Supreme NZYProof 2× Colourless Master Mix

Catalogue number: MB28601, 500 U
MB28602, 1000 U
MB28603, 5000 U

Description

Supreme NZYProof 2× Colourless Master Mix is a premixed ready-to-use solution containing Supreme NZYProof DNA polymerase (MB283), an engineered highly accurate, fast and sensitive variant of NZYProof DNA polymerase displaying a hot-start like PCR capacity. This feature is achieved by a novel hot-start technology, which inhibits both polymerase and 3'→5' exonuclease activities and thus avoids extension of non-specifically annealed primers or primer-dimers, as well as the degradation of primers and template DNA during PCR reaction setup. Thus, Supreme NZYProof DNA polymerase generates higher specificity, sensitivity and yield during the accurate amplification of DNA. The master mix 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. We recommend the purification of the PCR products using NZYGelpure (MB011) before employing nucleic acids in downstream protocols. Supreme 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).

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage Conditions

This master mix should be stored at -85 °C to -15 °C in a freezer without defrost cycles 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.

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 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 need to be optimized for long amplicons or difficult templates. It is strongly recommended to assemble all reaction components on ice and quickly transfer the reactions to a thermocycler preheated to the denaturing temperature to start the PCR.

1. Gently mix and briefly centrifuge the master mix after thawing. Set up the PCR reaction at room temperature or on ice. 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	0.5 µM
Template DNA	1 ng-0.5 µg
Supreme NZYProof 2× Colourless 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	96 °C	4 min	1
Denaturation	96 °C	30 s	25-35
Annealing	*	30 s	
Extension	72 °C	30 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.

¥ Use 40s/kb for PCR products >3 kb.

4. Analyse PCR products by agarose gel electrophoresis (0.7-1.2%, 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. Sequences longer than 30bp may improve PCR yield using Supreme 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 (T_m), 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 1 ng of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-50 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Quality control assays

Purity

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

Genomic DNA contamination

The product must be free of any detectable DNA contamination as evaluated through PCR. Thus, it is suitable for the amplification of bacterial and fungal DNA based on 16S and 18S rRNA PCR assays.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with Supreme NZYProof 2× Colourless 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

Supreme NZYProof 2× Colourless 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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For research use only.