Long Taq DNA Polymerase
(+buffer + loading dye)

Ref : NB-03-0110 250 U
NB-03-0111 250 U
NB-03-0112 1 000 U
NB-03-0113 1 000 U

Contents

	NB-03-0110	NB-03-0111	NB-03-01112	NB-03-0113
Long Taq DNA Polymerase (5 U/µl)	50 µl	50 µl	200 µl	200 µl
10×Long PCR Buffer I (Mg2+ Plus)	1,4 ml	1,4 ml	2x1,4 ml	2x1,4 ml
10×Long PCR Buffer II (Mg2+ Plus)	1,4 ml	1,4 ml	2x1,4 ml	2x1,4 ml
PCR Enhancer	500 µl	500 µl	2x500 µl	2x500 µl
6x loading buffer	1 ml	1 ml	1 ml	1 ml
DNTP (each 2,5mM)		1 ml		2x1 ml

Note

10X Long PCR Buffer I is classical Long Taq DNA Polymerase buffer, is good for long template especially above 10kb.
10X Long PCR Buffer II is a special buffer. It is for better fidelity but not good at long template above 10kb.Users could choose suitable buffer for different template.
Note:10xLong PCR Buffer I and 10xLong PCR Buffer II are Mg2+ plus.

Description

Long Taq DNA Polymerase, a combination of two thermostable DNA polymerases, Taq and Pfu, is a special formulation designed for amplifying large fragment.
This specially formulated Long Taq was shown to amplifiy long 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 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 nM of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Store all components at –20°C

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

Features

- High fidelity: three times fidelity of Taq DNA Polymerase.
- Longer fragment: amplify long templates as long as 40kb.
- Amplification of complex template (GC rich or repetitive sequence).
- Generates 3'-dA and blunt end PCR products.

Applications

- PCR amplification of DNA fragments as long as 5 kb
- DNA labelling
- DNA sequencing
- PCR for cloning

Storage Buffer

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

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 Taq DNA Polymerase, primers, Mg^{2+} and template DNA) vary and need to be optimized.

1. Add the following components to a sterile 50 µl microcentrifuge tube sitting on ice:

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

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

Recommandations with Template DNA in a 50 µl reaction volume

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

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℃	3 minutes
25-35 cycles	94℃ 55-68℃ 72℃	30 seconds 30 seconds 1 minute
Final extension	72℃	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 ethidium bromide staining. Use appropriate molecular weight standards.

Notes on cycling conditions:

- Initial denaturation can be performed over an interval of 1-5 min at 95℃ depending on the GC content of template.
- Denaturation for 30 sec to 2 min at 94-95 ℃ 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℃ 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℃.
- 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 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 5 µl Long Taq Polymerase with 1 µg of pBR322 DNA in 50 µl for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay
No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of5 µl Long Taq Polymerase with 1 µg of digested DNA in 50 µl for 4 hours at 37°C and at 70°C.

Ribonuclease Assay
0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 5 µl Long Taq Polymerase with1 µg of E.coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C. 0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 5 µl Long Taq Polymerase with 1 µg of E.coli [3H]-RNA (40000 cpm/µg) in 50 µl for 4 hours at 70°C.