

Long Taq Kit (2x)

Ref : NB-03-0116 5x 1 ml

Contents

NB-03-0116	200 reactions for 50ul PCR System
2×PCR Reaction Mix	5x 1 ml
Long Taq DNA Polymerase(2.5U/μl)	100 μl
PCR Enhancer	1 ml

Store all components at –20°C

Note

The Mix already contains 4 mM MgCl₂.

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 amplify 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.

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.

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.

2×PCR Reaction Mix

100 mM KCl, 20 mM Tris-HCl, 3 mM MgCl₂, 400 μM dNTPs, bromophenol blue, dd H₂O

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

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

Components	Volume	Final concentration
2X Long Reaction Mix	25 μl	1 x
Primer mix (10 μM each)	4 μl	0.4 μM each
Template DNA	1-10 μl	n/a
Long Taq DNA Polymerase (2.5U/μl)	0.5-1 μl	1.25-2.5U/50μl
Water, nuclease-free	To 50 μl	n/a

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.
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. Perform PCR using the following thermal cycling conditions.

Initial Denaturation	94°C	3 minutes
25-35 cycles	94°C	30 seconds
	55-68°C	30 seconds
	72°C	1 minute
Final extension	72°C	10 minutes

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°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.

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.