

Production Information

GeneDia™ One-Step RT-PCR 2x Probe Master Mix

Storage Temperature -20 C

GeneDia™ One-Step RT-PCR 2x Probe Master Mix	Without ROX
colour	clearance
Lot No.	MM06100
Content	0.5 ml

Product Description

GeneDia™ One-Step RT-PCR 2x Probe Master Mix is based on quantitative reverse transcription PCR (RT-qPCR) that uses RNA as starting material. It offers a convenient master mix to convert RNA to DNA and quantify in a one-step reaction. The kit is supplied with reverse transcriptase and a 5X master mix with Hot-Start Taq DNA polymerase, dNTP and all required buffer components, and it is universally compatible with all instrument platforms. This Master uses a reverse transcriptase to convert RNA to DNA, and an antibody-modified Taq DNA polymerase to avoid polymerase activity prior to thermal cycling. The optimized buffer system allows high amplification efficiency and specificity, as well as enhanced sensitivity of real time PCR reactions over a wide range of templates.

Precautions and Disclaimer

For Research Use Only.

Quality Control

GeneDia™ One-Step RT-PCR 2x PCR Master Mix is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

Storage/Stability

GeneDia™ One-Step RT-PCR 2x PCR Master Mix should be stored at -20°C. Thawed material kept on ice can be aliquoted and re-frozen up to two times.

Pre-procedure Considerations

Primers and Probes

The design of primers and probes is critical especially for successful multiplex PCR.

Design primers with similar annealing temperature.

Analyse primer and probe sequences to avoid primer/probe hairpins, homo- or heterodimers, or any primer/probe complementarity across the targets.

Optimization of primer and probe concentrations is highly recommended.

Preventing Template Cross-Contamination

Due to the high sensitivity of quantitative PCR there is a risk of contaminating the reactions with the products of previous runs.

Procedure:

This protocol serves as a guideline to ensure optimal PCR results when using GeneDia™ One-Step RT-PCR 2x Probe Master Mix. Optimal reaction conditions such as incubation times, temperatures, and amount of template RNA may vary and must be determined individually.

1. Thaw GeneDia™ One-Step RT-PCR 2x Probe Master Mix and primers and probes. It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts. Keep all components on ice.

2. Prepare a reaction mix. Table 1 shows the reaction set up for a final volume of 10 µL.

Component	Vol./reaction*	Final concentration*
GeneDia™ One-Step RT-PCR 2x Probe Master Mix	5 µl	1x
Forward Primer	0.5 µl	0.5 µl of 10 µM/µl final concentration (0.2 µM/µl)
Reverse Primer	0.5 µl	0.5 µl of 10 µM/µl final concentration (0.2 µM/µl)
Probe	1 µl	1 µl of 10 µM/µl final concentration (0.4 µM/µl)
PCR-grade H ₂ O	X µl	-
Template RNA	X µl	RNA: 50 ng (10 – 500 ng)
TOTAL volume	10 µl	-

3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the reaction mix up and down a few times.

4. Add template RNA to the individual tubes containing the reaction mix.

5. Program the thermal cycler according to the manufacturer's instructions. See table 2 for an example. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

Cycles	Duration of cycle	Temperature
1	5 minutes	65 ° C
1	2 minutes	25 ° C
1	30 minutes	47 ° C
1	2 minutes	95 ° C
40	30 seconds	95 ° C
	40 seconds	60° C

Set the qPCR instrument to detect and report fluorescence in 60° C of each cycle.

6. Place the tubes in the thermal cycler and start the reaction.

