Datasheet

One.Direct.Step RT-qPCR Kit with SybGreen High-ROX

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

Description:
The kit contains an enzyme mixture including a genetically engineered reverse transcriptase and an antibody-inhibited Taq polymerase. For an easy quantification.
The 2x conc. reaction mix contains ultrapure dNTPs and an optimized buffer system to resist various PCR inhibitors in impurified sample material.

One.Direct.Step RT-qPCR with SybrGreen fluorescent dye 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; SybrGreen intercalating dye, High-ROX

PCR-grade Water

Optional: ROX Dye (included in the mixture)

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.**

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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; extracted	-	-	1-2 µl	2-5 µl
Forward primer	10 µM	300 nM	0,6 µl	1,5 µl
Reverse Primer	10 µM	300 nM	0,6 µl	1,5 µl
optional ROX	25 µM	500nM	0,4 ml	1 µl
PCR- grade water	-	-	up to 20 µl	up to 50 µl

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)]	30 sec - 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	72 °C	1 min/kb	35-45x
final elongation	72 °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
105-570	One.Direct.Step RT-qPCR Kit with SybrGreen No-ROX	2x1,25 ml
105-572	One.Direct.Step RT-qPCR Kit with SybrGreen No-ROX	10x1,25 ml
105-574	One.Direct.Step RT-qPCR Kit with SybrGreen High-ROX	2x1,25 ml
105-576	One.Direct.Step RT-qPCR Kit with SybrGreen High-ROX	10x1,25 ml

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