



Product Information

REDTaq™ ReadyMix™ PCR Reaction Mix With MgCl₂

Product Code **R 2523**
Technical Bulletin No. MB-575

TECHNICAL BULLETIN

Product Description

REDTaq™ ReadyMix™ PCR Reaction Mix With MgCl₂ combines the performance and benefits of Sigma's REDTaq DNA polymerase with the convenience of ReadyMix Taq PCR Reaction Mix. ReadyMix REDTaq is a ready-to-use mixture of Taq DNA polymerase, 99% pure deoxynucleotides, reaction buffer, and an inert red dye in a 2x concentrate. ReadyMix REDTaq saves preparation time and reduces the risk of contamination from multiple pipetting steps. The ReadyMix REDTaq provides consistent reaction-to-reaction performance.

After the PCR reaction, the PCR product can be loaded directly onto an agarose gel. There is no need to add a loading buffer/tracking dye prior to electrophoresis. The inert red dye migrates at approximately the same rate as a 125 base pair fragment in a 1% agarose gel. Because the dye has no effect on the amplification process, a sample can be easily reamplified such as in "nested PCR".

For a typical PCR reaction, mix 25 µl of REDTaq ReadyMix PCR Reaction Mix with MgCl₂ with 25 µl of a mixture containing template DNA, primers, and water. Reaction volumes can be scaled down, if desired.

Reagents Provided

- REDTaq ReadyMix PCR Reaction Mix, Product Code R 2648
20 mM Tris-HCl, pH 8.3, with 100 mM KCl, 3 mM MgCl₂, 0.002 % gelatin, 0.4 mM dNTP mix (dATP, dCTP, dGTP, TTP), stabilizers, and 0.06 unit/µl of Taq DNA Polymerase.
Provided as 20 reactions or 5 × 20 reactions
- Water, PCR Reagent, Product Code W 1754
Provided as a 1.5 ml vial

Equipment and Reagents Required But Not Provided

(Product Codes have been given where appropriate).

- DNA template to be amplified
- Primers
- Mineral Oil, Product Code M 8662 (optional)
- Dedicated pipettes
- PCR pipette tips
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Product Codes P 3114 and P 3364
- Thermal cycler

Precautions and Disclaimer

Sigma's REDTaq ReadyMix PCR Reaction Mix is for R&D use only, not for drug, household or other uses. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. Refer to Material Safety Data Sheet.

Storage/Stability

Store at -20 °C. For short-term storage of one week or less, store at 2-8 °C, so there is no waiting for reaction components to thaw. Repeated freeze/thaw cycles do not affect the activity of the reactions.

Procedure

The optimal conditions for template DNA, primers, and cycling parameters will depend on the system being utilized. Sigma offers a separate PCR Optimization Kit (Product Code OPT-II) that contains a variety of buffers and adjuncts for optimizing the specificity, fidelity, and yield of a PCR product.

1. Add the following reagents to a 0.2 or 0.5 ml PCR tube in the following order:

Volume	Reagent	Final Concentration
25 μ l	2 \times REDTaq ReadyMix	1.5 units Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl ₂ , 0.001% gelatin, 0.2 mM dNTP, stabilizers
1 μ l	Forward Primer	0.1–1.0 μ M (15-30 bases in length)
1 μ l	Reverse Primer	0.1–1.0 μ M (15-30 bases in length)
X μ l q.s.	Template DNA Water	
50 μ l	Total Volume	

2. Mix gently and briefly centrifuge to collect all components to the bottom of the tube.
3. Add 50 μ l of mineral oil to the top of each tube to prevent evaporation if using a thermal cycler without a heated lid.
4. The amplification parameters should be optimized for individual primers, template, and thermal cycler.

Common cycling parameters:

- a. Denature the template at 94 °C for 1 minute
- b. Anneal primers at 55 °C for 2 minutes
- c. Extension at 72 °C for 3 minutes

25-30 cycles of amplification are recommended.

5. The amplified DNA can be loaded directly onto an agarose gel after the PCR process. It is not necessary to add a separate loading buffer/tracking dye. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase. The red tracer co-migrates with 125 bp fragment in a 1% agarose gel. If a more intense tracking dye is desired, an unused lane can be used to run any common tracking dye.

[†]The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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