

Speedy Supreme NZYTaQ DNA polymerase

Catalogue number	Presentation
MB39001	500 U (100 µL)
MB39002	1000 U (2 x 100 µL)
MB39003	2500 U (5 x 100 µL)

Description

Speedy Supreme NZYTaQ DNA polymerase is a recombinant modified form of Taq DNA polymerase that combines hot-start like PCR capacity and a fast polymerization reaction. The enzyme is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer-dimers and thus providing higher specificity and sensitivity of DNA amplification. The functional activity of the enzyme is restored during a short 5- minute incubation step at 95 °C. Only 5 seconds are required to successfully amplify a DNA fragment of 1 kb. The enzyme retains its speed when amplifying fragments up to around 2 kb. Successful amplification of higher DNA fragments up to 6 kb in size can be reached using a 10 sec/kb extension step. Faster PCR can be further achieved by increasing the primers melting temperature, which increases PCR annealing temperature, thus allowing combining the annealing and extension PCR steps during PCR cycling (see below).

Speedy Supreme NZYTaQ DNA polymerase is supplied with an optimized 10× Reaction Buffer and a 50 mM MgCl₂ solution. Minimal optimizations are required for successful DNA amplification. In addition, the hot-start-like capacity of Speedy Supreme NZYTaQ DNA polymerase not only leads to higher PCR sensitivity but also allows a room-temperature reaction setup. The enzyme lacks 3'→5' exonuclease activity and the PCR products generated have an A-overhang, being 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 Speedy Supreme NZYTaQ DNA polymerase.

Components

COMPONENT	MB39001 (500 U)		MB39002 (1000 U)		MB39003 (2500 U)	
	TUBES	VOLUME	TUBES	VOLUME	TUBES	VOLUME
Speedy Supreme NZYTaQ DNA polymerase	1	100 µL	2	100 µL	5	100 µL
10x Reaction Buffer for NZYTaqs	2	1000 µL	2	1000 µL	5	1000 µL
50 mM MgCl ₂	2	950 µL	3	950 µL	3	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 μ L 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 μ L
50 mM MgCl ₂ (provided)	2.5 (1.5-4.0) mM
dNTPs mix	0.4 (0.25-0.5) mM ^(a)
Forward and Reverse Primers (see below)	0.25 (0.1-0.5) μ M
Speedy Supreme NZYtaq DNA polymerase (5 U/ μ L) (provided)	0.5-1 μ L ^(b)
Template DNA (see below)	1 pg-0.5 μ g
Nuclease-free water	up to 50 μ L
FINAL VOLUME =	50 μ L

(a) Typically, 0.2 mM gives good results in a vast range of DNA sizes. Higher amounts of dNTPs could compromise sensitivity and impair amplification of long DNA fragments (>2kb).

(b) The sensitivity of enzyme could be compromised when using 0.5 μ L, especially for long DNAs (>2 kb). Thus, for long PCR protocols, it is recommended to use 1 μ L of enzyme.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Perform PCR using the cycling parameters below. For primers with a melting temperature (T_m) \geq 65 $^{\circ}$ C, the annealing and extension steps may be combined into a 2-step protocol. For primers with lower T_m , the 3-step protocol should be used.

CYCLES	2-step protocol		3-step protocol		STAGE
	TEMP.	TIME	TEMP.	TIME	
1	95 $^{\circ}$ C	5 min	95 $^{\circ}$ C	5 min	Initial Denaturation
25-35	94 $^{\circ}$ C	2 sec	94 $^{\circ}$ C	2 sec	Denaturation
	-	-	(*)	5 sec	Annealing
	68-72 $^{\circ}$ C (‡)	5 sec/kb (†)	72 $^{\circ}$ C	5 sec/kb	Extension
1	72 $^{\circ}$ C	2 min	72 $^{\circ}$ C	2 min	Final Extension

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

(‡) Extension temperature will depend on primer melting temperature (see below).

(†) For DNA fragments higher than 2-3 kb to 6 kb in size, it may be beneficial to use 10 s/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. 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 delete 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. For faster PCR protocols, primers should have a $T_m \geq 60$ $^{\circ}$ C. Please note that primer annealing and product extension can be combined into one step for a faster PCR reaction (see 2-step protocol above) if primers are designed to have a $T_m \geq 65$ $^{\circ}$ C.

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 pg 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.

Mg²⁺: The provided 50 mM MgCl₂ solution allows users to optimize Mg²⁺ concentration in different PCR set ups. In general, Speedy Supreme NZYtaq DNA polymerase works effectively with a 2.5 mM MgCl₂ 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

Speedy Supreme NZYtaq 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 Speedy Supreme NZYtaq DNA polymerase, in 1× reaction buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualized 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

Speedy Supreme NZYtaq DNA polymerase is extensively tested for performance in a fast polymerase chain reaction (PCR) of different-sized DNA fragments (1 and 2.5 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.

- **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.

- **Concentration of Mg²⁺ is too high**

Generally, 2-3 mM MgCl₂, 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 MgCl₂ in 0.5 increments up to 4 mM.

FOR REFERENCE USE ONLY

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