Long Taq Kit

#P3062, 5 ml

Contents:

2× Long Reaction Mix	1 ml × 5
Long Taq DNA Polymerase (2.5U/μl)	160 μl
PCR Enhancer	500 μl × 3

Store at -20°C

For research use only.

In total 9 vials.

Description

Long Taq Kit contains Long Taq DNA Polymerase and a premixed, ready-to-use Long Reaction Mix, which contains dNTPs, Mg^{2+} and other components at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only need to add primers and template DNA. Long Taq Kit contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. It also contributes to higher sensitivity by adding enhancer. Using the kit in your PCR reaction results in a mix of blunt-end and 3'-dA overhangs PCR products.

Long Taq DNA Polymerase, a combination of two thermostable DNA polymerases, Taq and Pfu, is a special formulation designed for amplifying large fragments. This specially formulated Long Taq was shown to amplify long templates from λ phage genome that up to 20 kb. The elongation rate is 3kb/min. It is also a better choice for amplifying complex template, such as GC-rich template. Long Taq is suitable as a direct replacement for ordinary Taq Polymerase in most applications. Using Long Taq in your PCR reactions results in 3´-dA overhangs PCR 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 (pH8.0), 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.5% NP-40, 0.5% TW 20, 50% (V/V) Glycerol

Composition of the 2× Long Reaction Mix

2× Long PCR Buffer, 0.4mM dNTPs, 3.2mM $MgCl_2$, 0.02% bromophenol blue.
Long Reaction Mix is a proprietary formulation optimized for robust performance in PCR.

Applications

- PCR for long templates of up to 20 kb
- High reproducible, high throughput PCR for complex template

Features

- **Convenient**: only primers and template DNA are added when prepare final PCR
- **Longer fragment**: amplify long templates as long as 20 kb
- **High efficiency**: saving your time by simplifying the process
- **Reproducible**: lower contamination and pipetting error risk
- Amplification of complex template (GC-rich or repetitive sequence)
- **Flexible**: the amount of polymerase is flexible and controllable.

Basic PCR Protocol

All solutions should be thawed on ice, gently vortex and briefly centrifuge.

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

Reagent	Quantity	Final concentration
2× Long Reaction Mix	25 µl	1×
Forward Primer	variable	0.4-1µM
Reverse Primer	variable	0.4-1µM
Template DNA	variable	10pg-1µg
Long Taq DNA Polymerase (2.5U/µl)	0.5-1 µl	1.25-2.5U/50µl
Water, nuclease-free	to 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.

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 in the 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 1-5 minutes
Final Extension	72°C	2 minutes

4. Incubate for an additional 2 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 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 primer-temperature DNA duplex. 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 product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.
- The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.

Guidelines for preventing contamination of PCR

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.