G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Taq PLUS DNA Polymerase

(Cat. # 786-850)

INTRODUCTION

Taq Plus DNA Polymerase is a mixture of Taq DNA Polymerase and Pfu, a proofreading DNA Polymerase, which allows for the amplification of long templates, up to 20kb, with high fidelity. The two enzymes act synergistically during PCR to generate more accurate and longer PCR products with greater yields compared to Taq DNA Polymerase alone. PCR products, amplified up to 20kb in length with Taq Plus DNA Polymerase, contain a mixture of blunt ends and single base (A) 3' overhang. The error rate of this PCR amplification is $7.5x10^{-5}$ per nucleotide per cycle.

ITEM(S) SUPPLIED

Description	Cat. # 786-850
Taq DNA PLUS Polymerase (2.5U/µl)	1000U
10X PCR Buffer (Mg^{2+} plus)	2 x 1.25ml

STORAGE CONDITIONS

The kit is shipped on blue ice. 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) 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 less than 10kb and 2U for DNA template greater than 10kb.
- **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

QUALITY CONTROL

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 10U Taq Plus DNA Polymerase with 1µg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 10U Taq Plus DNA Polymerase with 1µg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10U Taq Plus DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.

ADDITIONAL INFORMATION

- The half-life of enzyme is >40 minutes at 95°C.
- Taq PLUS 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 error rate of Taq Plus DNA Polymerase in PCR is about $1x10^{-5}$ errors per nucleotide per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error is $3.8x10^{-5}$
- The PCR products are the mixture of 3´-dA overhangs and blunt-ended products. But blunt-ended is the main product.
- •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.

PROTOCOL

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 *PLUS* DNA Polymerase (5U/µl)	0.5-1µl	1.25-2.5U/50µl
Template DNA	variable	10pg-1µg
Total		50µl

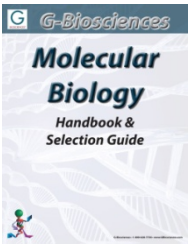
Recommendations for Template DNA Concentration in a 50µl reaction volume

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

Download our Molecular Biology Handbook.



http://info.gbiosciences.com/complete-molecular-biology-handbook/

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 12/17/2012 CMH

www.GBiosciences.com