Proof DNA polymerase

NB-60-0006

Proof DNA polymerase

Cat# NB-60-0006 Size : 1000U

Description

Proof DNA polymerase is a recombinant DNA polymerase that presents high fidelity and displays great performance in the majority of PCR applications. Neo Biotech Proof 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 Neo Biotech Proof DNA polymerase is similar to that of Pfu and Kod DNA polymerases and significantly lower than the error rate of Taq DNA polymerases. Neo Biotech Proof DNA polymerase generates blunt-ended PCR products that are suitable for cloning. Neo Biotech Proof DNA Polymerase is supplied with a 10× Reaction Buffer, which contains Mg2+ at an optimal concentration for standard PCR, and a 5× Stabilizer Solution, which maximizes yields especially when using lower template concentrations.

Storage Conditions

Proof DNA polymerase should be stored at -20 °C in a constant temperature freezer. Neo Biotech Proof 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 2.5 U/µL

Stabilizer Solution (5×)

A proprietary formulation that contains additives and stabilizers of Neo Biotech Proof 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)
- Neo Biotech Proof 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 Tm; typically it should be Tm-5 °C.

4. Analyse PCR products by agarose gel electrophoresis (0.71.2%, w/v) and visualize.

PCR designs

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 Proof 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 (Tm), 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

Proof DNA polymerase purity is >90% as judged by SDS-PAGE followed by Coomassie Blue staining.

Genomic DNA contamination

Proof DNA 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 Neo Biotech Proof DNA polymerase, in 1× reaction buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with Proof buffer and stabilizer solution.

Functional assay

Proof 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 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 Tm).

• 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, concentration, depending on initial DNA

• Concentration of Mg2+ too low

Mg2+ included in the 10× Reaction Buffer is at a final concentration of 2 mM, which is sufficient for most targets. Note that optimal Mg2+concentration can be affected by dNTP concentration and the type of template being used. For some targets, more Mg2+ may be required. Titrate from 2 mM to 3.5 mM (final concentration) in 0.25 mM increments.