MB358_IFU_EN_V2501

NZYTaq II 2× Green Master Mix

Catalogue number	Presentation
MB35801	2 x 1.25 mL (100 rxns of 50 µL)
MB35802	4 x 1.25 mL (200 rxns of 50 µL)
MB35803	20 x 1.25 mL (1000 rxns of 50 µL)
MB35805	1 x 50 mL (2000 rxns of 50 µL)

Description

NZYTaq II 2× Green Master Mix is a premixed ready-to-use solution containing NZYTaq II DNA polymerase (MB354), which belongs to a new generation of Taq-derived DNA polymerases optimized for standard PCR applications. The master mix contains dNTPs, reaction buffer and additives at optimal concentrations and supports the robust and reliable amplification of a wide range of DNA templates up to 6 kb. MgCl2 final concentration is 2.5 mM, allowing the implementation of a comprehensive range of PCR protocols. In addition, reactions assembled with NZYTaq II 2× Green Master Mix may be directly loaded onto agarose gels. The mix contains two dyes (blue and yellow) that allow monitoring the progress of the electrophoresis. NZYTaq II 2× Green Master Mix is not suitable when direct fluorescent or absorbance readings are required without prior purification of the amplified DNA from PCR. We recommend using the master mix version without dyes – NZYTaq II 2× Colourless Master Mix (MB357) – or purifying the PCR product using NZYGelpure (MB011) before performing such downstream protocols.

NZYTaq II DNA polymerase lacks 3'→5' exonuclease activity. Resulting PCR products have an A-overhang and are suitable for cloning with NZYtech's NZY-A PCR cloning kit (MB053) or NZY-A Speedy PCR cloning kit (MB137).

Shipping & Storage Conditions

The product can be shipped from dry ice to ambient temperature. Upon arrival, all components should be stored at -85 °C to -15 °C in a constant-temperature freezer to guarantee maximal shelf life. The high thermal stability of the enzyme mixture allows it to remain stable at 4 °C or even at room-temperature for up to 4 weeks. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	SKU	TUBES/BOTTLES	VOLUME
NZYTaq II 2× Green Master Mix	**MB35801**	2	1.25 mL
	MB35802	4	1.25 mL
	MB35803	20	1.25 mL
	MB35805	1	50 mL

Note: Consider preparing multiple aliquots of the master mix to reduce freeze/thaw cycles and minimize the risk of contamination.

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.

Procedure

The following protocol serves as a general guideline and a starting point for any PCR procedure. Optimal reaction conditions (*e.g*. incubation times, temperatures and template concentration) may vary and, in particular conditions, may require further optimization. In case you need to fine-tune primer concentrations, test recommended variations provided in brackets in the table below.

1. Thaw the master mix at room temperature or on ice. Mix the master mix thoroughly by flicking the tube and inverting it. Keep the master mix on ice. Set up the PCR reaction on ice and 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 VOLUME
NZYTaq II 2× Green Master Mix	25 µL
Primers	0.25 (0.1-0.5) µM
Template DNA	5 pg-0.5 µg
Nuclease-free water	up to 50 µL
FINAL VOLUME =	50 µL

2. Gently mix and centrifuge briefly to spin down the contents.

3. 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.

4. Analyse the PCR products through agarose gel electrophoresis (0.7-1.2%, w/v) and visualise with GreenSafe Premium (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 (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 5 pg 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.

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 T_m).
• **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.
• **Concentration of Mg^{2+} is too low**
Mg^{2+} is included in the Master Mix at a final concentration of 2.5 mM, which 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. (Note: $MgCl_2$ is not provided in separate tubes).
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.

Quality control assays

Genomic DNA contamination

The product must be free of any detectable DNA contamination as evaluated through PCR.

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the master mix for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the master mix for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

NZYTaq II 2× Green Master Mix is tested for performance in a polymerase chain reaction (PCR) for the amplification of different-sized DNA fragments (1 and 5 kb) from human genomic DNA. The resulting PCR products are visualised as single bands in a GreenSafe Premium-stained agarose gel.

This master mix is manufactured under stringent quality standards and complies with ISO 9001 and ISO 13485 certifications for research and diagnostic-grade reagents.