

Product Information

ReadyMix™ Taq PCR Reaction Mix with MgCl₂

Catalog Number **P4600**

TECHNICAL BULLETIN

Product Description

ReadyMix Taq PCR Reaction Mix with MgCl₂ is Sigma's high quality Taq DNA polymerase in a 2× concentrate containing all the nucleotides and reagents necessary to perform a standard PCR reaction. ReadyMix Taq PCR Reaction Mix with MgCl₂ saves preparation time, reduces the risk of contamination from multiple pipetting steps and provides consistent reaction-to-reaction performance.

For a typical PCR reaction, mix 25 µL of ReadyMix Taq 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

- ReadyMix Taq PCR Reaction Mix with MgCl₂, Catalog Number P0476. 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM MgCl₂, 0.002 % gelatin, 0.4 mM dNTP mix (dATP, dCTP, dGTP, TTP), stabilizers, 0.06 units Taq DNA Polymerase/µL. Provided as 20 reactions, 5 × 20 reactions
- Water, PCR Reagent, Catalog Number W1754. Provided as a 1.5 ml vial

Reagents and equipment required, not provided

- Primers
- DNA template
- Dedicated pipets
- PCR pipet tips
- 0.2 ml or 0.5 ml thin-walled PCR tubes, Catalog Numbers P3114 and P3364 or plates for specific thermal cycler
- Thermal cycler
- Mineral Oil, Catalog Number M8662 (optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed.

Storage/Stability

Store ReadyMix Taq PCR Reaction Mix with MgCl₂ at 2-8 °C so there is no waiting for reaction components to thaw. It can also be stored at -20 °C. Repeated freeze thaws 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, Catalog Number OPT2, 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 µL	2× ReadyMix Taq PCR Reagent Mix	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 µL	Forward Primer	0.1–1.0 µM (15-30 bases in length)
1 µL	Reverse Primer	0.1–1.0 µM (15-30 bases in length)
x µL	Template DNA	
q.s.	Water	
50 µL	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 evaluated by agarose gel electrophoresis and subsequent ethidium bromide staining. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

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