MB359_IFU_EN_V2601

# Supreme NZYTaq II 2x Colourless Master Mix

| Catalogue number | Presentation |
|---|---|
| MB35901 | 2 x 1.25 mL (100 rxns of 50 µL) |
| MB35902 | 4 x 1.25 mL (200 rxns of 50 µL) |
| MB35903 | 20 x 1.25 mL (1000 rxns of 50 µL) |

## Description

Supreme NZYTaq II 2× Colourless Master Mix is a premixed ready-to-use solution containing Supreme NZYTaq II DNA polymerase (NZYtech, Cat. No. MB355), a robust DNA polymerase derived from Taq DNA polymerase that was engineered to exhibit high processivity and to provide high PCR sensitivity. The enzyme, displaying, a hot-start-like PCR capacity, is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer-dimers and providing a highly specific DNA amplification. The functional activity of the enzyme is restored during a short 5-minute incubation step at 95 °C. In addition, the master mix contains dNTPs, reaction buffer and additives at optimal concentrations for the efficient amplification of a wide range of DNA templates of up to 6 kb. $MgCl_2$ final concentration is 2.5 mM, allowing the implementation of a variety of PCR protocols. For highly sensitive downstream applications it is recommended to purify the amplified PCR product using NZYGelpure (NZYtech, Cat. No. MB011) before usage in subsequent protocols. Supreme 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 (NZYtech, Cat. No. MB053) or NZY-A Speedy PCR cloning kit (NZYtech, Cat. No. MB137).

## 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 |
|---|---|---|---|
| **Supreme NZYTaq II 2x Colourless Master Mix** | **MB35901** | 2 | 1.25 mL |
| | **MB35902** | 4 | 1.25 mL |
| | **MB35903** | 20 | 1.25 mL |

## 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, 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/or 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. Thaw the master mix at room temperature or on ice. Mix the master mix thoroughly by flicking the tube and inverting it.
2. Set up the PCR reaction at room temperature. A single reaction mixture of 50 µL should combine the following components: (**Note**: template DNA should be the last component to be added to the reaction mixture).

| | **1 REACTION** |
|---|---|
| Forward and Reverse Primers | 0.25 (0.1-0.5) µM each (*) |
| Supreme NZYTaq II 2x Colourless Master Mix | 25 µL |
| DNA Template | 1 pg-0.5 µg |
| Nuclease-free water | up to 50 µL |
| FINAL VOLUME = | 50 µL |

*(*) Final concentrations less than 0.25 µM may be beneficial to improve sensitivity in some conditions.*

3. Gently mix and centrifuge briefly to spin down the contents.
4. Perform PCR using the following cycling parameters:

| CYCLES | TEMP. | TIME | STAGE |
|---|---|---|---|
| 1 | 95 °C | 5 min | Initial Denaturation |
| 25-35 | 94 °C | 30 sec (¥) | Denaturation |
| | ** | 30 sec | Annealing |
| | 72 °C | 15-30 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.*
***(¥)** 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.*

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

## Technical Notes

**Primers:** 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 (Tm), allowing their annealing with the denatured template DNA 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 5 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 1 pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 0.5-20 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.

## Quality control assays

**Genomic DNA contamination**

The product must comply to internal standards of DNA contamination as evaluated through real-time qPCR.

**Nuclease assays**

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the master mix for 14-16 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

**Functional assay**

Supreme NZYTaq II 2× Colourless Master Mix is tested for performance in a polymerase chain reaction (PCR) for the amplification of different-sized DNA fragments (1 and 5 kb) from human genomic DNA. The resulting PCR products are visualized as single bands in a GreenSafe Premium 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. |
| • **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. |
| • **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.***