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Description

The kit is recommended for use with Dual Labelled Fluorescent Probes, e.g. TaqMan®, Molecular Beacons or FRET probes but can also be used without fluorescent probes in standard PCR assays. The kit contains an enzyme mixture including a genetically engineered reverse transcriptase and an antibody-inhibited Taq polymerase. The 2x conc. reaction mix contains ultrapure dNTPs and a unique buffer system optimized to resist various PCR inhibitors in unpurified sample material.
One.Step RT-qPCR for Probes is designed for quantitative real-time analysis of target RNA directly from whole blood, swabs and animal- or plant tissue without the requirement of any prior RNA purification steps

Features

The RT-qPCR kit ensures fast and easy preparation with a minimum of pipetting steps and is highly recommended for:

- direct detection of RNA viral pathogens in various tissues
- direct amplification of target RNA from sample materials
- point-of-care Diagnostics

Content

- Extraction Buffer: 10x concentrated
- Direct Enzyme: Mix of engineered reverse transcriptase, antibody-inhibited hot start polymerase and RNase inhibitor in storage buffer with 50 % glycerol (v/v)
- Direct Reaction Mix: 2x conc. buffer system containing dNTPs, enhancer and stabilizer
- PBS (phosphate buffered saline): 10x concentrated
- PCR-grade Water

Shipping and storage

transportation with blue ice; storage @ -20°C for at least 16 months (stable @ +4°C up to 4 weeks), avoid frequent freeze/thaw cycles

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Sample preparation:

1. Whole Blood or Salvia (heparin-, EDTA- or citrate-treated whole blood) •

• Add 1-5 _l of the sample without any pre-treatment directly to the RT-PCR assay.

2. Swab Samples

• Place the swab brush into a 1.5 ml microcentrifuge tube containing 270 µl PCR-grade Water and 30 µl PBS, 10x conc.
• Rotate the brush 5-10 times.
• Squeeze the brush and remove it from the tube.
• Centrifuge at 12,000 g for 3 min at room temperature.
• Discard the supernatant.
• Add 90 µl PCR-grade Water and 10 µl Extraction Buffer to the harvested sample.
• Briefly mix the sample by vortexing and make sure that the sample is soaked with Extraction Buffer.
• Incubate for 3 min at room temperature for tissue lysis and RNA releasing.
• Centrifuge briefly and transfer 1-5 _l of the supernatant to the RT-PCR assay.
• The lysate (supernatant) can be stored at -20°C for several weeks.

Animal or Plant Tissue

• Prepare a small piece from animal or plant tissue not exceeding 6 mm in diameter.
• Crack plant seeds to less than 1 mm in diameter using a BeadBeater, TissueLyser or small hammer.
• Add Extraction Buffer to the tissue sample as following:

Sample size (diameter)	1-2 mm	3-4 mm	5-6 mm
PCR-grade Water	45 µl	90 µl	135 µl
Extraction Buffer	5 µl	10 µl	15 µl

Mix briefly by tapping or vortexing. Make sure that the sample is soaked with Extraction Buffer.
• Incubate for 3 min at room temperature for tissue lysis and RNA releasing.
• Centrifuge briefly and transfer 1-5 _l of the supernatant to the RT-PCR assay.
• The lysate (supernatant) can be stored at -20°C for several weeks.

Preparation of the RT-PCR Assay

Add the following components to a nuclease-free microtube. Pipette on ice and mix the components by pipetting gently up and down.

component	stock conc.	final conc.	20 µl assay	50 µl assay
direct reaction mix	2x	1x	10 µl	25 µl
sample	-	-	1-2 µl	1-5 µl
forward primer	10 µM	400 nM	0,8 µl	2 µl
reverse Primer	10 µM	400 nM	0,8 µl	2 µl
dual labeled probe	10 µl	200 nM	0,4 µl	1 µl
direct enzyme mix 1)	25x	1x	0,8 µl	2 µl
PCR- grade water	-	-	up to 20 µl	up to 50 µl

[1)] Direct Enzyme Mix already contains RNase inhibitor that is recommended and may be essential when working with low amounts of starting RNA.

Reverse transcription and thermal cycling: Place the vials into a real-time PCR cycler and start the following program.

reverse transcription	50 °C	30 min	1x
initial denaturation	95 °C	3-5 min	1x
denaturation	95 °C	15 sec	35-45x
annealing and elongation	60-65 °C 2)	1 min 3)	35-45x

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Protocol for standard PCR cycler combined with gel - based DNA analysis the following cycling protocol is recommended:

reverse transcription	50 °C	30 min	1x
initial denaturation	95 °C	3-5 min	1x
denaturation	95 °C	15 sec	35-45x
annealing	55-65 °C 2)	1 min 3)	35-45x
elongation	72 °C	1 min/kb	35-45x
final elongation	72 °C	5 min	1x

Note: For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary. Note that optimal reaction times and temperatures should be adjusted for each particular sample/primer pair.

Order information

Prod. No.	Description	Quantity
1905-540	One.Step RT-qPCR Probe Kit	20 rcs x 50 µl
1905-542	One.Step RT-qPCR Probe Kit	100rcs x 50 µl
1905-544	One.Step RT-qPCR Probe Kit	1000rcs x 50 µl