## 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 | 0.7 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 DNA 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 20 µL.

| Component | Vol./reaction* | Final concentration* |
|---|---|---|
| **GeneDia™ One-Step RT-PCR 2x Green Master Mix** | 7 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 $H_2O$ | X µl | - |
| Template DNA | X µl | genomic DNA: 50 ng (10 – 500 ng)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>bacterial DNA: 5 ng (1 – 10 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 DNA 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**<br>**40 seconds** | **95 ° C**<br>**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.

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