MB331_IFU_EN_V2601

Supreme NZYLong DNA polymerase

Catalogue number	Presentation
MB33101	125 U (25 µL)
MB33102	500 U (100 µL)
MB33103	1000 U (2 x 100 µL)

Description

Supreme NZYLong DNA polymerase is an engineered version of NZYLong DNA polymerase designed to amplify longer PCR products, generally of 25 kb and beyond, which displays a higher specificity provided by its 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 degradation of primers and template DNA during PCR reaction setup. The functional activity of the enzyme is restored during a short 5-minute incubation step at 94 °C. The increased processivity of Supreme NZYLong DNA polymerase combined with the hot-start-like PCR capacity results in higher specificity, sensitivity and yield during the amplification of long nucleic acids. A wide range of long PCR products, typically of 25 kb and up to 40 kb, can be generated usimg lambda DNA or human genomic DNA as starting template. Supreme NZYLong DNA polymerase displays a higher fidelity than conventional Taq DNA polymerases. The provided 10x reaction buffer protects DNA from depurination and nicking during long thermal cycling. The enzyme generates a mixture of A-overhang-ended (predominantly) and blunt-ended PCR products, being suitable for cloning with NZYtech's TA PCR cloning kits (Cat. No. MB053 or MB137).

Shipping & Storage Conditions

The product can be shipped in a range of temperatures from dry ice to room temperature. Upon arrival, all the components should be stored at -85 °C to -15 °C in a constant-temperature freezer to guarantee maximal shelf life. The high thermal stability of the enzyme allows it to remain stable at 4°C or even at room-temperature for up to 1 week, so, if you forget your enzyme on your lab bench, no harm is done. The product will remain stable till the expiry date if stored as specified.

Components

	MB33101 (125 U)		MB33102 (500 U)		MB33103 (1000 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
Supreme NZYLong DNA polymerase	1	25 µL	1	100 µL	2	100 µL
10x Reaction Buffer for Supreme NZYLong	1	1000 µL	2	1000 µL	4	1000 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, in controlled assay conditions.

Enzyme concentration: 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. Avoid excessive pipetting and mixing of the DNA template when amplicons above 15 kb are desired.
- 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 NZYLong DNA polymerase. Optimal reaction conditions (e.g. concentration of DNA Polymerase, primers, dNTPs, magnesium and template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune PCR conditions, recommended variations of each PCR component are provided in brackets in the table below.

1. Gently mix and briefly centrifuge the master mix after thawing.
2. 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. A single reaction mixture of 50 µL should combine the following components:

	1 REACTION
Reaction Buffer, 10x (*provided*)	5 µL
dNTPs mix (*)	0.3 (0.2-0.5) mM
Forward and Reverse Primers (see below)	0.35 (0.25-0.5) µM
Supreme NZYLong DNA polymerase (5 U/µL) (*provided*)	1 µL
Template DNA (see below)	1 ng-0.5 µg
Nuclease-free water	up to 50 µL
FINAL VOLUME =	50 µL

(*) Use high-quality dNTPs and avoid repeated freeze cycles. We recommend preparing small-volume working aliquots from the stock solution.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Perform PCR using the following cycling parameters:

CYCLES	**TEMP.**	**Time**	**STAGE**
1	94 °C	5 min	Initial denaturation
25-35	94 °C	20 sec	Denaturation
	(**)	30 sec	Annealing
	68 °C	1 min/kb	Extension
1	68 °C	1.5 min/kb	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 (0.6-0.8%, w/v) and visualise with GreenSafe Premium (NZYtech, Cat. No. MB132) or any other means

Technical Notes

Primer design: Optimal primer design is critical for long-range amplifications. PCR primers should be designed to have 18–35 bases in length and a GC content of 45-60%. Pay special attention to avoid sequences that might produce internal secondary structures. The 3'-ends of the primers should not be complementary to avoid the production of primer-dimers, and it is recommended to have at least 2 Cs or Gs. Ideally, both primers should have nearly identical melting temperatures (Tm), allowing their annealing with the denatured template DNA at roughly the same temperature. For long PCRs avoid using primers that have been previously subjected to multiple freezing-thawing cycles. Note that primer annealing and DNA extension can be combined into one step if primers are designed to have a Tm ≥ 70 °C.

DNA template: The amplification of long PCR products requires high quality gDNA retaining long DNA fragments (it is not possible to amplify a 20 kb product from damaged gDNA with an average fragment size of 5 kb, for example). The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 10 ng to 500 ng 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.

Try to add the DNA as the latest component to the PCR reaction and avoid pipetting after this. To retain DNA integrity, avoid multiple freeze-thawing cycles stock DNA solutions and keep working DNA at small aliquots.

Mg^{2+}: This cofactor is already incorporated in the reaction buffer provided for a 2.5 mM final concentration.

Cycling conditions: It is recommended to use incubation and extension temperatures optimized for the enzyme and experimental conditions. An extension step performed at 68 °C favours the accumulation of long PCR products without compromising enzyme performance. In addition, a slightly lower initial denaturation temperature (94 °C instead of the conventional 95 °C) is recommended to minimize DNA damage for long 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

Supreme NZYLong 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 NZYLong DNA polymerase, in 1× Reaction Buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with the buffer.

Functional assay

Supreme NZYLong DNA polymerase is extensively tested for performance in a fast polymerase chain reaction (PCR) of different-sized DNA fragments (10 and 15 kb) from human genomic DNA in the presence of 10× Reaction Buffer. The resulting PCR products are visualized as single bands in a GreenSafe Premium-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
• **Incomplete activation of the hot-start-like system**
Ensure the initial denaturation/activation step is performed following instructions provided above: 5 minutes at 95 °C. Do not shorten this step unless validated.
• **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 of large 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 2.5 mM, which is sufficient for most targets. For some targets, higher Mg^{2+} concentration may be required. Titrate from 2.5 mM to 4 mM (final concentration) in 0.5 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.