Long Taq DNA Polymerase

#P1061, 250U

Concentration: 5U/μl
Contents:

Long Taq DNA Polymerase	50 μl
10× Long PCR Buffer Ⅰ (Mg^{2+} Plus)	1.25 ml
10× Long PCR Buffer Ⅱ (Mg^{2+} Plus)	1.25 ml
PCR Enhancer	500 μl
6× Loading Buffer	1 ml

Store at -20°C

For research use only.

In total 5 vials.

Description

Long Taq DNA Polymerase, a combination of two thermostable DNA polymerases, Taq and Pfu, is a special formulation designed for amplifying long DNA fragments. This specially formulated Long Taq DNA Polymerase was shown to amplify long DNA templates from λ phage genome of up to 20 kb. It is also a better choice for amplifying complex template, such as GC-rich template.
Long Taq DNA Polymerase is suitable as a replacement for ordinary Taq Polymerase in most applications. The elongation rate is 3kb/min. The products of Long Taq DNA Polymerase is a mix of 3´-dA overhangs and blunt-ends products, which can be used in TA clone.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

20mM Tris-HCl (pH 8.0), 100mM KCl, 3mM $MgCl_2$, 1mM DTT, 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (V/V) glycerol.

10× Long PCR Buffer Ⅰ with Mg^{2+}

500mM Tris-HCl (pH 8.8), 160mM $(NH_4)_2SO_4$, 25mM $MgCl_2$, 1% Triton X-100.

10× Long PCR Buffer Ⅱ with Mg^{2+}

200mM Tris-HCl (pH 8.8), 100mM KCl, 100mM $(NH_4)_2SO_4$, 16mM

$MgSO_4$, 1% Triton X-100.

Note:

10× Long PCR Buffer Ⅰ is classical Long Taq DNA Polymerase buffer, and it's good for long template DNA, especially above 10 kb.

10× Long PCR Buffer Ⅱ is a special buffer optimized by Dongsheng Biotech. It is for better fidelity but not good at long template DNA above 10 kb.

Users could choose suitable buffer for different template.

Features

- High fidelity: three times fidelity of Taq DNA Polymerase.
- Longer fragment: amplify long templates as long as 20 kb.
- Amplification of complex template (GC rich or repetitive sequence).
- Generates 3'-dA and blunt-end PCR products.

Applications

- PCR amplification of complex template DNA
- PCR amplification of long DNA sequences
- DNA sequencing
- PCR for cloning

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Long Taq DNA Polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

1.1 Recommended PCR assay with Long PCR Buffer (Mg^{2+} plus)

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

4-16 μl PCR Enhancer can be added to the reaction system of 50 μl. By reducing the dissociation temperature of DNA template and promoting the effective amplification of DNA template, PCR Enhancer can increase the sensitivity and specificity of PCR reaction.

1.2 Recommended PCR assay with Long PCR Buffer (Mg^{2+} free)

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× Long PCR Buffer (Mg^{2+} free)	5 μl	1×
dNTPs (10mM each)	1 μl	0.2mM each
Primer I	variable	0.4-1μM
Primer II	variable	0.4-1μM

25mM Mg^{2+}	variable	1.0-4.0mM
Long Taq DNA Polymerase (5U/μl)	0.25-0.5 μl	1.25-2.5U/50 μl
Template DNA	variable	10pg-1μg
Total		50 μl

Table for selection volume of 25 mM $MgCl_2$ solution in a 50 μl reaction mix:

Final Mg^{2+} Conc. (mM)	1.0	1.5	2.0	2.5	3.0	4.0
Mg^{2+} (25mM)	2 μl	3 μl	4 μl	5 μl	6 μl	8 μl

Recommendation amounts of template DNA in a 50 μl reaction mix:

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

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 μl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1-10 minutes
Final Extension	72°C	10 minutes

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- Initial denaturation can be performed over an interval of 1-5 min at 94°C-95°C depending on the GC content of template.
- Denaturation for 30 sec to 2 min at 94-95°C is sufficient. If the amplified DNA has a very high GC content, denaturation time may be increased up to 3-4 min.
- Optimal annealing temperature is 5°C lower than the melting temperature of duplex primers. If nonspecific PCR products are obtained, optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2°C.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR products. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.
- The final extension step can extend amplicons that will be cloned into T/A vectors.

Guidelines for preventing contamination of PCR reaction

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

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 Long Taq 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 Long Taq 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 Long Taq DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.