Datasheet

One.Direct.Step RT-qPCR Kit for Probes without ROX

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

Cat.-No.: 105-542, 2x1,25 ml

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: 1x concentrated

Direct Enzyme: Mix of engineered reverse transcriptase, mAB-inhibited hot start polymerase, dNTPs, reaction buffers, enhancers and additives

PCR-grade Water

Optional: ROX Dye (not included)

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

Easy Preparation (e.g. 50 µl reaction volume)

1. Whole Blood (not treated heparin-, EDTA- or citrate-treated whole blood)

- Add 2-5 µl whole blood (for 50 µl reaction volume) without any pre-treatment directly to the **RT-PCR assay.**

2. Swab Samples from nasal or swaps

- fill 200 µl Extraction Buffer in a 1,5 ml Tube
- Cut the tip with nasal or throat swap and put to micro tube and vortex about 15-20 sec
- let absorb and incubate at room temperature for about 3 min
- press the tip of the swap to the wall of the microtube and take it out
- centrifuge the tube extensively and transfer, for a 50µl reaction volume, 2-5 µl of the supernatant to your **RT-PCR assay.**

.. a good decision ..

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3. Animal or Plant Tissue samples

- Prepare a small piece from animal or plant tissue not exceeding 8 mm in diameter.
- Crack plant seeds to less than 1 mm in diameter using a cell-disrupter, Tissue-lyser or small hammer.
- Add 1x Extraction Buffer to the tissue sample using:

Sample size

1-2 mm - 50 µl
3-4 mm - 100 µl
5-8 mm - 200 µl

- mix Extraction Buffer and sample briefly and incubate for about 3 min at room temperature
- Centrifuge extensively and and transfer 2-5 µl (for 50 µl reaction volume) of the supernatant to **your RT-qPCR assay**

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**
RT-qPCR enzyme mix	2x	1x	10 µl	25 µl
Sample, whole blood or extracted	-	-	1-2 µl	2-5 µl
Forward primer Xn 1.)	10 µM	300 nM	0,6 µl	1,5 µl
Reverse Primer Xn 1.)	10 µM	300 nM	0,6 µl	1,5 µl
Dual labelled probe / TaqMan Xn 1.)	10 µl	200 nM	0,4 µl	1 µl
optional ROX	25 µM	500 nM	0,4 µl	1 µl
PCR- grade water	-	-	up to 20 µl	up to 50 µl

1.) for each PCR-Target (Multiplex PCR) take Xn+1 primers and the related amount of TaqMan probe

Note: For each primer on have to optimize the best assay parameters. The optimal primer can vary from 100 - 500 nM.

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

reverse transcription	50-55 °C	10-15 min	1x
initial denaturation	95 °C	5 min	1x
denaturation	95 °C	15 sec	35-45x
annealing and elongation	60-65 °C [2)]	1 min [3)]	35-45x

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

reverse transcription 2.)	50 °C	up to 30 min	1x
initial denaturation	95 °C	3-5 min	1x
denaturation	95 °C	15 sec	35-45x
annealing 3.) 4.)	55-65 °C [)]	1 min [3)]	35-45x
elongation	67 °C	1 min/kb	35-45x
final elongation	67 °C	5 min	1x

2.) 10 min for amplicons < 200 bp; each 100 bp fragment length need about 3 min longer incubation time

3.) The annealing temperature depends on the melting temperature of the primers.

4.) 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
105-542	One.Direct.Step RT-qPCR Kit for Probes No-ROX	2x1,25 ml
105-544	One.Direct.Step RT-qPCR Kit for Probes No-ROX	10x1,25 ml
105-546	One.Direct.Step RT-qPCR Kit for Probes High-ROX	2x1,25 ml
105-548	One.Direct.Step RT-qPCR Kit for Probes High-ROX	10x1,25 ml

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