MB287_IFU_EN_V2601

NZYProof 2x Green Master Mix

Catalogue number	Presentation
MB28701	2 x 1.25 mL (100 rxns of 50 µL)
MB28702	4 x 1.25 mL (200 rxns of 50 µL)
MB28703	20 x 1.25 mL (1000 rxns of 50 µL)

Description

NZYProof 2× Green Master Mix is a premixed ready-to-use solution containing NZYProof DNA polymerase, an enzyme that presents high fidelity and displays great performance in most PCR applications. NZYProof DNA polymerase possesses 3´→5´ exonuclease proofreading capacity which enables the polymerase to amplify DNA with increased accuracy. The enzyme is highly efficient in the amplification of longer (≤10 kb) PCR products and site-directed mutagenesis. In addition, it is the recommended polymerase for routine cloning that requires precision. The master mix was optimized to allow high yields and enhanced sensitivity due the presence of stabilizer solution. It contains dNTPs, reaction buffer and additives at optimal concentrations for the efficient amplification of DNA templates by PCR. Mg^{2+} final concentration is 1.5 mM, allowing the implementation of a variety of PCR protocols. In addition, reactions assembled with NZYProof 2× Green Master Mix may be directly loaded onto agarose gels. NZYProof 2× Green Master Mix contains two dyes (blue and yellow) that allow monitoring the progress of the electrophoresis. NZYProof 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 the purification of the PCR products using NZYGelpure (NZYtech, Cat. No. MB011) before employing nucleic acids in downstream protocols. NZYProof DNA polymerase possesses 3'→5' exonuclease proofreading activity. Resulting PCR products have blunt-ends and are suitable for cloning with NZYtech's NZY-blunt PCR cloning kit NZYtech (NZYtech, Cat. No. MB121).

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. Minimize the number of freeze-thaw cycles by storing in working aliquots. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	SKU	TUBES/BOTTLES	VOLUME
NZYProof 2x Green Master Mix	**MB28701**	2	1.25 mL
	MB28702	4	1.25 mL
	MB28703	20	1.25 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: Stringent precautionary measures must be imposed to mitigate the risk of carry-over contamination of DNA. We recommend using DNase-free plasticware/reagents and working in a DNase-free area (Nucleases & Nucleic Acid Cleaner, NZYtech, Cat. No. MB48301, or DNA & RNA Cleaner, Cat. No. MB46201, can help remove DNases from surfaces and materials).
- Handling instructions: To help prevent any carry-over DNA contamination, you should assign independent areas for reaction set-up, PCR amplification and any post-PCR gel analysis. Any tubes containing amplified PCR product mustn't be opened in the PCR set-up area. Use sterile filtered tips. Minimize exposure by keeping reaction and components capped whenever possible.
- 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 (incubation times and temperatures, concentration of primers and template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune primer concentrations, test the recommended variations provided in brackets in the table below.

1. Gently mix and briefly centrifuge the master mix after thawing.
2. 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
Forward and Reverse Primers	0.4 (0.3-0.5) µM each
DNA Template	5 ng-0.5 µg
NZYProof 2× Green Master Mix	25 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 initiate the PCR by transferring the PCR mixtures to the thermocycler with the block pre-heated to 95 °C and following the below cycling parameters:

CYCLES	TEMP.	TIME	STAGE
1	95 °C	3 min	Initial Denaturation
20-40	95 °C	30 sec	Denaturation
	*	30 sec	Annealing
	72 °C	60 sec/kb	Extension
1	72 °C	5-10 min	Final Extension

(*) The annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm - 5 °C.

5. Analyse the PCR products by agarose gel electrophoresis (0.7-1.2%, w/v) and visualise with GreenSafe Premium (NZYtech, Cat. No. MB132) or any other mean.

Technical Notes

Primers: PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Sequences longer than 30bp may improve PCR yield using NZYProof DNA polymerase since its 3´→5´ exonuclease activity may degrade primers. In addition, to overcome primer degradation, the 3´ termini of primers may be protected with phosphorothioate modifications. Primers should contain 40–60% GC and 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 increasing the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (Tm); in this manner, the two primers will anneal at roughly the same temperature.

Template: The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 50 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 5 ng of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 10-50 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.

Cycling conditions: It is highly recommended to use incubation and extension temperatures as high as required by the experiment. An extension performed at 68 °C favours the accumulation of long PCR products without compromising enzyme performance.

Intercalating Dyes: The master mix contains both a blue and a 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 faster than primers (<50 bp) in a 1% (w/v) agarose gel. This mix is not suitable for direct fluorescence or absorbance measurements without prior purification of the amplified DNA from the PCR.

Quality control assays

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 NZYProof 2× Green Master Mix 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.

Functional assay

NZYProof 2× Green Master Mix is tested for performance in a PCR reaction using 1.25 units of enzyme for the amplification of different-sized DNA fragments (1 and 2.5 kb) from human genomic DNA. The resulting PCR products are visualized as a single band in a GreenSafe 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.
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.

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