Production Information

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

1.5mM $MgCl_2$final concentration

Storage Temperature -20 C

GeneDia 2x PCR Master Mix	1.5 mM $MgCl_2$
colour	RED
Lot No.	MM04100
Content	0.5 ml

Product Description

GeneDia™ One-Step RT-PCR 2x PCR Master Mix is a ready-to-use solution that contains components required for RT-PCR amplification of RNA templates. The mix includes reverse transcriptase, *Taq* DNA polymerase, dNTPs, reaction buffer, $MgCl_2$, KCl, and a PCR enhancer/stabilizer. The user needs only to add the template, the primer set and water to the Master Mix to set up the RT-PCR reaction. This convenient **One-Step RT-PCR 2x PCR Master Mix** reduces the time required to set up PCR reactions and reduces the possibility of contamination, particularly when preparing large numbers of reactions. The optimized master mix allows for robust amplification of RNA templates with high yields of PCR products.

Taq DNA Polymerase is a highly thermostable DNA polymerase that possesses a 5´→ 3´ polymerase activity and a very low 5´→ 3´ exonuclease activity. Reverse Transcriptase is an RNA-directed DNA polymerase that can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. There is no need to buy and use separate loading dyes. Simply load a portion of the reaction product onto an agarose gel for electrophoresis and subsequent visualization. The red dye front runs at 1000 – 2000 bp on a 0.5 – 1.5% agarose gel.

Precautions and Disclaimer

For Research Use Only.

Composition of the 2x PCR Master Mix RED

(1.5 mM $MgCl_2$ final concentration)

- Tris-HCl pH 8.5, (NH4)2S04, 3 mM MgCl2, KCl
- Reverse transcriptase
- 0.4 mM of each dNTP
- Taq DNA polymerase
- Inert red dye
- Stabilizer
- Enhancer

Quality Control

GeneDia™ One-Step RT-PCR 2x PCR 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 PCR 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 2x PCR Master Mix RED. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw 2x PCR Master Mix RED 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 PCR Master Mix	5 µl	**1x**
Forward Primer	0.5 µl	0.5µl of 10 µM/µl final concentration (0.2µM/µl)
Reverse Primer	0.5 µl	0.5 µl of 10 µM/µl final concentration (0.2µM/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	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 40 seconds	95 ° C 60° C

6. Place the tubes in the thermal cycler and start the reaction.

7. At the end of the run, simply load a portion of the reaction product (e.g. 10µl) onto an agarose gel for analysis.

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