Production Information

GeneDia™ 2x PCR Master Mix for Probe

Storage Temperature -20

GeneDia™ 2x PCR Master Mix for Probe	
colour	Clearance
Lot No.	MM03100
Content	1 ml

Product Description

The GeneDia™ 2x PCR Master Mix for Probe is a single-tube 2x reagent including all components necessary to perform probe based real-time DNA amplification. The GeneDia™ 2x PCR Master Mix for Probe is suitable for multiplexing for up to four DNA targets in the same tube, thereby saving PCR consumables, time, workload and valuable DNA. Just add your probes, primers and DNA.

The GeneDia™ 2x PCR Master Mix for Probe promote high specificity and low background by using TEMPase Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

Detection limit of GeneDia™ 2x PCR Master Mix for Probe without ROX™ is approximately 5 copies (~0.01 ng of human gDNA). Quantification limit is approximately 25 copies (0.01 ng of human gDNA).

Precautions and Disclaimer

For Research Use Only.

Composition of the GeneDia™ 2x PCR Master Mix for Probe

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

Quality Control

Genedia™ 2x PCR Master Mix For Probe 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 for Probe 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 real-time 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.

Amplicon size

Recommended amplicon size is less than 150 bp.

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.

Instrument compatibility

Real-time instruments which does not require ROX™ internal reference dye such as: Bio-Rad CFX96 Touch™, CFX384 Touch™, and CFX Connect™.

Procedure:

This protocol serves as a guideline to ensure optimal PCR results when using GeneDia™ 2x PCR Master Mix for Probe. 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 for Probe 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 For Probe	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) 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.

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