



## Product Information

### REDTaq™ Genomic DNA Polymerase SuperPak™ Recombinant From *E. coli*

Product Code **D 6063**  
Technical Bulletin No. MB-815

## TECHNICAL BULLETIN

### Product Description

REDTaq™ DNA Polymerase SuperPak™ is a convenient package that includes all the necessary components for a PCR<sup>†</sup> 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 one unit per microliter 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.

The enzyme is supplied at 1 unit/µl.

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, Product Code D 0688  
1 unit/µl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Igepal CA-630, 50% glycerol
- 10× PCR Buffer, Product Code P 2192  
100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM MgCl<sub>2</sub>, and 0.01% gelatin
- 10 mM Deoxynucleotide Mix, Product Code D 7295  
10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP

Reagents and Equipment Required but Not Provided  
(Sigma Product Numbers have been given where appropriate.)

- Water, PCR Reagent, Product Code W 1754
- Mineral Oil, Product Code M 8662 (optional)
- Primers
- DNA to be amplified
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Product Codes P 3114 and P 3364
- Thermal cycler

### Precautions and Disclaimer

Sigma's Taq DNA Polymerase is for R&D use only. Not for drug, household or other uses. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. Refer to Material Safety Data Sheet.

### Storage

Store all components at -20 °C.

### Procedure

The optimal conditions for the concentration of Taq DNA polymerase, template DNA, primers, and MgCl<sub>2</sub> 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<sub>2</sub> concentration. It is recommended the enzyme and the MgCl<sub>2</sub> be titrated to determine the optimal efficiency. Sigma offers a separate PCR Optimization Kit (Product Code OPT-2) 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:

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.

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

### References

1. Innis, M. A., et al. (Eds.) *PCR Strategies*, Academic Press, New York (1995) (Product Code Z36,445-2).
2. Innis, M., et al. (Eds.) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, California (1990) (Product Code P 8177).
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) (Product Code Z36,491-6).
6. Sambrook, J., et al. *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, New York (2000) (Product Code M 8265).

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