Biomatik
Tel: (519) 489-7195, (800) 836-8089
Fax: (519) 231-0140, (877) 221-3515
Email: info@biomatik.com
http://www.biomatik.com

Product Information

Version 5.1, Revision 2012-07-21

Product: **Code:** **Concentration:** **Contents:**	**Long Taq DNA Polymerase** A4186 5U/μl Long Taq DNA Polymerase 50μl 10xLong PCR Buffer Ⅰ 1.25ml 10xLong PCR Buffer Ⅱ 1.25ml PCR Enhancer 500μl 6xLoading Buffer 1ml
Unit Definition:	One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 70°C using herring sperm DNA as substrate.
Description:	**Long Taq DNA Polymerase** is a combination of two thermostable DNA polymerases, Taq and Pfu. It is a special formulation designed for amplifying large fragments. This specially formulated Long Taq has been shown to amplify long templates from λ phage genome of up to 20kb. It is also a better choice for amplifying complex templates, such as GC-rich templates. 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 overhang/blunt end PCR products, which can be used in TA cloning. • 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 complex templates PCR amplification of long templates DNA sequencing PCR for cloning
Storage Buffer:	20mM TrisCl (pH8.0), 100mM KCl, 3mM $MgCl_2$ 1mM DTT， 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol.
10X Long PCR Buffer I with Mg2+:	500mM Tris-HCl pH 8.8, 160mM $(NH_4)_2SO_4$, 25mM $MgCl_2$, 1% Triton X-100 **Note:** 10xLong PCR Buffer I is classical Long Taq DNA Polymerase buffer, is good for long template especially above 10kb. Users could choose suitable buffer for different template.
10X Long PCR Buffer II with Mg2+:	200mM Tris-HCl PH8.8, 100mM KCl, 100mM $(NH_4)_2SO_4$, 16mM $MgSO_4$, 1% Tritonx-100 **Note:** 10xLong PCR Buffer II is a special buffer optimized by Biomatik. It is for better fidelity but not good at long template above 10kb. Users could choose suitable buffer for different template.
25mM $MgCl_2$:	In general 2mM is recommended to start; this may vary with different conditions and primer sets.
Storage:	-20°C

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.

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.

PCR Reaction Setup

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

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

Reagent	Quantity, for 50µl reaction	Final concentration
Sterile deionized water	variable	-
10X Long PCR Buffer (Mg^{2+} plus)	5µl	1X
dNTPs （10mM each）	1µl	0.2 mM 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		50µl

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

Reagent	Quantity, for 50µl reaction	Final concentration
Sterile deionized water	variable	-
10X Long PCR Buffer (Mg^{2+} free)	5µl	1X
dNTPs （10mM each）	1µl	0.2 mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
25mM Mg^{2+}	variable	1-4mM
Long Taq DNA Polymerase（5U/µl）	0.25-0.5 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50µl

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

Final Mg^{2+} conc.	1.0mM	1.5mM	2.0mM	2.5mM	3mM	4mM
Mg^{2+} Stock	2µl	3µl	4µl	5µl	6µl	8µl

Recommendations with Template DNA in a 50µl 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 at 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 1-10 minutes
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

1. 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.

2. 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

3. 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 Polymerase with 1µg pBR322 DNA in 50µl for 4 hours at 37°C and 70°C.

4. Exodeoxyribonuclease Assay

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

5. Ribonuclease Assay

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