MB364_IFU_EN_V2601

NZYTaq II with 5× Gel Load Reaction Buffer

Catalogue number	Presentation
MB36401	500 U (100 µL)
MB36402	1000 U (2 x 100 µL)
MB36403	2500 U (5 x 100 µL)

Description

NZYTaq II DNA polymerase belongs to a new generation of Taq-derived DNA polymerases that was engineered to produce high DNA yields in shorter PCR running times (15-30 s/kb extension) under minimal optimization conditions. NZYTaq II DNA polymerase supports the robust and reliable amplification of a wide range of DNA templates up to 6 kb. NZYTaq II is provided with 5× Gel Load Reaction Buffer allowing reactions to be loaded directly into gels without the extra adding of loading dye. This buffer contains two tracking dyes: a blue dye that co-migrates with ~3–5 kb DNA fragments and a yellow dye that migrates faster than primers (<50 bp) in a 1% (w/v) agarose gel. NZYTaq II DNA polymerase lacks 3'→5' exonuclease activity. Resulting PCR products have an A overhang and are suitable for cloning with NZYtech's TA PCR cloning kits (NYtech, Cat. No. MB053 or MB137).

Shipping & Storage Conditions

The product can be shipped in a range of temperatures from dry ice to room temperature. This product should be stored at -85 °C to -15 °C in a freezer without defrost cycles 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 4 weeks, so, if you forget your enzyme on your lab bench, no harm is done. Repeated freeze-thaw cycles will affect the stability of the Gel Load Reaction Buffer (the buffer will remain stable at 4 °C for at least one month). The product will remain stable till the expiry date if stored as specified.

Components

	MB36401 (500 U)		MB36402 (1000 U)		MB36403 (2500 U)	
COMPONENT	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**	**TUBES**	**VOLUME**
NZYTaq II DNA polymerase	1	100 µL	2	100 µL	5	100 µL
5x Gel Load Reaction Buffer for NZYTaq II	2	2000 µL	3	2000 µL	7	2000 µL
50 mM $MgCl_2$	2	1000 µL	3	1000 µL	7	1000 µL

Note: Consider preparing multiple aliquots of the 5x Gel Load Reaction Buffer to reduce freeze/thaw cycles and minimize the risk of contamination.

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.
- 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, $MgCl_2$ 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:

	1 REACTION
Gel Load Reaction Buffer, 5x (*provided*)	10 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
Template DNA (see below)	5 pg-0.5 µg
NZYTaq II DNA polymerase (5 U/µL) (*provided*)	0.25-1 µL
Nuclease-free water	up to 50 µL
FINAL VOLUME =	50 µL

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

CYCLES	**TEMP.**	**Time**	**STAGE**
1	95 °C	3 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 Tm; typically, it should be Tm- 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 (Tm), 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 10 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 5pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-20 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, NZYTaq II DNA polymerase works effectively with a 2.5 mM $MgCl_2$ concentration. Vortex the $MgCl_2$ solution thoroughly after thawing.

Gel Load Reaction Buffer, 5x: Proprietary formulation supplied at pH 8.8. Vortex the reaction buffer thoroughly after thawing and prior to use. It is composed by a blue and yellow dye. The blue dye migrates at the same rate as a 3-5 kb DNA fragment in a 1% (w/v) agarose gel. The yellow dye migrates at a rate faster than primers (<50 bp) in a 1% (v/v) agarose gel. The 5× Gel Load Reaction Buffer is not suitable when direct fluorescent or absorbance readings are required without prior purification of the amplified DNA from PCR.

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

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 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 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 $MgCl_2$ solution.

Functional assay

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 $MgCl_2$ 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
• **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 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
• **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.