Multiplex PCR NZYTaq 2× Colourless Master Mix

Catalogue number: MB33601, 500 U
MB33602, 1000 U
MB33603, 5000 U

Description

Multiplex PCR NZYTaq 2× Colourless Master Mix is a premixed ready-to-use solution designed for the simultaneous amplification of multiple DNA fragments (up to 15 targets) in a single tube. The Master Mix contains dNTPs and reaction buffer at optimal concentrations for efficient multiplexing of targets ranging in size from 70 bp to 2.5 kb over a broad range of primer and template concentrations. $MgCl_2$ final concentration is 2.5 mM, allowing the implementation of a comprehensive variety of PCR protocols. Amplification efficiency is further improved by the inclusion of a recombinant modified enzyme derived from *Taq* DNA polymerase, which was engineered to display a hot-start-like PCR capacity and high processivity (fast polymerisation). The enzyme is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer-dimers, and thus ensuring highly specific and sensitive multiplex PCR amplification.

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

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. Minimize the number of freeze-thaw cycles by storing in working aliquots. The Mix may be stored at 4 °C for up to 7 days. The product will remain stable till the expiry date if stored as specified.

Important Notes

- Ensure that the DNA fragments you want to simultaneously amplify differ in size sufficiently enough to be distinguished through electrophoresis.
- Primers producing a single product of correct size are pivotal for successful multiplex assays. Thus, special care must be taken when designing multiplex PCR primers. See below general recommendations for primer design.
- Prior to any multiplex assay, we recommend performing single PCR reactions to test the functionality and specificity of each primer pairs.
- For easy handling of multiple primers used in multiplex assays, we recommend preparing a primer mix by combining all primers at equimolar concentrations.
- The enzyme provided in the Multiplex PCR NZYTaq 2× Colourless Master Mix is inactive at room temperature, but its functional activity is restored during the 5-min incubation step at 95 °C.
- 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).

Standard Protocol

The following protocol serves as a general guideline and a starting point for standard multiplex PCR amplifications. Depending on the complexity of the PCR reaction (such as multiplex reactions with more than 10 targets, amplification of fragments longer than 2 kb or when using very low amounts of starting template), reaction conditions (e.g. incubation times and temperatures, concentration of primers and/or template DNA) vary and may need to be optimised.

1. 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):

(Note: reaction setup should be performed at room temperature. Incubation of enzyme mixture with primers for 2-3 min at room temperature before adding DNA could increase yield and specificity)

Multiplex PCR NZYTaq 2× Colourless Master Mix	25 µL
Primer mix	0.2 µM [1]
Template DNA	1 ng-0.5 µg
Nuclease-free water	up to 50 µL

[1] Recommended final primer concentration is 0.2 µM. However, the primer concentration may be optimised in a range of 0.1-0.3 µM. If a same primer is used to amplify more than one target, it may be beneficial to use this primer at 0.4 µM final concentration.

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	5 min	1
Denaturation	94 °C	30 s	25-40 †
Annealing	*	30-60 s	
Extension	72 °C	30-60 s/kb ¥	
Final Extension	72 °C	5-10 min	1

***** Care must be taken when selecting the annealing temperature because a low annealing temperature may yield nonspecific PCR products while a high value is likely not to amplify the target. The optimal annealing temperature should be optimised for the primer mixture. We recommend to use 3-5 °C below the lowest melting temperature (T_m) of the primer mixture. If necessary, perform a gradient PCR to find the optimal annealing temperature.

† 30 cycles is optimal for most protocols. A larger number of cycles may be required to increase PCR sensitivity when using low levels of template DNA. Smaller number of cycles may be used when amplifying microsatellites or short amplicons.

¥ Calculate extension time based on the size of the largest fragment you want to amplify. for the majority of protocols, a sucefull PCR is achieved using a extension time of 30 s/kb; Increase the time of extesion for 60 s/kb when have larger amplicons (≥ 2 kb).

4. Analyse PCR products using an appropriate detection system. When separating samples through agarose gel electrophoresis, please take into account that both concentration and grade of agarose are important when working with closely-sized fragments. NZYtech offers a vast portfolio of agaroses with different specifications to cover a wide range of needs. Please visit

www.nzytech.com to identify the agarose that most suits your experiment.

Primer Design

Special care must be taken when designing multiplex PCR primers. In general, primers should range in length from 20–34 bases and should contain 40–60% of GC residues. Minimize the number of consecutive G's in the primers. Preferably, G and C residues should be distributed uniformly along the primer. Avoid sequences that might produce internal secondary structures. Also, avoid significant homology between primers. The 3´-ends of the primers should not be complementary to avoid the production of primer-dimers. 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, and increased synthesis of undesirable reaction products. All primers should have nearly identical melting temperatures (T_m). The recommended T_m of all primers is between 55-60 °C and should not differ by more than 5-6 °C. To design primers for multiplex PCR, it is important to adjust the amplification conditions for each primer pair so that they produce a single PCR product of correct size. Another critical factor for successful multiplexing is the quality of primers. Primers should be of high-quality, purchased desalted or purified. We recommend dissolving primers in TE buffer and to prepare small-volume aliquots of a primer mix containing all primer pairs at equimolar concentrations. Store at -20 °C and avoid repeated freezing and thawing.

DNA template

The optimal amount of starting material may vary depending on the template DNA quality and complexity, as well on the range of targets size. In general, we recommend using 500-20 ng of genomic DNA template. Lower amounts of DNA template (typically 20-1 ng) can be used for amplification of lambda or plasmid DNA or even 50-10 ng for amplification of multicopy chromosomal genes.

Quality control assays

Purity

The purity of NZYtech's Multiplex PCR DNA polymerase is > 90% as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (MB15201).

Genomic DNA contamination

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

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with Multiplex PCR NZYTaq 2× Colourless Master Mix for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe Premium (MB13201)-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

Multiplex PCR NZYTaq 2× Colourless Master Mix is tested for performance in a PCR assay for the simultaneously amplification of 12 fragments (sizes ranging between 200-2500 bp) from human genomic DNA. The resulting PCR products are visualized as single bands in a 2.5% agarose gel (ultrapure grade) stained with GreenSafe Premium (MB13201).

Troubleshooting

No product amplification or low yield
• Inadequate annealing temperature/ primer concentration
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 (3 ° to 5 °C lower than Tm). Optimise annealing temperature using temperature gradient PCR. Increase primer concentration for lower yield amplicons.
• Inadequate cycling conditions
Increase extension time and/or number of cycles.
• 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.
• Inadequate amount of template DNA
Titrate template amount. We recommend starting with 20-50 ng of genomic DNA.
• 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 0.25 mM increments. (Note: $MgCl_2$ is not provided).
Presence of non-specific bands
• Non-specific annealing of primers
Adjust annealing conditions and/or design another pool of primers, avoiding complementary sequences. Ensure that each primer pair originates a single product without primer-dimers in individual PCR reactions. If necessary, reduce primer concentration.
• Inadequate cycling conditions
Reduce the number of cycles.

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