

Product Information

REDTaq® Genomic DNA Polymerase without MgCl₂

Catalog Number **D2812**

Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

REDTaq Genomic DNA Polymerase is Sigma's high quality Taq DNA Polymerase mixed with an inert red dye. It 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/μL for more accurate volume measurement and less waste.

Reactions using REDTaq Genomic DNA Polymerase, 10× PCR 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, Catalog Number D0688 – 1 unit/μL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1mM EDTA, 1 mM DTT, 0.5% Tween® 20, inert dye, 50% glycerol. Provided as 50, 250, 1,000, or 2,500 units
- 10× PCR Buffer without MgCl₂, Catalog Number P2317, 100 mM Tris-HCl, pH 8.3, 500 mM KCl, and 0.1% gelatin. Provided as 1.5 ml package.
- Magnesium chloride solution, 25 mM, Catalog Number M8787. Provided as 1.5 ml package.

Equipment and reagents required but not provided

- Deoxynucleotide Mix, Catalog Number D7295 10 mM each dATP, dCTP, dGTP, TTP
- Water, PCR Reagent, Catalog Number W1754
- Mineral Oil, Catalog Number M8662 (optional)
- Primers
- DNA template
- Dedicated pipettes
- PCR pipette tips
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Catalog Numbers P3114 and P3364
- Thermal cycler

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.

Storage/Stability

Store at -20 °C.

Procedure

It is recommended the REDTaq Genomic DNA Polymerase and the $MgCl_2$ be titrated to determine the optimal efficiency. A range of 1.0 to 4.0 mM final $MgCl_2$ concentration in 0.5 mM increments is recommended. Optimal concentrations of template DNA, $MgCl_2$, 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 μ L	10 \times PCR Buffer	1 \times
- μ L	25 mM magnesium chloride	Typically 1.0 to 4.0 mM
1 μ L	Deoxynucleotide Mix	200 μ M
- μ L	Sense primer	0.1-0.5 μ M
- μ L	Antisense primer	0.1-0.5 μ M
2.5 μ L	REDTaq Genomic DNA polymerase	0.05 unit/ μ L
- μ L	Template DNA (typically 10 ng)	200 pg/ μ L
q.s.	Water	
50 μ L	Total reaction volume	

2. Mix gently by vortex 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 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 μ L 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 μ L 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.

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Tween is a registered trademark of Croda International PLC

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AF,CR,PHC 03-10-1