Supreme NZY Taq II Pink Master Mix 2x

Catalogue number	Presentation
MB48801	2 x 1.25 mL (100 rxns of 50 µL)

Description

Supreme NZY Taq II Pink Master Mix 2x is a premixed, ready-to-use solution containing Supreme NZYTaq II DNA polymerase (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 and supports the robust and reliable amplification of a wide range of DNA templates up to 6 kb. The final concentration of $MgCl_2$ is 2.5 mM, allowing for the implementation of a comprehensive range of PCR protocols. Additionally, reactions assembled with Supreme NZY Taq II Pink Master Mix 2x may be directly loaded onto agarose gels. The mix contains two dyes that allow for visual monitoring of DNA migration distance and control of the gel run time during electrophoresis. Each dye will migrate to the same point as dsDNA of determined sizes (400 and <50 bp). Supreme NZY Taq II Pink Master Mix 2x 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 – Supreme NZYTaq II 2× Colourless Master Mix (Cat. No. MB359) – or purifying the PCR product using NZYGelpure (Cat. No. MB011) before performing such downstream 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 (Cat. No. MB053) or NZY-A Speedy PCR cloning kit (Cat. No. MB137).

Shipping & Storage Conditions

This product can be shipped at a range of temperatures from dry ice to blue ice. Upon receipt, store Supreme NZY Taq II Pink Master Mix 2x immediately at -85 °C to -15 °C in a constant temperature freezer. Minimize the number of freeze-thaw cycles by storing in working aliquots. The product will remain stable until the expiry date if stored as specified.

Components

Supreme NZY Taq II 2x Pink Master Mix is supplied in enough volume to perform 100 ligation reactions of 50 µL each.

COMPONENT	TUBES	VOLUME
Supreme NZY Taq II 2x Pink Master Mix	2	1250 µL

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. MB483, or RNase & DNase Cleaner, Cat. No. MB463, 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 post-PCR gel analysis.
  - Thaw the Supreme NZY Taq II 2x Pink Master Mix on ice. Then, to guarantee uniformity of all its components, gently flick the tube before using.
  - The PCR can be set up at room temperature. Add the template DNA in the last step.

Procedure

The following standard protocol serves as a general guideline and astarting point for any PCR amplification. Optimal reactionconditions (incubation times and temperatures, concentration ofprimers 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 inbrackets in the table below.

1. In a sterile, nuclease-free microcentrifuge tube, on ice, prepare a reaction mixture by judiciously combining the following components for a 50 µL reaction:
   Note1: *If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed*
   Note2: *If a higher volume of reaction is required, scale up the components of the reaction, accordingly, ensuring that NZY Taq II Pink Master Mix 2x in the reaction is 1x.*

COMPONENT	AMOUNT/REACTION
Primer Forward	0.25 µL (0.1-0.5) µM (*)
Primer Reverse	0.25 µL (0.1-0.5) µM (*)
Supreme NZY Taq II Pink Master Mix 2x	25 µL
Template DNA	1 pg-0.5 µg
Nuclease-free water	up to 50 µL

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

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.
3. Perform PCR using the following cycling parameters:

<table>
<tr><th>CYCLES</th><th>TEMP.</th><th>TIME</th><th>STAGE</th></tr>
<tr><td>1</td><td>95 °C</td><td>5 min</td><td>Initial denaturation</td></tr>
<tr><td rowspan="3">25-35</td><td>94 °C</td><td>30 sec (¥)</td><td>Denaturation</td></tr>
<tr><td>(*)</td><td>30 sec</td><td>Annealing</td></tr>
<tr><td>72 °C</td><td>15-30 sec/kb (¥)</td><td>Extension</td></tr>
<tr><td>1</td><td>72 °C</td><td>5-10 min</td><td>Final Extension</td></tr>
</table>

(*) 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 (Cat. No. MB132) or any other means.

Technical Notes

Primers design

PCR primers generally range in length from 15–30 bases and aredesigned to flank the region of interest. Primers should contain 40–60% GC and care should be taken to avoid sequences that mightproduce internal secondary structure. The 3'-ends of the primersshould not be complementary to avoid the production of primerdimers. Primer-dimers unnecessarily delete primers from thereaction and result in an unwanted polymerase reaction thatcompetes with the desired reaction. Avoid three G or C nucleotidesin a row near the 3'-end of the primer, as this may result in nonspecific primer annealing. Ideally, both primers should have nearlyidentical melting temperatures (Tm), allowing their annealing withthe denatured template DNA at roughly the same temperature.

DNA template

The DNA template must be purified and devoid of contamination by PCR inhibitors (e.g. EDTA). 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

Genomic DNA contamination

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

Nucleases assay

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. To test for RNase contamination, 1 µg of RNA is incubated with the master mix for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

Supreme NZY Taq II 2x Pink 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 visualised 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 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).
• **Concentration of primers and template are not optimized**
Adjust the concentrations of primers and template DNA following recommendations described in the Technical Notes above. Too low or too high concentrations can inhibit amplification.
• **Degraded or damaged DNA**
Analyse DNA on a denaturing gel to verify integrity. Use aseptic conditions while working with DNA to prevent nuclease contamination. Replace water and plasticware if necessary. Ina addition, if required, re-purify DNA.
PRESENCE OF NON-SPECIFIC BANDS
• **Non-specific annealing of primers**
Optimize annealing temperature, by performing a temperature gradient PCR or by systematically testing different annealing temperatures. If necessary, design another set of primers, by increasing the length and avoiding complementary sequences. In some conditions, a pre-incubation of 1-2 minutes of the Master Mix with primers before adding the template DNA may be beneficial.
• **Inadequate extension time**
Increase the extension time during the PCR cycling program. This can help ensure complete amplification of the target sequence.
• **Presence of contaminants**
Contamination with genomic DNA, PCR products, or other contaminants can lead to nonspecific amplification or PCR failure. Use precautions such as using separate work areas for pre- and post-PCR steps, regularly changing gloves, and using dedicated pipettes and reagents.

For life science research only. Not for use in diagnostic procedures.