Supreme NZYProof DNA polymerase

Catalogue number: MB28301, 125 U
MB28302, 500 U
MB28303, 1000 U

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 (MB121).

Storage Conditions

Supreme NZYProof DNA polymerase should be stored at -20°C at all times in a constant temperature freezer. Keep it on freezer while perform PCR set up until use. Supreme NZYProof 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

Reaction buffer (5×)

The enzyme reaction buffer contains Mg^{2+} (1.5 mM at the final, 1×, reaction concentration). Presence of a sugar alcohol in its composition decreases freezing temperatures. The buffer remains stable at 4 °C up to one month.

Standard 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 all components after thawing. At room temperature, or 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:

Component	Volume	Final conc.
5× Reaction buffer	10 µL	1×
25 mM dNTPs	0.4 µL	0.2 mM
10 µM Forward primer	2.5 µL	0.5 µM
10 µM Reverse primer	2.5 µL	0.5 µM
Template DNA	X µL	(*)
Supreme NZYProof DNA Polymerase (2.5 U/µL)	0.5 µL	0.025 U/µL
Nuclease-free water	to 50 µL	-

(*) Typically, add DNA at a range of 1ng-0.5µg. See below general recommendations for DNA template.

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°C and following the below cycling parameters:

Cycle step	Temp.	Time	Cycles
Initial denaturation	96 °C	4 min	1
Denaturation	96 °C	30 s	25-35
Annealing	*	30 s	
Extension	72 °C	30 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.

¥ Use 40s/kb for PCR products >3 kb.

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 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 (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 10ng to 500ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 1ng of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-50ng). 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

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

Genomic DNA contamination

Supreme NZYProof DNA polymerase 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. 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.

V1902

Certificate of Analysis

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

Approved by:

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