## Production Information

**GeneDia™ 2x PCR Master Mix Green**

Storage Temperature -20 C

| GeneDia™ 2x PCR Master Mix Green | without ROX™ |
|---|---|
| colour | Clearance |
| Lot No. | MM02100 |
| Content | 1 ml |

### Product Description

The GeneDia™ 2x PCR Master Mix Green are available with high (MM07) or without ROX™ for optimal performance on most of the commonly used real-time PCR instruments. The GeneDia™ 2x PCR Master Mix Green promote high specificity and low background by using TEMPase Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

The GeneDia™ 2x PCR Master Mix Green is a single tube 2x reagent including all components necessary to perform real-time DNA amplification for DNA-binding dye based PCR. Just add your primers and DNA.

**Precautions and Disclaimer**

For Research Use Only.

**Composition of the GeneDia™ 2x PCR Master Mix Green**

- TEMPase Hot Start DNA Polymerase
- Optimized buffer system
- dNTPs
- green dye
- Stabilizer

**Quality Control**

**Genedia™ 2x PCR Master Mix Green** is tested for contaminating activities, with no trace of endonuclease activity, nicking activity, exonuclease activity or priming activity, efficiency and absence of contaminating human genomic DNA.

**Storage/Stability**

**Genedia™ 2x PCR Master Mix Green** should be stored at -20˚C. Thawed material kept on ice can be aliquoted and re-frozen up to two times.

### Procedure:

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

1. Thaw GeneDia™ 2x PCR Master Mix Green and primers.
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™ 2x PCR Master Mix Green** | 10 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 | 20 µl | - |

3. Gently mix without creating bubbles* (do not vortex).

* Bubbles interfere with detection of fluorescence.

4. Place the reaction in the instrument and run the appropriate program according to the manufacturer's instructions.

**Three-step PCR Program**

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| **1** | **15 minutes** | **95 ° C** |
| **30** | **15-30 seconds**<br>**30 seconds**<br>**30 seconds** | **95 ° C**<br>**60-55 ° C**<br>**72 ° C** |

**Two-step PCR Program**

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| **1** | **15 minutes** | **95 ° C** |
| **30** | **15-30 seconds**<br>**30 seconds** | **95 ° C**<br>**60-55 ° C** |

**Depending on the type of primer and probe designed, set the qPCR instrument to detect and report fluorescence in 60° C or 72 ° C of each cycle.**

1. Cycle one for activation of the TEMPase hot start enzyme.
2. Denaturation time is varying between thermocyclers.
3. Choose an appropriate annealing temperature for the primer set used.

Unit 1, Ground Floor, No.14 Building, End of the Taghavi Alley,South Bahar St, Kaveh Blvd ,Tehran, Iran.
E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584