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 DNA polymerases derived from *Taq* DNA polymerase with high processivity and robustness. The master mix contains dNTPs and reaction buffer at optimal concentrations for efficient 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. There are two dyes (blue and yellow) present on the mix that allow monitoring the progress of 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 any other protocol.

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).

Storage temperature

NZYTaq II 2× Green Master Mix should be stored at -20 °C, in a constant temperature freezer. Minimize the number of freeze-thaw cycles by storing pre-prepared working aliquots of the master mix. The Mix may be 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.

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/or template DNA) vary and may need to be optimised.

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture for the appropriate number of samples to be amplified. A single reaction mixture should combine the following components (for a 50 µL reaction):

NZYTaq II 2× Green Master Mix	25 µL
Primers	0.2-0.5 µM
Template DNA	5 pg-0.5 µg
Nuclease-free water	up to 50 µL

2. If using a thermal cycler without a heated lid, overlay the reaction mix with 1-2 drops of mineral oil to prevent evaporation during the thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.

3. Perform PCR using the following parameters:

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	94 °C	30-50 s	25-35
Annealing	*	30-60 s	
Extension	72 °C	15-30 s/kb	
Final Extension	72 °C	5-10 min	1

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

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 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 DNAat 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 500-50 ng of genomic DNA template, although the enzyme may function with as little as 5 pg of gDNA. Lower amounts of DNA template (typically 50-1 ng) can be used for amplification of lambda or plasmid DNA or even 100-10 ng for amplification of multicopy chromosomal genes. For a cDNA synthesis reaction mixture, 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

NZYTaq II 2× Green Master Mix must be free of any detectable genomic DNA contamination as evaluated through PCR.

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.

Revised 01/17

Certificate of Analysis

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

*These assays were performed exclusively with the enzyme

Approved by: José Prates

José Prates
Senior Manager, Quality Systems

nzytech genes & enzymes
Estrada do Paço do Lumiar, Campus do Lumiar, Edifício E, R/C, 1649-038 Lisboa
Tel: +351.213643514 Fax: +351.217151168 www.nzytech.com