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Product Information

REDTaq® Genomic DNA Polymerase without $MgCl_2$

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 $MgCl_2$, and $MgCl_2$ 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 $MgCl_2$ for a greater degree of control when optimization of $MgCl_2$ 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, stabilizers, inert dye, 50% glycerol. Provided as 50, 250, 1,000, or 2,500 units
- 10× PCR Buffer without $MgCl_2$, Catalog Number P2317, 100 mM Tris-HCl, pH 8.3 and 500 mM KCl. 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 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.

Related Products

Reagents

- Lambda DNA *Hind* III Digest, Catalog No. D9780
- Enhanced Avian HS RT-PCR kits, Catalog No HSRT100 (100 reactions).

Equipment

- PCR Multiwell Plate, 96-well, Catalog No. Z374903
- PCR Multiwell Plate, 384-well, Catalog No. Z374911
- PCR Microtubes, 0.2 ml, attached caps, Catalog No. Z374873
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Catalog No. Z374962
- Sealing accessory for PCR vessels, Micro Mats, Catalog No. Z374938
- PCR Workstation, 120V, Catalog No. Z376213
- PCR Workstation, 240V, Catalog No. Z376221

Trademarks
The following trademarks and registered trademarks are accurate to the best of our knowledge at the time of printing. Please consult individual manufacturers and other sources for specific information.

REDTaq is a registered trademark of Sigma-Aldrich Co. LLC

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Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: US 8,404,464 and US 7,972,828. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims.
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