Smart One RT-PCR Master Mix

Description: The ready-to-use Master Mix is designed for all applications that require probe-based One Tube RT-qPCR (Reverse Transcription and Real-Time PCR).

One-tube one-step: This 2x Master Mix can efficiently perform the Reverse Transcription of RNA into cDNA and the subsequent PCR amplification. Pipetting steps and contaminations can be minimized by only pipetting primers and sample RNA to the mix.

The enzymes: The Reverse Transcription step is directed by a Reverse Transcriptase with a heat stability up to 55°C. PCR amplification of synthesised cDNA is performed by a HotStart Taq DNA polymerase.

Kit content

Ref No.	**105610**	**color**
Smart One RT-PCR Master Mix (2x)	**100 reactions**	**white**
Datasheet	**1**	**--**

Application: Analysis of RNA samples with Real-Time PCR for probe-based PCR assays. For example for the pathogen diagnostic analysis of RNA viruses.

For research use only. Not approved for use in clinical or in-vitro diagnostics.

Kit components: Reverse Transcriptase, HotStart Taq DNA polymerase, dNTPs, $MgCl_2$, reaction buffer, RNAsin, stabiliser.

Concentration: 2x liquid master mix with blue dye for convenient visual pipetting. Volume: 1 ml

Assays sizes: 10 - 20 µl

Sensitivity: high, low concentration of sample RNA template is required

Storage condition: -20 °C

Shelf life: 12 months

Quality control:

- Probe-based real-time PCR of MS2 with MS2 phage RNA
- No detectable exo-/endonuclease and RNase activities
- Probe-based real-time PCR of human GAPDH with total human RNA

Standard RT-qPCR Protocol

Prepare dilutions of RNA sample in water or TE-buffer and prepare reactions as described below.

Before starting the procedure:

- Thaw the tube and invert the Master Mix 5 - 6 times to ensure mixing of the solution.
- Do not vortex!
- After thawing spin the tube briefly!

Tests can be set up at room temperature.

Protocol:

Component	Apply for 20 µl assay	Apply for 10 µl assay	Final concentration
2x Smart Script-1 MM	10 µl	5 µl	1x
Primer reverse/forward	Variable (0.5 µl) each	Variable (0.25 µl) each	0.1 – 0.4 µM each
Fluorescent probe	Variable (0.5 µl)	Variable (0.25 µl)	0.1 – 0.4 µM
Template RNA	Variable	Variable	10 fg – 10 ng
Sterile water	Fill up to 20 µl	Fill up to 10 µl	

After mixing by pipetting, centrifuge briefly and place the tube into a standard thermocycler or Real Time PCR equipment. We recommend to use one of the following programs:

RT + 3-step qPCR protocol

Step	Time	Temperature	Number of cycles
Reverse Transcription	10 - 30 minutes	50-55 °C	1
Inactivation of reverse transcriptase	1 - 3 minutes	95 °C	1
Denaturation	5-10 seconds	92 °C	40-45
Annealing	5-10 seconds	58 – 60°C*	
Extension	10 – 20 seconds	72 °C	

* temperature is depending on used primers and probes

RT + 2-step qPCR protocol

Step	Time	Temperature	Number of cycles
Reverse Transcription	10 - 30 minutes	50-55 °C	1
Inactivation of reverse transcriptase	1-3 minutes	95 °C	1
Denaturation	5 -10 seconds	92 °C	40-45
Annealing /extension	10 – 20 seconds	60 °C *	

* temperature is depending on used primers and probes

Note: For some applications the addition of ROX is required. The ***2x One-Step RT-qPCR Master Mix*** is also available with low or high concentrations of ROX. Please enquire.

Note: For maximum efficiency and specificity for all kind of assays please optimize:
the annealing temperatures,
extension times,
primer/probe concentrations and template RNA concentration.