3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Taq DNA Polymerase SuperPak™
Recombinant From *E. coli*

Product Code **D 5938**
Technical Bulletin No. MB-720

TECHNICAL BULLETIN

Product Description

Taq DNA Polymerase SuperPak™ 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 approximately 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, Product Code D 6677
  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, 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 $MgCl_2$, 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 $MgCl_2$ 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 (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
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.

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

General 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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