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A Geno Technology, Inc. (USA) brand name

Taq DNA Polymerase

(Cat. # 786-447, 786-448)

INTRODUCTION

Taq DNA Polymerase is a highly thermostable recombinant DNA polymerase derived from the thermophile, *Thermus aquaticus*. The molecular weight of the recombinant protein is 94kD. The Taq polymerase is able to amplify DNA up to 5kb with an elongation velocity of 0.9-1.2kb/min at 70-75°C. The error rate of this Taq polymerase is ~$2.2x10^{-5}$ nucleotide^{-1} cycle^{-1}. Taq DNA polymerase catalyzes the 5'→3' synthesis of DNA. The enzyme has no detectable 3'→5' proofreading exonuclease activity, and possesses low 5'→3' exonuclease activity, which results in a 3'-dA overhang on the PCR product.

SOURCE

A recombinant protein expressed in *E. coli* that carries the *pol* gene for *Thermus aquaticus*.

ITEM(S) SUPPLIED

Cat. #	**786-447**	**786-448**
Taq DNA Polymerase (5U/µl)	1000U	5 x 1000U
10X PCR Buffer (Mg^{2+} plus)	2 x 1.25ml	10 x 1.25ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store at -20°C. Storage buffer is 20mM Tris.HCl (pH8.0), 100mM KCl, 3mM $MgCl_2$, 1mM DTT, 0.1% Nonidet® P-40, 0.1% Tween® 20, 0.2mg/ml BSA, 50% (v/v) glycerol.

10X PCR BUFFER

100mM Tris-HCl (pH8.8), 500mM potassium chloride, 1% Triton® X-100, 16mM $MgCl_2$.

UNIT DEFINITION

One unit (U) of Taq polymerase is defined as the amount of enzyme needed to catalyze the incorporation of 10 nanomoles of deoxyribonucleotides into acid-insoluble material in 30 minutes at 70°C using herring sperm DNA as a substrate.

GENERAL PCR GUIDELINES

The following protocol is a general guideline and starting point for PCR amplification, however, as with all polymerases, optimal reaction conditions for incubation times, temperatures and reagent concentrations for all components vary and require precise optimization.

- **Primers:**
  - Normally 15-30 nucleotides long.
  - G-C content of 40-60%.
  - Not self-complementary or complementary to other primers in reaction.
  - Melting temperature of primer pairs should not differ by >5°C.
- **Polymerase:**
  - Recommend 1-2U polymerase/50µl reaction. 1U for DNA template les than 10kb and 2U for DNA template greater than 10kb. Higher polymerase concentrations may result in amplification of non-specific products.
- **PCR Program**
  - Denaturation
    - 0.5- 2min at 94-98°C is normally sufficient.
  - Annealing
    - Optimal annealing temperature is ~5°C lower than the melting temperature of primer-template DNA duplex
  - Extension
    - Normally performed at 72-75°C.
    - Extension time is 1 minute for <2kb fragments.
    - For larger DNA fragments, increase extension time by 1 minute/kb
  - Cycle Number
    - For less than 10 copies of template DNA use 40 cycles
    - If >10 copies, use 25-35 cycles

Additional Information

- Recombinant Taq DNA Polymerase is the enzyme of choice for most PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

The PCR reactions should be assembled in a DNA-free environment. We recommend setting up a control reaction that is performed in the absence of DNA to ensure no DNA contamination. Briefly centrifuge the Taq polymerase tubes to collect all the enzyme at the tube bottom.

1. On ice, add the following components to a sterile 0.2ml thin walled PCR tube. For multiple reactions we recommend preparing a master mix.

Recommended PCR assay with 10X PCR Buffer (Mg^{2+} plus)

Reagent	**Quantity, for 50µl of reaction mixture**	**Final concentration**
Sterile deionized water	variable	-
10X PCR Buffer (Mg^{2+} plus)	5µl	1X
dNTPs (10mM each)	1µl	0.2mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
Taq DNA Polymerase (5U/µl)	0.25-0.5µl	1.25-2.5U/50µl
Template DNA	variable	10pg-1µg
Total		50µl

Recommendations for Template DNA Concentration in a 50µl reaction volume

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

2. Mix the contents in the tube and centrifuge briefly.
3. If the thermocycler has a heated lid, proceed with the PCR amplification. If not, overlay the PCR reaction mix with mineral oil or an appropriate amount of wax. Place sample in thermocycler and proceed with amplification.
4. Denature the DNA by heating in a thermocycler for 3 minutes at 94°C
5. Perform ~30 cycles of:
   a. Denature at 94°C for 30 seconds
   b. Anneal at 55°C for 30 seconds
   c. Extend: 72°C for 1min/1kb template
6. Incubate for additional 10 minutes at 72°C and then maintain the reaction at 4°C or store at -20°C until required.

RELATED PRODUCTS

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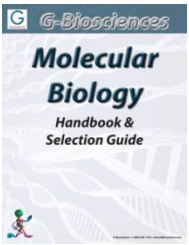

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