NZYLong DNA polymerase

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

Description

NZYLong DNA polymerase is an optimized DNA polymerase designed to amplify target DNA sequences up to 20 kb in size. NZYLong DNA polymerase displays a 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).

Storage temperature

NZYLong DNA polymerase should be stored at -20 °C, in a constant temperature freezer. NZYLong DNA polymerase 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.

Enzyme concentration: 5 U/µL

Reaction Buffer (10×): Contains Mg^{2+} (2 mM at the final, 1×, reaction concentration). Prior to use, mix the Reaction Buffer solution thoroughly after thawing.

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of DNA Polymerase, primers, $MgCl_2$, and template DNA) vary and may need to be optimized.

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture for the appropriate number of samples to be amplified. A single reaction mixture should combine the following components (for a 50 µL reaction):

10x Reaction Buffer (provided)	5 µL
dNTPs mix [1]	0.2-0.5 mM
Primers	0.25-0.5 µM
Template DNA	5 ng-0.5 µg
NZYLong (5 U/µL)	0.5-1.0 µL
Nuclease-free water	up to 50 µL

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

2. If using a thermal cycler without a heated lid, overlay the reaction mix with 1-2 drops of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.

3. Perform PCR using standard parameters.

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	94 °C	10-30 s	25-35
Annealing	*	30-60 s	
Extension	68 °C	60-120 s/kb	
Final Extension	68 °C	10 min	1

*Annealing temperature should be optimised 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 l 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 ≥ 68 °C.

DNA template

The optimal amount of starting material may vary depending on the quality and complexity of template DNA. In general, we recommend using 500-50 ng of genomic DNA template. Lower amounts of DNA template (typically 60-10 ng) can be used for amplification of lambda or plasmid DNA. For a cDNA synthesis reaction mixture, do not exceed 10% of the final PCR reaction volume.

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
NZYLong polymerase must be free of any detectable genomic DNA contamination as evaluated through PCR.

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) using 5 U of enzyme for the amplification of different sized PCR fragments (15 and 20 kb) from human genomic DNA. The resulting PCR product is visualized as a single band in a GreenSafe Premium-stained agarose gel. Similar functional tests are performed with the buffer.

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.
• Concentration of Mg^{2+} is too low
Mg^{2+} is included in the 10× Reaction Buffer at a final concentration of 2 mM, which is sufficient for most targets. For some targets, a higher Mg^{2+} concentration may be required. Titrate from 2 mM to 4 mM (final concentration) in 0. 5 mM increments.
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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Certificate of Analysis

Test	Result
Enzyme purity*	Pass
Genomic DNA contamination*	Pass
DNase contamination	Pass
Functional assay	Pass

*These assays were performed exclusively with the enzyme

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