NZYProof 2× Green Master Mix

Catalogue number: MB28701, 500 U
MB28702, 1000 U
MB28703, 5000 U

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 the majority of 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 (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 (MB121).

Storage temperature

NZYProof 2× Green Master mix should be stored at -20 °C, in a constant temperature freezer. Minimize the number of freeze-thaw cycles by storing in working aliquots. The Mix maybe stored at 4 °C for up to 7 days.

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, under the following assay conditions: 50 mM Tris-HCl, pH 9.0, 50 mM NaCl, 2.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabelled and α-[^{32}P]-labelled), 10 µg activated salmon sperm DNA in a final volume of 50 µL.

Enzyme concentration: 0.2 U/µL

Standard 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. 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:

Forward and Reverse Primers	0.4 (0.3-0.5) µM
Template DNA	10 ng-0.5 µg
NZYProof 2× Green Master Mix	25 µL
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reactions.

3. 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:

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	30 s	20-40
Annealing	*	30 s	
Extension	72 °C	60 s/kb	
Final Extension	72 °C	5-10 min	1

*Annealing temperature should be optimized for each primer set based on the primer Tm; typically it should be Tm-5 °C.

4. Analyse PCR products by agarose gel electrophoresis (0.6-0.8%, w/v) and visualize with GreenSafe Premium (MB132) or any other mean.

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 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 (T_m); in this manner, the two primers will anneal at roughly the same temperature.

DNA template

The optimal amount of starting material may vary depending on the quality and complexity of template DNA. In general, we recommend using 50-500 ng of high quality genomic DNA templates. Lower amounts of DNA template (typically 10-50 ng) can be used for the amplification of lambda or plasmid DNA or even 20-100 ng for the amplification of multicopy chromosomal genes. When using a cDNA synthesis reaction as template, do not exceed 10% of the final PCR reaction volume.

Quality control assays

Purity
NZYProof DNA polymerase purity is >90% as judged by SDS-PAGE followed by Coomassie Blue staining.

Genomic DNA contamination
NZYProof 2× Green Master Mix 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

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.

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Certificate of Analysis

Test	Result
Enzyme purity*	Pass
Genomic DNA contamination*	Pass
DNase contamination	Pass
Functional assay	Pass

*These assays were exclusively performed with the enzyme

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