NZYLong DNA polymerase

Catalogue number: MB00301, 125 U
MB00302, 500 U
MB00303, 1000 U

Description

NZYLong DNA polymerase is an engineered DNA polymerase designed to amplify long target DNA sequences, generally of 20 kb and beyond. A wide range of long PCR products can be generated using lambda DNA or human genomic DNA as starting template. The provided 10x reaction buffer protects DNA from depurination and nicking during long thermal cycling. NZYLong DNA polymerase offers a fivefold higher fidelity than conventional *Taq* DNA polymerases. The enzyme generates a mixture of A-overhang-ended (predominantly) and blunt-ended PCR products, being suitable for cloning with NZYtech's TA PCR cloning kits (MB053 or MB137).

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to room-temperature.

Storage Conditions

This product should be stored at -85°C to -15°C in a freezer without defrost cycles to guarantee maximal shelf life. The high thermal stability of the enzyme allows it to remain stable at 4°C or even at room-temperature for up to 1 week, so, if you forget your enzyme on your lab bench, no harm is done. The product will remain stable till the expiry date if stored as specified.

Unit definition

One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nmoles of dNTPs into acid insoluble material in 30 minutes at 72 °C, in controlled assay conditions.

Enzyme concentration 5 U/µL, in glycerol

Reaction Buffer (10×)

The enzyme reaction buffer contains Mg^{2+} (2 mM at the final, 1×, reaction concentration). Mix the Reaction Buffer solution thoroughly after thawing.

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for a long PCR amplification. Optimal reaction conditions (e.g., concentration of DNA Polymerase, primers, magnesium, and template DNA) vary and may need to be optimized. In case you need to fine-tune PCR conditions, recommended variations of each PCR component are provided in brackets in the table below. Avoid pipetting samples containing target DNA when amplicons above 15 kb are desired.

1. Gently mix and briefly centrifuge all components after thawing. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a mixture for the appropriate number of PCR reactions. Add water first and the remaining components in the order specified in the table below. A single reaction mixture of 50 µL should combine the following components:

10x Reaction Buffer (provided)	5 µL
dNTPs mix *	0.3 (0.2-0.5) mM
Primers	0.35 (0.25-0.5) µM
NZYLong (5 U/µL)	1 µL
Template DNA (see below)	5 ng-0.5 µg
Nuclease-free water	up to 50 µL

* Use high-quality dNTPs and avoid repeated freeze cycles. We recommend to prepare small-volume working aliquotes from the stock solution.

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters:

Cycle step	Temp.	Time	Cycles
Initial denaturation	94 °C	2 min	1
Denaturation	94 °C	20 s	25-35
Annealing	*	30 s	
Extension	68 °C	1 min/kb	
Final Extension	68 °C	1.5 min/kb	1

*Annealing temperature should be optimized for each primer set based on the primer T_m; typically it should be T_m-5 °C.

4. Analyse the PCR products through agarose gel electrophoresis (0.6-0.8%, w/v) and visualise with GreenSafe Premium (MB132) or any other means.

Primer Design

Optimal primer design is critical for long-range amplifications. PCR primers should be designed to have 18–35 bases in length and a GC content of 45-60%. Pay special attention to avoid sequences that might produce internal secondary structures. The 3´-ends of the primers should not be complementary to avoid the production of primer-dimers, and it is recommended to have at least 2 Cs or Gs. Ideally, both primers should have nearly identical melting temperatures (T_m) allowing their annealing with the denatured template DNA at roughly the same temperature. For long PCRs avoid using primers that have been previously subjected to multiple freezing-thawing cycles. Note that primer annealing, and DNA extension can be combined into one step if primers are designed to have a Tm ≥ 70 °C.

DNA template

The amplification of long PCR products requires high quality gDNA retaining long DNA fragments (it is not possible to amplify a 20 kb product from damaged gDNA with an average fragment size of 5 kb, for example). The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 50ng to 500ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 5ng of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 10-50ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Try to add the DNA as the latest component to the PCR reaction and avoid pipetting after this. To retain DNA integrity, avoid multiple freeze-thawing cycles stock DNA solutions and keep working DNA at small aliquots.

Cycling conditions

It is highly recommended to use incubation and extension temperatures as high as required by the experiment. An extension performed at 68 °C favours the accumulation of long PCR products without compromising enzyme performance.

Quality control assays

Purity

NZYLong DNA polymerase purity is > 90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

The product must be free of any detectable DNA contamination as evaluated through PCR. Thus, it is suitable for the amplification of bacterial and fungal DNA based on 16S and 18S rRNA PCR assays.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of NZYLong DNA polymerase, in 1× Reaction Buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with the buffer.

Functional assay

NZYLong DNA polymerase is tested for performance in a polymerase chain reaction (PCR) of different-sized DNA fragments (until 15kb) from human genomic DNA. The resulting PCR products are visualized as single bands in a GreenSafe Premium-stained agarose gel.

Troubleshooting

No product amplification or low yield
• Inadequate annealing temperature
The reaction mix composition may affect the melting properties of primers and DNA. Adjust the annealing temperature to accommodate the primer with the lowest melting temperature (5 ° to 10 °C lower than T_m).
• Presence of PCR inhibitors
Some DNA isolation procedures, particularly genomic DNA isolation, can result in the co-purification of PCR inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to the reaction. Diluting samples even 1:10,000 has been shown to be effective in improving results, depending on initial DNA concentration.
• Template DNA damaged or degraded
An intact, high-quality template is essential to achieve a reliable amplification of large fragments. Extreme care must be taken in the preparation and handling of DNA. Always use purified high-quality DNA as template.
Presence of non-specific bands
• Non-specific annealing of primers
Adjust annealing conditions and/or design another set of primers, by increasing the length and avoiding complementary sequences.
• Primer degradation
Check the quality and concentration of primer solutions. We recommend to prepare small-volume working aliquots from the stock solution. Avoid using primers subjected to multiple freezing-thawing cycles.

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