MB146_IFU_EN_V2601

NZYProof DNA polymerase

Catalogue number	Presentation
MB14601	125 U (50 µL)
MB14602	500 U (200 µL)
MB14603	1000 U (2 x 200 µL)

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 (Cat. No. MB121). NZYProof DNA Polymerase is supplied with a 10x Reaction Buffer, which contains Mg^{2+} at an optimal concentration for standard PCR, and a 5x Stabilizer Solution, which maximizes yields especially when using lower template concentrations.

Shipping & Storage Conditions

The product can be shipped from dry ice to blue ice. 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 it in working aliquots. The product will remain stable till the expiry date if stored as specified.

Components

	MB14601 (125 U)		MB14602 (500 U)		MB14603 (1000 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
NZYProof DNA polymerase	1	50 µL	1	200 µL	2	200 µL
10x Reaction Buffer for NZYProof	1	1000 µL	2	1000 µL	4	1000 µL
5x Stabilizer Solution	1	1500 µL	3	1500 µL	6	1500 µL

Specifications

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.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: The quality and integrity of DNA templates are critical for achieving reliable PCR results. Use high-quality DNA extraction kits to isolate clean, contaminant-free DNA. To avoid degradation or contamination, handle DNA samples with care and work in designated clean areas. Consider using NZYtech Nucleases & Nucleic Acid Cleaner (Cat. No. MB48301) or DNA & RNA Cleaner (Cat. No. MB46201) to remove nucleases or residual contaminants from surfaces and materials.
- Handling instructions: To prevent carry-over contamination, dedicate separate areas for reaction setup, PCR amplification, and any post-PCR analysis. Never open tubes containing amplified PCR products in the PCR setup area. Always use sterile, filtered pipette tips and avoid reusing any consumables.
- Preventing Contamination: Implement stringent laboratory practices to avoid false positives caused by contaminants. Use sterile equipment, clean workstations regularly, and monitor assay integrity by including No-Template Controls (NTC) in each PCR run.

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 the master mix after thawing.
2. 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 50 µL reaction mixture should combine the following components:
   ***Note**: 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*.

	1 REACTION
Reaction Buffer, 10x (*provided*)	5 µL
5x Stabilizer Solution (*provided; optional**)	(10 µL) **(*)**
dNTPs mix	0.2 mM
Forward and Reverse Primers (see below)	0.4 (0.3-0.5) µM
Template DNA (see below)	5 ng-0.5 µg **(*)**
NZYProof DNA polymerase (2.5 U/µL) (*provided*)	0.5 µL
Nuclease-free water	up to 50 µL
FINAL VOLUME =	50 µL

(*) When using template DNA at lower concentrations (≤ 10 ng), add the 5× Stabilizer Solution provided.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Immediately initiate the PCR by transferring the PCR mixtures to the thermocycler with the block pre-heated to 95 °C and following the cycling parameters described below:

CYCLES	**TEMP.**	**Time**	**STAGE**
1	95 °C	3 min	Initial denaturation
20-40	95 °C	30 sec	Denaturation
	(******)	30 sec	Annealing
	72 °C	60 sec/kb	Extension
1	72 °C	5-10 min	Final Extension

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

5. Analyse the PCR products through agarose gel electrophoresis (0.7-1.2%, w/v) and visualise with GreenSafe Premium (NZYtech, Cat. No. MB132) or any other means

Technical Notes

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 (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, especially 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).

Mg^{2+}: This cofactor is already incorporated in the reaction buffer provided for a 2 mM final concentration.

Stabilizer Solution (5x): 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.

PCR controls: The reliability of the data may be affected by the presence of contaminating DNA. It is strongly recommended to include a no-template control reaction in the PCR design, replacing template DNA with nuclease-free PCR-grade water. Additionally, include a positive control to serve as a reference for ensuring the correct functioning of the PCR reaction. The positive control should exhibit the expected amplification profile, confirming the assay's ability to accurately amplify the target sequence.

Quality control assays

Purity

NZYProof 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.

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 reaction 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

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

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, depending on initial DNA concentration.
• **Template DNA damaged or degraded**
An intact, high-quality template is essential to achieve a reliable amplification, especially from large DNA fragments. Extreme care must be taken in the preparation and handling of DNA. Always use purified high-quality DNA as template.
• **Contamination with DNases**
Ensure that all labware, including pipettes, tubes, and containers, is clean and free from residual DNase contamination. Disinfect laboratory surfaces with the RNase & DNase Cleaner (NZYtech, Cat. No. MB463). Use autoclaved or sterile equipment whenever possible. Use DNase-free water. Change gloves frequently.
• **Concentration of Mg^{2+} is 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.
• **Additives required**
Adding the 5x Stabilizer Solution provided may improve yield while allowing the amplification of low-copy templates.
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 preparing small volume working aliquots from the stock solution. Avoid using primers subjected to multiple freezing-thawing cycles.