MB362_IFU_EN_V2601

Speedy NZYTaq 2× Green Master Mix

Catalogue number	Presentation
MB36201	2 x 1.25 mL (100 rxns of 50 µL)
MB36202	4 x 1.25 mL (200 rxns of 50 µL)
MB36203	20 x 1.25 mL (1000 rxns of 50 µL)

Description

Speedy NZYTaq 2× Green Master Mix is a premixed ready-to-use solution containing Speedy NZYTaq DNA polymerase (NZYtech, Cat. No. 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 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. In addition, reactions assembled with Speedy NZYTaq 2× Green Master Mix may be directly loaded onto agarose gels. The mix contains two dyes (blue and yellow) that allow monitoring the progress of the electrophoresis. Speedy NZYTaq 2× Green Master Mix 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 – Speedy NZYTaq 2× Colourless Master Mix (NZYtech, Cat. No MB361) – or purifying the PCR product using NZYGelpure (NZYtech, Cat. No MB011) before performing any other protocol. 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 (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 room temperature. Upon arrival, all components should be stored at -85 °C to -15 °C in a constant temperature freezer 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.

Components

COMPONENT	SKU	TUBES/BOTTLES	VOLUME
Speedy NZYTaq 2× Green Master Mix	**MB36201**	2	1.25 mL
	MB36202	4	1.25 mL
	MB36203	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 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 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.

	1 REACTION
Forward and Reverse Primers	0.25 (0.1-0.5) µM each
DNA Template	50 pg-0.5 µg
Speedy NZYTaq 2× Green Master Mix	25 µL
Nuclease-free water	up to 50 µL
FINAL VOLUME =	50 µL

3. Gently mix and centrifuge briefly to spin down the contents.
4. Perform PCR using the cycling parameters below. For primers with a melting temperature (Tm) ≥ 65 °C, the annealing and extension steps may be combined into a 2-step protocol. For primers with lower Tm, the 3-step protocol should be used.

CYCLES	2-step protocol		3-step protocol		STAGE
	TEMP.	TIME	TEMP.	TIME	
1	95 °C	1 min	95 °C	1 min	Initial Denaturation
25-35	94 °C	2 sec	94 °C	2 sec	Denaturation
	-	-	**(*)**	5 sec	Annealing
	68-72 °C (¥)	5 sec/kb **(ŧ)**	72 °C	5 sec/kb	Extension
1	72 °C	2 min	72 °C	2 min	Final Extension

(*) *Annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm -5 °C.*
(¥) *Extension temperature will depend on primer melting temperature (see below).*
(ŧ) *For DNA fragments higher than 2-3 kb to 6 kb in size, it may be beneficial to use 10 s/kb.*

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. For faster PCR protocols, primers should have a Tm ≥ 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 Tm ≥ 65 °C.

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.

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.

Intercalating Dyes: The master mix contains both a blue and a yellow dye. The blue dye migrates at the same rate as a 3–5 kb DNA fragment in a 1% (w/v) agarose gel. The yellow dye migrates faster than primers (<50 bp) in a 1% (w/v) agarose gel. This mix is not suitable for direct fluorescence or absorbance measurements without prior purification of the amplified DNA from the PCR.

Quality control assays

Genomic DNA contamination

The product must be free of any detectable DNA contamination as evaluated through PCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with Speedy NZYTaq 2× Green 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× Green Master Mix is tested for performance in a polymerase chain reaction (PCR) for the amplification of two DNA fragments from human genomic DNA (1 kb and 2.5 kb). 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 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.