NZYTaq II 2× Green Master Mix

Catalogue number: MB35801, 500 U
MB35802, 1000 U
MB35803, 5000 U

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. $MgCl_2$ 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 Conditions

The product can be shipped in a range of temperatures from dry ice to room-temperature.

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. 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.

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: 0.2 U/µL, in glycerol

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/or template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune primer concentrations, test 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:

Primers	0.25 (0.1-0.5) µM
Template DNA	5 pg-0.5 µg
NZYTaq II 2× Green Master Mix	25 µL
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters:

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	94 °C	30 sec **(¥)**	25-35
Annealing	**(*)**	30 sec	
Extension	72 °C	15-30 sec/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.

(¥) 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.

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 (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 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.

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. 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 NZYTaq II 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

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 2.5 kb) from human genomic DNA. The resulting PCR products are visualised as single bands in a GreenSafe Premium-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.
• 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.

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