MB436_IFU_EN_V2601

Speedy Supreme NZYProof DNA polymerase

Catalogue number	Presentation
MB43601	125 U (50 µL)
MB43602	500 U (200 µL)

Description

Speedy Supreme NZYProof DNA polymerase belongs to a new generation of DNA polymerases displaying a faster polymerization rate than any other conventional non-proofreading enzyme. Less than 5 seconds are required for the successful synthesis of a 1 kb size DNA fragment. In addition to a high-speed performance, the enzyme retains high sensitivity for low-copy templates. Speedy Supreme NZYProof DNA polymerase is supplied with an optimized 5× Reaction Buffer, which already contains Mg^{2+}. The enzyme supports robust and reliable reactions while tolerating a wide range of templates. It is useful for fast, routine and sensitive DNA amplifications that require fidelity, as well as for genotyping. PCR products generated by Speedy Supreme NZYProof DNA polymerase have blunt-ends and are suitable for cloning with NZYtech´s NZY-blunt PCR cloning kit (NZYtech, 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

	MB43601 (125 U)		MB43602 (500 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
Speedy Supreme NZYProof DNA polymerase	1	50 µL	1	200 µL
5x Reaction Buffer for Speedy Supreme NZYProof	1	1000 µL	2	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.

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 serves as a general guideline and a starting point for PCR amplification and contemplates amplification at 5 or 2 s/kb. Optimal reaction conditions (e.g., concentration of DNA polymerase, primers, 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. 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:
***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	0.4 (0.25-0.5) mM
Forward and Reverse Primers (see below)	0.3 (0.1-0.5) µM
Template DNA (see below)	50 pg-0.5 µg
Speedy Supreme NZYProof DNA polymerase (2.5 U/µL) (*provided*)	0.5-1 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 perform PCR using the cycling parameters below. For primers with a melting temperature (Tm) ≥ 65 °C, the annealing and extension steps may be combined into a 2-step protocol. For primers with lower Tm, the 3-step protocol should be used:
***Note**: Speedy Protocol (extension at 5 sec/kb) is the recommended protocol for the majority of templates; a faster protocol (Speedy Supreme Protocol with extension at 2 sec/kb) is also provided for ultra-rapid PCRs; although it does not compromise sensitivity, this protocol can however result in lower yields when compared with the Speedy Protocol depending on the template used.*

Speedy Protocol (5 sec/ kb)

CYCLES	**2-step protocol**		**3-step protocol**		**STAGE**
	TEMP.	**TIME**	**TEMP.**	**TIME**	
1	95 °C	1 min	95 °C	1 min	Initial Denaturation
25-35	95 °C	2 sec	94 °C	2 sec	Denaturation
	-	-	**(*)**	2 sec	Annealing
	72 °C	5 sec/kb	72 °C	5 sec/kb	Extension
1	72 °C	2 min	72 °C	2 min	Final Extension

Speedy Supreme Protocol (2 sec/ kb)

CYCLES	**2-step protocol**		**3-step protocol**		**STAGE**
	TEMP.	**TIME**	**TEMP.**	**TIME**	
1	95 °C	1 min	95 °C	30 sec	Initial Denaturation
25-35	95 °C	1 sec	94 °C	1 sec	Denaturation
	-	-	**(*)**	1 sec	Annealing
	72 °C	2 sec/kb	72 °C	2 sec/kb	Extension
1	72 °C	30 sec	72 °C	30 sec	Final Extension

***(*)** Annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm -5 °C.*
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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 20–40 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 (Tm), allowing their annealing with the denatured template DNA at roughly the same temperature. For faster PCR protocols, primers should have a Tm ≥ 60 °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 Tm ≥ 70 °C.

DNA template: The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 20ng to 500ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 50pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 5-20ng). 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 2.5 mM final 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 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 Speedy Supreme NZYProof 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.

Functional assay

Speedy Supreme NZYProof DNA polymerase is tested for performance in a polymerase chain reaction (PCR) using 5 U of enzyme for the amplification of a 1 kb fragment from human genomic DNA. The resulting PCR product is visualized as a single band 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
• **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 2.5 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 2.5 mM to 4 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.