MB404_IFU_EN_V2601

Speedy NZYProof DNA polymerase

Catalogue number	Presentation
MB40401	125 U (50 µL)
MB40402	500 U (200 µL)

Description

Speedy NZYProof DNA polymerase belongs to a new generation of DNA polymerases displaying a fast polymerization rate. Only 5 seconds are required for the successful synthesis of a 1 kb size DNA fragment. 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 (see below). Speedy NZYProof DNA polymerase is supplied with an optimized 5× Reaction Buffer and a 50 mM $MgCl_2$ solution. The enzyme supports robust and reliable reactions while tolerating a wide range of templates. It is useful for fast and routine DNA amplifications thar require fidelity, as well as for genotyping. PCR products generated by Speedy 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

	MB40401 (125 U)		MB40402 (500 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
Speedy NZYProof DNA polymerase	1	50 µL	1	200 µL
5x Reaction Buffer for Speedy NZYProof	1	1000 µL	2	1000 µL
50 mM $MgCl_2$	1	1000 µL	1	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. Optimal reaction conditions (e.g., concentration of DNA polymerase, primers, $MgCl_2$, 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
50 mM $MgCl_2$ (*provided)*	2.5 µL
dNTPs mix	0.3 (0.25-0.5) µM
Forward and Reverse Primers (see below)	0.3 (0.1-0.5) µM
Template DNA (see below)	50 ng-0.5 µg
Speedy 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.

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	94 °C	2 sec	94 °C	2 sec	Denaturation
	-	-	**(*)**	5 sec	Annealing
	68-72 °C (¥)	5 sec/kb **(ŧ)**	72 °C	5 sec/kb	Extension
1	72 °C	2 min	72 °C	2 min	Final Extension

***(*)** Annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm -5 °C.*
***(¥)** Extension temperature will depend on primer melting temperature (see below).*
***(ŧ)** 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 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 20 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 50 pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 5-20 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

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 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 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 and $MgCl_2$ solution.

Functional assay

Speedy NZYProof DNA polymerase is tested for performance in a polymerase chain reaction (PCR) using 5 U of enzyme for the amplification of a 1 kp 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**
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
• **Additives required**
Adding the 5x Stabilizer Solution provided may improve yield while allowing the amplification of low-copy templates.
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.
• **Concentration of Mg^{2+} 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