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Product Information

***Taq* SuperPak™ DNA Polymerase**

Catalog Number **D5938**

TECHNICAL BULLETIN

Product Description
Taq 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 Taq DNA polymerase, 10 mM ultrapure deoxynucleotide mix, and 10× reaction buffer.

Taq DNA polymerase is a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus*. It is able to withstand repeated heating to 95 °C without significant loss of activity. The enzyme is ~94 kDa by SDS-PAGE with no detectable endonuclease or exonuclease activity. It has 5'→3' DNA polymerase activity and 5'→3' exonuclease activity. Each lot of *Taq* DNA Polymerase is tested for PCR amplification.

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
- Taq DNA Polymerase, Catalog Number D6677
  5 units/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% TWEEN® 20, 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 dATP, 10 mM dCTP, 10 mM dGTP, 10 mM 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
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 $MgCl_2$ 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 Taq 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. Sigma offers a separate PCR Optimization Kit, Catalog Number OPT2, 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
0.5 µL	*Taq* 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 and subsequent ethidium bromide staining. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

General References

1. Innis, M. A., et al. (Eds.) *PCR Strategies*, Academic Press, New York (1995), Catalog Number Z364452.
2. Innis, M., et al. (Eds.) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, California (1990), Catalog Number P8177.
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), Catalog Number M8265.

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