

Production Information

GeneDia™ 2x PCR Master Mix Green

Storage Temperature -20 C

GeneDia™ 2x PCR Master Mix Green	High ROX™
colour	Clearance
Lot No.	MM07100
Content	1 ml

Product Description

The GeneDia™ 2x PCR Master Mix Green are available with high or without ROX™ (MM02) 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 High ROX™ 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. The ROX™ fluorescence does not change during the course of the PCR reaction nor does it influence the PCR reaction.

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
- ROX™
- 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 ₂ O	X µl	-
Template DNA	X µl	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) 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 30 seconds 30 seconds	95 ° C 60-55 ° C 72 ° C

Two-step PCR Program

Cycles	Duration of cycle	Temperature
1	15 minutes	95 ° C
30	15-30 seconds 30 seconds	95 ° C 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.

