# Long Taq Kit

Catalog No: P3011 (5x1ml)

**Contents:** 2X Long Reaction Mix 5x1ml
PCR Enhancer 3x500µl
Long Taq DNA Polymerase(2.5U/µl) 160µl

**Store at -20°C**
In total 9 vials.

***Description***

Long Taq Kit is a premixed, ready-to-use solution containing Long Taq DNA Polymerase, dNTPs and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. In this kit, Long Taq DNA Polymerase is stored separately from PCR reaction Mix. To prepare the final PCR, please mix the polymerase and the reaction buffer in appropriate proportion, and then add the primers and template DNA needed. Long Taq Mix 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 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.

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<u>PRODUCT USE LIMITATION.</u>

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.

*Unit Definition*

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

*Storage Buffer*

20 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5 % TW 20, 0.5 % NP 40, 50 % Glycerol

*Composition of 2X Long Reaction Mix*

2X Long PCR buffer, 0.4mM dNTPs, 3.2 mM $MgCl_2$, 0.02% bromophenol blue.

Long Taq mix buffer is a proprietary formulation optimized for robust performance in PCR..

*Features*

- Convenient: only primers and template are needed
- Efficiency: simplifying the operation and saving your time
- Reproducible: reduce the risk of pipetting errors,

  miscalculation and contamination
- The amount of polymerase is flexible and controllable.

*Applications*

- PCR for long templates of up to 40kb
- High reproducible ,high throughput amplification reaction of

  complex templates
- Generate PCR product for TA cloning.

*Basic PCR Protocol*

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. Add in a thin walled PCR tube on ice:

For a total 50µl reaction volume

| Component of sample | Volume | Final concentration |
|---|---|---|
| 2X Long Reaction Mix | 25 µl | 1X |
| Forward Primer | variable | 0.1-1 µM |
| Reverse Primer | variable | 0.1-1 µM |
| Template DNA | variable | 10 pg-1 µg |
| Long Taq DNA Polymerase (2.5U/µl) | 0.5-1 µl | 1.25-2.5U/50µl |
| Water, nuclease-free | to 50 µl | – |

**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. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube. Overlay the sample with mineral oil or add an appropriate amount of wax.**

This step may be omitted if the thermal cycler is equipped with a heated lid.

**3. Overlay the sample with mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid.**

**4. Preform PCR using the following thermal cycling conditions.**

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

**5. 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.**

**6. 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°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 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 Polymerase with 1µg pBR322 DNA in 50µl 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 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 Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.