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ipsogen[®] RT Kit

Instructions for Use (Product Sheet)

Version 2

IVD



REF

679823



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Intended Use

For In Vitro Diagnostic Use

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

The *ipsogen* RT Kit is intended to be used only by professionals specially 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

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, 0.1M

Materials Required but Not Provided

Consumables

- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 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; 20–200µl; 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*
- Heating block
- Ice or microtube and PCR plate cooler

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

Shipping and Storage

The *ipsogen* RT Kit is shipped on dry ice and must be stored at -30°C 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°C 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°C to -15°C or lower (-90°C 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 transcription 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 input 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 the 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 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°C to 8°C or on ice (or on a microtube and PCR plate cooler) until use.
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 (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 premix volume for n + 1 reactions, where n is the number of 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

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

- Centrifuge briefly to collect the cDNA at the bottom of the tube.
- Keep at 2°C to 8°C or on ice (or on a microtube and PCR plate cooler) and proceed to the qPCR.

Symbols



This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.



In vitro diagnostic medical device



Catalog number



Material number



Lot number



Global Trade Item Number



Unique Device Identifier



Contains



Component



Number

Rn

R is for revision of the Product Sheet and n is the revision number



To be used by



Temperature limitations



Legal manufacturer



Consult instructions for use downloadable from

resources.qiagen.com/679823



<N>

Contains reagents sufficient for <N> reactions

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.

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

DTT

Warning! Causes mild irritation of eyes, skin and mucous membranes. If irritation occurs: get medical advice/attention.

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

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.

Ordering Information

Product	Contents	Cat. no.
<i>ipsogen</i> [®] 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 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.

Document Revision History

Date	Changes
08/2022	Initial revision

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