Batch NP081
Expiry date:12/2024

NZYProof DNA polymerase

Catalogue number: MB14601, 125 U
MB14602, 500 U
MB14603, 1000 U

Description

NZYProof DNA polymerase is a recombinant DNA polymerase that presents high fidelity and displays great performance in the majority of PCR applications. NZYProof DNA polymerase possesses 3´→5´ exonuclease proofreading capacity which enables the polymerase to amplify DNA with increased accuracy. The enzyme is highly efficient in the amplification of longer (≤10 kb) PCR products and site-directed mutagenesis. In addition, it the recommended polymerase for routine cloning that requires precision. The error rate of NZYProof DNA polymerase is similar to that of *Pfu* and *Kod* DNA polymerases and significantly lower than the error rate of *Taq* DNA polymerases. NZYProof DNA polymerase generates blunt-ended PCR products that are suitable for cloning with NZYtech's NZY-blunt PCR cloning kit (MB121). NZYProof DNA Polymerase is supplied with a 10× Reaction Buffer, which contains Mg^{2+} at an optimal concentration for standard PCR, and a 5× Stabilizer Solution, which maximizes yields especially when using lower template concentrations.

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage Conditions

This product should be stored at -85°C to -15°C in a freezer without defrost cycles to guarantee maximal shelf life. Keep the enzyme on freezer while perform PCR set up until use. Minimize the number of freeze-thaw cycles by storing in working aliquots. 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.

Enzyme concentration 2.5 U/µL

Stabilizer Solution (5×)

A proprietary formulation that contains additives and stabilizers of NZYProof DNA polymerase allowing to increase PCR yield and sensitivity of detection for low-copy templates.

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (e.g., concentration of DNA Polymerase, primers, 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. It is strongly recommended to assemble all reaction components on ice and quickly transfer the reactions to a thermocycler preheated to the denaturing temperature to start the PCR.

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. It is strongly advisable that the enzyme is the last component to add to the reaction in order to minimize primer degradation due to the 3'→5'exonuclease activity. A single 50 µL reaction mixture should combine the following components:

10× Reaction buffer	5 µL
5× Stabilizer Solution (optional)[1]	(10 µL)
dNTPs mix	0.2 mM
Primers (see below)	0.4 (0.3-0.5) µM
Template DNA (see below)	5 ng-0.5 µg [(1)]
NZYProof DNA Polymerase (2.5 U/µL)	0.5 µL
Nuclease-free water	up to 50 µL

[(1)] When using template DNA at lower concentrations (≤ 10 ng), add 5× Stabilizer Solution.

2. Mix and quickly pulse the reactions.

3. Immediately initiate the PCR by transferring the PCR mixtures to the thermocycler with the block pre-heated to 95 ºC and following the below cycling parameters:

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	30 s	20-40
Annealing	*	30 s	
Extension	72 °C	60 s/kb	
Final Extension	72 °C	5-10 min	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 PCR products by agarose gel electrophoresis (0.7-1.2%, w/v) and visualize with GreenSafe (MB088) or any other means.

Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Sequences longer than 30bp may improve PCR yield using NZYProof DNA polymerase since its 3´→5´ exonuclease activity may degrade primers. In addition, to overcome primer degradation, the 3´ termini of primers may be protected with phosphorothioate modifications. Primers should contain 40–60% GC, and care should be taken to avoid sequences that might produce internal secondary structure. The 3´-ends of the primers should not be complementary to avoid the production of primer-dimers. Primer-dimers unnecessarily remove primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3´-end of the primer, as this may result in non-specific primer annealing. Ideally, both primers should have nearly identical melting temperatures (T_m), allowing their annealing with the denatured template DNA at roughly the same temperature.

DNA template

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.

Enzyme concentration

In general, we recommend using 1.25 U of enzyme (0.5µL) in a 50 µL reaction. You may increase the volume of enzyme to a maximum of 2.5 U (1 µL) in a 50 µL reaction when amplifying abundant templates (>50 ng gDNA). Do not exceed this enzyme concentration in particular for longer PCR products (>5 kb). For convenience during PCR assembly, enzyme may be dilute in water (for example, dilute 1/10 in water to add 5 µl of diluted enzyme instead of 0.5 µl of undiluted preparation).

Quality control assays

Purity

NZYProof DNA polymerase purity is >90% as judged by SDS-PAGE 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 NZYProof DNA polymerase, in 1× reaction buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with NZYProof buffer and stabilizer solution.

Functional assay

NZYProof DNA polymerase is extensively tested for performance in a PCR reaction using 1.25 units of enzyme for the amplification of different-sized DNA fragments (1 and 2.5 kb) from human genomic DNA. The resulting PCR products are visualized as a single band in a GreenSafe 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.
• Concentration of Mg^{2+} too low
Mg^{2+} included in the 10× Reaction Buffer is at a final concentration of 2 mM, which is sufficient for most targets. Note that optimal Mg^{2+}concentration can be affected by dNTP concentration and the type of template being used. For some targets, more Mg^{2+} may be required. Titrate from 2 mM to 3.5 mM (final concentration) in 0.25 mM increments.

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For research use only.