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Product Information

REDTaq® SuperPak™ DNA Polymerase

Catalog Number **D6063**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

REDTaq SuperPak DNA Polymerase is a convenient package that includes all the necessary components for a PCR reaction except primers, DNA template and water. The SuperPak includes Sigma's high quality REDTaq DNA polymerase, 10 mM ultrapure deoxynucleotide mix and 10× PCR reaction buffer.

REDTaq DNA polymerase is Sigma's Taq DNA Polymerase mixed with an inert red dye. The dye provides quick recognition of reactions to which enzyme has been added as well as visual confirmation of complete mixing. The enzyme is provided at 1 unit/µL for more accurate volume measurement and less waste. Reactions using REDTaq are formulated as any PCR mixtures. There are no additional reaction preparation steps or protocol changes required. These formulations allow aliquots (5-10 µL) from the PCR to be directly loaded onto an agarose gel without addition of electrophoresis loading buffers. The inert dye co-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 reamplified, such as in nested PCR. If necessary, the dye can be removed from the amplicon by routine purification methodologies. The presence of the dye has no effect on automated DNA sequencing, ligase mediated ligations, exonucleolytic PCR product digestion and transformation. Though exceptions may exist, the dye is generally inert in restriction enzyme digestions.

Ultrapure dNTPs are HPLC tested (≥99% pure, <0.9% dNDP). Qualified for use in standard and long PCR, sequencing, RT-PCR, and cDNA synthesis, DNA labeling and mutagenesis reactions.

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

Reagents Provided

- REDTaq DNA Polymerase, Catalog Number D0688
  1 unit/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% glycerol
- 10× PCR Buffer, Catalog Number P2192
  100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% gelatin
- 10 mM Deoxynucleotide Mix, Catalog Number D7295
  10 mM each dATP, dCTP, dGTP, TTP

Reagents and equipment required but not provided

- Water, PCR Reagent, Catalog Number W1754
- Mineral Oil, Catalog Number M8662 (optional)
- Primers
- DNA to be amplified
- 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.

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

Storage/Stability

Store all components at –20 °C.

Procedure

The optimal conditions for the concentration of Taq DNA polymerase, template DNA, primers, and $MgCl_2$ will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component. This is especially true for the REDTaq DNA polymerase, cycling parameters, and the $MgCl_2$ concentration. It is recommended the enzyme and the $MgCl_2$ be titrated to determine the optimal efficiency.

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

Amount	Component	Final Concentration
q.s.	Water	-
5 µL	10× PCR Buffer	1×
1 µL	10 mM dNTP mix	200 µM of each dNTP
- µL	Forward primer	0.1-0.5 µM
- µL	Reverse primer	0.1-0.5 µM
2.5 µL	REDTaq DNA Polymerase	0.05 units/µL
- µL	Template DNA (typically 10 ng)	200 pg/µL
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 using a thermal cycler without a heated lid.
4. The amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system 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 by loading 5-10 µL of the PCR reaction onto the gel without the addition of gel loading buffers.

Note: a minimum of 1.5 units of REDTaq DNA polymerase must be added per 50 µL reaction for unencumbered gel loading. 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.

References

1. Innis, M. A., et al. (Eds.) *PCR Strategies*, Academic Press, New York (1995)
2. Innis, M., et al. (Eds.) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, California (1990)
3. Innis, M., et al., *Proc. Natl. Acad. Sci. USA* **85,** 9436-9440 (1988).
4. Mytelka, D. S., and Chamberlin, M. J., *Nucleic Acids Res.*, **24** (14), 2774-2781 (1996).
5. Newton, C. R., (Ed.) *PCR: Essential Data*, John Wiley & Sons, New York (1995)
6. Sambrook, J., et al. *Molecular Cloning: A Laboratory Manual,* Third Edition, Cold Spring Harbor Laboratory Press, New York (2000)

Related Products

Reagents

- Lambda DNA *Hind* III Digest, Catalog No. D9780
- Enhanced Avian HS RT-PCR kit, 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

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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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