DATA SHEET

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# RT-qPCR Probe Master Multiplex with UDG

RT-real-time-PCR Multiplex mix optimized for detection of RNA viruses

| Cat. Nº. | Amount |
|---|---|
| ☐ PRT-301S | 100 reactions |
| ☐ PRT-301M | 200 reactions |
| ☐ PRT-301L | 400 reactions |
| ■ PRT-301XL | 1.000 reactions |

**Concentration:**
Master Mix 2x concentrated

**Shipping:**
Shipped on blue ice

**Storage Conditions:**
Store at -20 °C (Avoid freeze/thaw cycles, store in dark).

**For** in vitro **use only!**

**Form:**
Liquid

**Shelf Life:**
12 months

**Description:**
RT-qPCR Probes Master Multiplex is designed for quantitative reverse transcription real-time analyses of up to 5 RNA targets simultaneously using Dual Labeled Fluorescent Probes (suitable for TaqMan system and other probes). It is a fast, accurate and optimized mix for several approaches.

The kit is based on a genetically engineered reverse transcriptase with enhanced thermal stability providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments and contains all reagents required for RT-qPCR (EXCEPT TEMPLATE, PRIMERS AND FLUORESCENT PROBE) to ensure fast and easy preparation. The premium quality SCRIPT Reverse Transcriptase and Taq DNA Polymersae Hot Start antibody-coupled in a optimized reaction buffer containing ultrapure dNTPs, ensure superior real time PCR results for multiplex reaction.

RT-qPCR Probes Master Multiplex is used to amplify double-stranded DNA from singlestranded RNA templates to allow a rapid real-time quantification of RNA targets. In the reverse transcription step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template.

In the first cycle of the PCR step hot-start DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. The hot-start polymerase activity is blocked at ambient temperature by antibody and switched on automatically at the onset of the initial denaturation.

The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup. RT-qPCR Probes Master Multiplex offers tremendous convenience when applied to analysis of targets from multiple samples of RNA and minimizes the risk of contaminations.

**Kit contents:**

**RT-qPCR Probes Master Multiplex 2x (red cap)**
Script Reverse Transcriptase, Taq DNA Polymerase Hot Start (antibody-coupled), RNAse Inhibitor, UDG, dNTP mix with dUTP and stabilizers.

**RNase-free Water (white cap)**

**Sensitivity:**
Targets can be detected from 1 copy of the genes. The UDG system prevent from cross-contamination which is indispensable for diagnostic detection.

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## 1) Preparation of the RT-qPCR Assay

Add the following components to a nuclease-free microtube. Pipett on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers and probes should be mixed together before the remaining components are added.

| Component | 25 µL assay | Final [ ] |
|---|---|---|
| **2x RT- qPCR Multiplex Mix**[1] | 12,5 µL | 1x |
| **Primer forward (10 µM)** | 1 µL | 400 nM |
| **Primer reverse (10 µM)** | 1 µL | 400 nM |
| **dual-labeled Probe (10 µM)** | 0,5 µL | 200 nM |
| **RNA Template** | 5 µL | < 100 ng |
| **ROX Passive Reference Dye**[2] | Optional | - |
| **RNAse free water** | to 25 µL | - |

1) RT-qPCR Probes Master already contains RNase inhibitor that may be essential when working with low amounts of starting RNA.
2) ROX passive reference is not supplied in this kit but is available as #PCK-121. The use of ROX Dye should be followed to the intrument recommendation.

**Continue with reverse transcription and thermal cycling as recommended.**

**1.**Program a real-time PCR instrument as follow to synthesize cDNA and for PCR amplification. Set up the excitation and emission maxima suitable to the fluorescent probe chemistry.

| Step | Temp. | Time | Cycle |
|---|---|---|---|
| **Reverse Transcription** | 50 °C | 30 min | 1x |
| **Initial denaturation** | 95 °C | 5 min | 1x |
| **Denaturation** | 95 °C | 15 sec | 40x |
| **Annealing and elongation** | 60 °C | 1 min | |

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 RNA / primer pair.