Speedy NZYTaq 2× Colourless Master Mix

Catalogue number: MB36101, 500 U
MB36102, 1000 U
MB36103, 5000 U

Description

Speedy NZYTaq 2× Colourless Master Mix is a premixed ready-to-use solution containing Speedy NZYTaq DNA polymerase (MB403), a recombinant DNA polymerase displaying a faster polymerization reaction than any other conventional non-proofreading enzyme. Only 5 seconds are required for the successful synthesis of 1 kb size DNA. The enzyme retains its speed (5 sec/kb) when amplifying fragments up to around 2-3 kb. Successful amplification of higher DNA fragments up to 6 kb in size can be reached using a 10 sec/kb extension step. Faster PCR can be further achieved by increasing the primers melting temperature, which increases PCR annealing temperature, thus allowing combining the annealing and extension PCR steps during PCR cycling (see below). The master mix contains dNTPs, reaction buffer and additives at optimal concentrations for the efficient amplification of a wide range of DNA templates. $MgCl_2$ 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 (MB011) before usage in subsequent protocols. NZYSpeedy 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).

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to room-temperature.

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. The high thermal stability of the enzyme mixture allows it to remain stable at 4°C or even at room-temperature for up to 4 weeks. The product will remain stable till the expiry date if stored as specified.

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, in controlled assay conditions.

Enzyme concentration: 0.2 U/µL, in glycerol

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) 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. Gently mix and briefly centrifuge the master mix after thawing. Set up the PCR reaction on ice and add water first and the remaining components in the order specified in the table below. A single reaction mixture of 50 µL should combine the following components:

Primers	0.25 (0.1-0.5) µM
Template DNA	50 pg-0.5 µg
Speedy NZYTaq 2× Colourless Master Mix	25 µL
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters:

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	95 °C	1 min	95 °C	1 min	1
Denaturation	94 °C	2 s	94 °C	2 s	25-35
Annealing	-	-	**(*)**	5 s	
Extension	68-72 °C **(¥)**	5 s/kb **(†)**	72 °C	5 s/kb	
Final Extension	72 °C	2 min	72 °C	2 min	1

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

(¥) Extension temperature will depends on primer melting temperature (see below).

(†) For DNA fragments higher than 2-3 kb to 6 kb in size, it may be beneficial to use 10 s/kb.

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 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 (T_m), allowing their annealing with the denatured template DNA at roughly the same temperature. For faster PCR protocols, primers should have a $T_m \geq 60$ °C. Please note that primer annealing, and product extension can be combined into one step for a faster PCR reaction (see 2-step protocol above) if primers are designed to have a $T_m \geq 65$ °C.

DNA template

The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 20 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 50 pg of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 5-20 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Quality control assays

Purity

Speedy NZYTaq DNA polymerase purity is > 90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

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.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with Speedy NZYTaq 2× Colourless 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

Speedy NZYTaq 2× Colourless Master Mix is tested for performance in a polymerase chain reaction (PCR) for the amplification of a 1000 bp fragment 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+} concentrations 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.

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