



Product Information

REDTaq™ Genomic DNA Polymerase without MgCl₂ Recombinant from *E. coli*

Product Code **D 2812**
Technical Bulletin No. MB-790

TECHNICAL BULLETIN

Product Description

REDTaq™ Genomic DNA Polymerase is Sigma's high quality Taq DNA Polymerase mixed with an inert red dye. REDTaq Genomic DNA polymerase is a formulation of Sigma's REDTaq DNA polymerase designed to provide an enhanced amplification of more complex or genomic templates. The dye allows quick recognition of reactions to which enzyme has been added as well as visual confirmation of complete mixing. The enzyme is provided at one unit per microliter for more accurate volume measurement and less waste.

Reactions using REDTaq Genomic DNA polymerase, 10× PCR reaction buffer without MgCl₂, and MgCl₂ are formulated as any PCR[†] mixtures when optimizing individual components. There are no additional preparation steps or protocol changes required. The formulation allows aliquots (5-10 μl) from the PCR to be directly loaded onto an agarose gel without addition of electrophoresis loading buffers. The dye migrates at the same rate as a 125 bp fragment in a 1% agarose gel. Because a gel loading buffer is not added to the reaction mix, a sample can be re-amplified, such as in nested PCR.

If desired, the dye can be removed from the amplicon by any standard purification method. The presence of the dye has no effect on manual or automated DNA sequencing, ligation, and transformations. Though exceptions may exist, the dye is generally inert in restriction enzyme digestions.

The 10× PCR buffer is supplied without MgCl₂ for a greater degree of control when optimization of MgCl₂ is required.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 min at 74 °C.

Reagents Provided

- REDTaq Genomic DNA Polymerase, Product Code D 0688. 1 unit/μl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Igepal CA-630, inert dye, 50% glycerol. Provided as 50, 250, 1,000, or 2,500 units
- 10× PCR Reaction Buffer, Product Code P 2317, 100 mM Tris-HCl, pH 8.3, 500 mM KCl, and 0.1% gelatin. Provided as 1.5 ml package.
- 25 mM Magnesium Chloride, Product Code M 8787. Provided as 1.5 ml package.

Equipment and Reagents Required but not Provided

- Deoxynucleotide Mix, Product Code D 7295
10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM dTTP
- Water, PCR Reagent, Product Code W 1754
- Mineral Oil, Product Code M 8662 (optional)
- Primers
- DNA template
- 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

Storage/Stability

Store all components at -20 °C.

Precautions and Disclaimer

Sigma's REDTaq Genomic DNA Polymerase without MgCl₂ is for R&D use only. Not for drug, household or other uses.

Procedure

It is recommended the REDTaq Genomic DNA polymerase and the MgCl₂ be titrated to determine the optimal efficiency. A range of 1.0 to 4.0 mM final MgCl₂ concentration in 0.5 mM increments is recommended. Optimal concentrations of template DNA, MgCl₂, KCl, and PCR adjuncts as well as pH are often target specific. It may be necessary to determine the optimal concentration of each of these components as well.

1. Add the following reagents to a 0.2 ml or 0.5 ml microcentrifuge tube. A master mix is highly recommended when performing multiple PCR reactions.

Amount	Component	Final Concentration
5 µl	10× PCR Buffer	1×
- µl	25 mM magnesium chloride	Typically 1.0 to 4.0 mM
1 µl	Deoxynucleotide Mix	200 µM
- µl	Sense primer	0.1-0.5 µM
- µl	Antisense primer	0.1-0.5 µM
2.5 µl	REDTaq Genomic DNA polymerase	0.05 unit/µl
- µl	Template DNA (typically 10 ng)	200 pg/µl
q.s.	Water	
50 µl	Total reaction volume	

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Add 50 µl of mineral oil to the top of each tube to prevent evaporation if not using a thermal cycler with a heated lid.
4. The amplification parameters should be optimized for individual primers, template, and thermal cycler.

Typical cycling parameters for 0.2–2 kb fragments:

Initial denaturation 94 °C for 2 min

25-30 cycles:

Denaturation 94 °C for 30 sec
Annealing 55 °C to 68 °C for 30 sec
Extension 72 °C for 2 min

Final extension 72 °C for 5 min

Hold 4 °C

5. The amplified DNA can be evaluated by loading 5-10 µl of the PCR reaction directly onto agarose gel. It is not necessary to add a separate loading buffer/tracking dye. Amplification products can be visualized by standard methodologies such as ethidium bromide staining.

Note: A minimum of 1.5 units of REDTaq Genomic DNA polymerase must be added per 50 µl reaction to ensure enough glycerol is present for direct gel loading. The red dye migrates as a 125 bp fragment in a 1% agarose gel.

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

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