MB283_IFU_EN_V2601

Supreme NZYProof DNA polymerase

Catalogue number	Presentation
MB28301	125 U (50 µL)
MB28302	500 U (200 µL)
MB28303	1000 U (2 x 200 µL)

Description

Supreme NZYProof DNA polymerase is an engineered highly accurate, fast and sensitive variant of NZYProof DNA polymerase displaying a hot-start like PCR capacity. This feature is achieved by a novel hot-start technology, which inhibits both polymerase and 3'→5' exonuclease activities and thus avoids extension of non-specifically annealed primers or primer-dimers, as well as the degradation of primers and template DNA during PCR reaction setup. Thus, Supreme NZYProof DNA polymerase generates higher specificity, sensitivity and yield during the accurate amplification of DNA. Supreme NZYProof DNA polymerase was also engineered for higher processivity, thus allowing fast PCR reactions of longer PCR products.

This highly robust version of NZYProof DNA polymerase is a broad range enzyme suitable for a variety of applications, including amplification of longer (≤10 kb) and difficult PCR products, as well as site-directed mutagenesis. Supreme NZYProof DNA polymerase possesses 3'→5' exonuclease proofreading activity that enables the polymerase to amplify DNA with increased accuracy. The error rate of Supreme NZYProof DNA polymerase is similar to that of Pfu and KOD DNA polymerases and significantly lower than the error rate of Taq DNA polymerases. It generates blunt-ended PCR products that are suitable for cloning with NZYtech's NZY-blunt PCR cloning kit (Cat. No. MB121).

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

	MB28301 (125 U)		MB28302 (500 U)		MB28303 (1000 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
Supreme NZYProof DNA polymerase	1	50 µL	1	200 µL	2	200 µL
5x Reaction Buffer for Supreme NZYProof	1	2000 µL	3	2000 µL	6	2000 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 is provided to ensure successful PCR amplification using Supreme NZYProof DNA polymerase. Optimal reaction conditions (e.g. concentration of DNA Polymerase, primers, dNTPs and template DNA) may need to be optimized for long amplicons or difficult

templates. 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, 5x (*provided*)	10 µL
dNTPs mix	0.2 mM
Forward and Reverse Primers (see below)	0.5 µM
Template DNA (see below)	1 ng-0.5 µg
Supreme NZYProof DNA polymerase (2.5 U/µL) (*provided*)	0.5 µL
Nuclease-free water	up to 50 µL
FINAL VOLUME =	50 µL

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	96 °C	4 min	Initial denaturation
25-35	96 °C	30 sec	Denaturation
	(*)	30 sec	Annealing
	72 °C	30 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.
(**) Use 40s/kb for PCR products > 3 kb.

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 Supreme 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 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 increasing the synthesis of undesirable reaction products. 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 10ng to 500ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 1 ng of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-50 ng). 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 (0.5 µL) 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 1.5 mM final concentration.

Reaction Buffer (5x): Presence of a sugar alcohol in its composition decreases freezing temperatures. The buffer remains stable at 4 °C up to one month.

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

Supreme 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 Supreme 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.

Functional assay

Supreme 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. In addition, the enzyme is tested in a Site-directed Mutagenesis Assay in a standard protocol. The resulting PCR products are visualized as expected bands 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 5× Reaction Buffer is at a final concentration of 1.5 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 1.5 mM to 3.5 mM (final concentration) in 0.25 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 preparing small volume working aliquots from the stock solution. Avoid using primers subjected to multiple freezing-thawing cycles.