 µg of RNA per sample.

Things to do before starting

- Clean the bench area dedicated for reverse transcription (RT) mix preparation to ensure no template or nuclease contamination.
- Thaw all necessary components - except the reverse transcriptase and the RNase inhibitor, which must be kept in the freezer when they are not being used - and place them on ice or in a microtube and PCR plate cooler.

  Note: Do not exceed 30 minutes for the thawing step to avoid any material degradation.
- Mix gently by inverting the tubes several times (do not vortex) and centrifuge briefly to collect liquid at the bottom of the tube.
- Adjust RNA samples to 0.1 µg/µL with nuclease-free water.

  Note: Please consult the handbook of the QIAGEN products for which the *ipsogen* RT Kit is listed as an accessory kit, to see whether a specific water reference shall be used for RNA

samples normalization, as well as for the RNA quantitation, qualification, and normalization protocols when applicable.

Procedure

1. Incubate 1 µg of each RNA sample to be tested (10 µL) for 5 min at 65°C using a heating block.
2. Cool the sample at 4°C in a heating block and keep at 2–8°C or on ice (or on a microtube and PCR plate cooler).
3. Centrifuge briefly to collect the liquid at the bottom of the tube. Keep on ice (or on a microtube and PCR plate cooler).
4. Prepare the reverse transcription premix on ice or in a microtube cooler, and keep on ice (or on a microtube and PCR plate cooler). See Table 1.

Table 1. Preparation of reverse transcription premix*

Premix component	Volume per sample (µL)	Final concentration
5x Reverse transcriptase buffer	5.0	1x
dNTP (10mM 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 premix volume for n+1 reactions, where n is the number of or RNA samples to be tested.

5. Mix with care by pipetting up and down (do not vortex), centrifuge briefly, and add 15 µL of the premix to each RNA sample and to the water control (RT-Neg). Keep on ice (or on a

microtube and PCR plate cooler).

6. Mix each tube with care by pipetting up and down several times (do not vortex) and centrifuge briefly.
7. Run the reverse transcription program (see Table 2) using a thermal cycler.

Table 2. Reverse transcription program

Step	Time
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 to collect the cDNA at the bottom of the tube.
9. Keep at 2–8°C or on ice (or on a microtube and PCR plate cooler) and proceed to the qPCR.

Symbols

The following symbols appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
	Contains reagents sufficient for <N> reactions
	Use by
	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Global Trade Item Number
	Unique Device Identifier
	Contains

Symbol	Symbol definition
	Component
	Number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Manufacturer
	Consult instructions for use downloadable from **resources.qiagen.com/679823**
	Caution, consult accompanying documents

Ordering Information

Product	Contents	Cat. no.
ipsogen® RT Kit	For 24 samples: Reverse transcriptase, 5x RT buffer, dNTP mix, Random primer, RNase Inhibitor, DTT	679823

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit Instructions for Use. QIAGEN kit Instructions for Use are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Revision	Description
R1, August 2022	Initial release
R2, July 2024	Added clarification in the Intended Use section that the *ipsogen*RT Kit is not an automated device Updated Safety Information section to update DTT warning and add CHEMTREC emergency information

Limited License Agreement for *ipsogen*® RT Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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