

NZY Taq II Pink Master Mix 2x

Catalogue number	Presentation
MB48701	2 x 1.25 mL (100 rxns of 50 µL)

Description

NZY Taq II Pink Master Mix 2x is a premixed, ready-to-use solution containing NZYTaq II DNA polymerase (Cat. No. 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. The final concentration of MgCl₂ is 2.5 mM, allowing for the implementation of a comprehensive range of PCR protocols. Additionally, reactions assembled with 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). 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 – NZYTaq II 2x Colourless Master Mix (Cat. No. MB357) – or purifying the PCR product using NZYGelpure (Cat. No. 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 (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 room temperature. Upon receipt, store NZY Taq II Pink Master Mix 2x immediately at -85 °C to -15 °C in a constant temperature freezer. The high thermal stability of the enzyme mixture allows it to remain stable at 2-8 °C or even at room-temperature for up to 4 weeks. The product will remain stable until the expiry date if stored as specified.

Components

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

COMPONENT	TUBES	VOLUME
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 NZY Taq II 2x Pink Master Mix on ice. Then, to guarantee uniformity of all its components, gently flick the tube before using.
 - Set up the PCR reaction on ice and add water first and the remaining components in the order specified in the Procedure described below.

Procedure

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. 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
NZY Taq II Pink Master Mix 2x	25 µL
Template DNA	5 pg-0.5 µg
Nuclease-free water	up to 50 µL

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.

3. Perform PCR using the following cycling parameters:

CYCLES	TEMP.	TIME	STAGE
1	95 °C	3 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

(*) Annealing temperature should be optimized for each primer set based on the primer T_m ; typically, it should be $T_m - 5$ °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 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 nonspecific 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 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 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.

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

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
<ul style="list-style-type: none">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).
<ul style="list-style-type: none">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
<ul style="list-style-type: none">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).
<ul style="list-style-type: none">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.
<ul style="list-style-type: none">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. In addition, if required, re-purify DNA.
PRESENCE OF NON-SPECIFIC BANDS
<ul style="list-style-type: none">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.
<ul style="list-style-type: none">Inadequate extension time
Increase the extension time during the PCR cycling program. This can help ensure complete amplification of the target sequence.
<ul style="list-style-type: none">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.