One.Direct.Step RT-qPCR Kit for Probes, DLP-Line

RT-PCR Kit for highly sensitive and specific amplification directly from blood or cell material

Description:
The kit is recommended for use with Dual Labeled 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 an unique buffer system optimized to resist various PCR inhibitors in unpurified sample material

One.Direct.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

Performance:
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 frequently freeze/thaw cycles

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.

GeneON .. a good decision ..
Contact Phone +49-(0)-621- 5720 864 Fax: +49-(0)-621-5724 462
E-Mail: mailto:info@geneon.net WEB: http://www.GeneOn.net **Version: 10.2009 UK**
Unless specified otherwise, all products of GeneON are sold for research use only.

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

Unless specified otherwise, all products of GeneON are sold for research use only.

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

[2)] The annealing temperature depends on the melting temperature of the primers.
[3)] The elongation time depends on the length of the amplicon. A time of 1 min for a fragment of 1,000 bp is recommended.

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.

Orderdetails:

Cat.-no	Description	Amount
105-540	One.Direct.Step RT-qPCR Kit for Probes	20 rcs x 50 µl
105-542	One.Direct.Step RT-qPCR Kit for Probes	100 rcs x 50 µl
105-544	One.Direct.Step RT-qPCR Kit for Probes	1000 rcs x 50 µl

GeneON .. a good decision ..
Contact Phone +49-(0)-621- 5720 864 Fax: +49-(0)-621-5724 462
E-Mail: mailto:info@geneon.net WEB: http://www.GeneOn.net **Version: 10.2009 UK**
Unless specified otherwise, all products of GeneON are sold for research use only.