

Supreme NZYTaQ II DNA polymerase

Catalogue number	Presentation
MB35501	500 U (100 µL)
MB35502	1000 U (2 x 100 µL)
MB35503	2500 U (5 x 100 µL)

Description

Supreme NZYTaQ II DNA polymerase is an engineered version of NZYTaQ II DNA polymerase displaying a hot-start-like PCR capacity. The enzyme activity at room temperature is limited, avoiding extension of non-specifically annealed primers or primer-dimers and thus providing higher specificity, sensitivity and yield during DNA amplification. The functional activity of the enzyme is restored during a short 5-minute incubation step at 95 °C. Once Supreme NZYTaQ II DNA polymerase is activated, it displays high performance leading to high DNA yields in shorter extension times (15-30 s/kb) of DNA templates up to 6 kb. Supreme NZYTaQ II DNA polymerase requires minimal optimization. In addition, the hot-start-like capacity of Supreme NZYTaQ II DNA polymerase not only leads to higher PCR sensitivity but also allows a room-temperature reaction setup. Supreme NZYTaQ II DNA polymerase lacks 3'→5' exonuclease activity. Thus, resulting PCR products have an A-overhang and are suitable for cloning with Nzytech's TA PCR cloning kits (Cat. No. MB053 or MB137).

Shipping & Storage Conditions

The product can be shipped from dry ice to blue ice. Upon arrival, all components should be stored at -85 °C to -15 °C in a constant-temperature freezer to guarantee maximal shelf life. The product will remain stable till the expiry date if stored as specified. Minimize the number of freeze-thaw cycles by storing in working aliquots the Supreme NZYTaQ II DNA polymerase.

Components

COMPONENT	MB35501 (500 U)		MB35502 (1000 U)		MB35503 (2500 U)	
	TUBES	VOLUME	TUBES	VOLUME	TUBES	VOLUME
Supreme NZYTaQ II DNA polymerase	1	100 µL	2	100 µL	5	100 µL
10x Reaction Buffer for NZYTaqs	2	1000 µL	4	1000 µL	10	1000 µL
50 mM MgCl ₂	2	950 µL	3	950 µL	7	950 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: 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, MgCl₂ 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. In a sterile, nuclease-free microcentrifuge tube, prepare a mixture for the appropriate number of PCR reactions. Set up the PCR reaction at room temperature. A single reaction mixture of 50 μL should combine the following components:

Note: template DNA should be the last component to be added to the reaction mixture.

	1 REACTION
Reaction Buffer, 10x (provided)	5 μL
50 mM MgCl_2 (provided)	2.5 (1.5-4.0) mM
dNTPs mix	0.4 (0.25-0.5) mM
Forward and Reverse Primers (see below)	0.25 (0.1-0.5) μM (*)
Supreme NZYtaq II DNA polymerase (5 U/ μL) (provided)	0.25-1 μL
Template DNA (see below)	1 μg -0.5 μg
Nuclease-free water	up to 50 μL
FINAL VOLUME =	50 μL

(*) Final concentrations less than 0.25 μM may be beneficial to improve sensitivity in some conditions.

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

CYCLES	TEMP.	Time	STAGE
1	95 °C	5 min	Initial denaturation
25-35	94 °C	30 sec (¥)	Denaturation
	(**)	30 sec	Annealing
	72 °C	15-30 sec/kb (¥)	Extension
1	72 °C	5-10 min	Final Extension

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

(¥) For DNA fragments higher than 3 kb to 6 kb in size, it may be beneficial to use 20 sec for denaturation and 30-60 sec/kb for extension.

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. 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 (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 5 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 1 μg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 0.5-20 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Mg^{2+} : The provided 50 mM MgCl_2 solution allows users to optimize Mg^{2+} concentration in different PCR set ups. In general, Supreme NZYtaq II DNA polymerase works effectively with a 2.5 mM MgCl_2 concentration.

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 NZYtaq II DNA polymerase purity is > 90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of Supreme NZYtaq II 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 reaction buffer and MgCl₂ solution.

Functional assay

Supreme NZYtaq II DNA polymerase is extensively tested for performance in a polymerase chain reaction (PCR) of different-sized DNA fragments (1 and 5 kb) from human genomic DNA in the presence of 10× Reaction Buffer and MgCl₂ solution. 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
<ul style="list-style-type: none">• 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.
<ul style="list-style-type: none">• 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).
<ul style="list-style-type: none">• 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.
<ul style="list-style-type: none">• 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.
<ul style="list-style-type: none">• 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.
<ul style="list-style-type: none">• Concentration of Mg²⁺ is too low
Adding MgCl ₂ at a final concentration of 2.5 mM 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.
<ul style="list-style-type: none">• Additives required
Adding PCR-enhancing agents (NZYtaq 5× Optimizer Solution – NZYtech, Cat. No. MB060 or NZYtaq 2× GC-Enhancer Solution – NZYtech, Cat. No. MB143) may improve yield while allowing the amplification of difficult templates.
PRESENCE OF NON-SPECIFIC BANDS
<ul style="list-style-type: none">• Non-specific annealing of primers
Adjust annealing conditions and/or design another set of primers, by increasing the length and avoiding complementary sequences.
<ul style="list-style-type: none">• 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.
<ul style="list-style-type: none">• Concentration of Mg²⁺ is too high
Generally, 2-3 mM MgCl ₂ , typically 2.5 mM final concentration, works well for most PCR reactions. Optimal concentration depends on target template, buffer and dNTPs. Optimize magnesium concentration by supplementing MgCl ₂ in 0.5 increments up to 4 mM.

For life science research only. Not for use in diagnostic procedures.