

## Production Information

### GeneDia™ One-Step RT-PCR 2x Green Master Mix

Storage Temperature -20 C

GeneDia 2x PCR Master Mix	Without ROX
colour	Clearance
Lot No.	MM05100
Content	0.5 ml

## Product Description

**GeneDia™ One-Step RT-PCR 2x PCR Master Mix** is a convenient and highly sensitive solution for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using SYBR Green I dye detection and gene-specific primers. cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. The system has been optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity. The proprietary reaction buffer has been specifically formulated to maximize activities of both reverse transcriptase and Taq DNA polymerase while minimizing the potential for primer-dimer and other non-specific PCR artifacts. The kit is compatible with both fast and standard qPCR cycling protocols.

### Precautions and Disclaimer

For Research Use Only.

### Quality Control

**GeneDia™ One-Step RT-PCR 2x Green 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 Green 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 Green 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 Green Master Mix and primers. 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 Green 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)
PCR-grade H <sub>2</sub> O	X µl	-
Template	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.

