MB333_IFU_EN_V2601

Supreme NZYLong 2x Green Master Mix

Catalogue number	Presentation
MB33301	2 x 1.25 mL (100 rxns of 50 µL)
MB33302	4 x 1.25 mL (200 rxns of 50 µL)
MB33303	20 x 1.25 mL (1000 rxns of 50 µL)

Description

Supreme NZYLong 2× Green Master Mix is a premixed ready-to-use solution containing Supreme NZYLong DNA polymerase (NZYtech, Cat. No. MB331), an engineered version of NZYLong DNA polymerase designed to amplify longer PCR products, generally of 25 kb and beyond, that displays a higher specificity provided by its hot-start-like PCR capacity. This feature is achieved by a novel hot-start technology, which inhibits both polymerase and 3'→5' exonuclease activities and thus avoids extension of non-specifically annealed primers or primer-dimers, as well as degradation of primers and template DNA during PCR reaction setup. The functional activity of the enzyme is restored during a short 5-minute incubation step at 94 °C. The increased processivity of Supreme NZYLong DNA polymerase combined with the hot-start-like PCR capacity results in higher specificity, sensitivity and yield during the amplification of long nucleic acids. 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 Supreme NZYLong 2× Green Master Mix may be directly loaded onto agarose gels. There are two dyes (blue and yellow) in the mix that allow monitoring the progress of the electrophoresis. Supreme NZYLong 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 – Supreme NZYLong 2× Colourless Master Mix (NZYtech, Cat. No. MB334) – or purifying the PCR product using NZYGelpure (NZYtech, Cat. No. MB011) before performing any other protocol. Supreme NZYLong DNA polymerase generates a mixture of A-overhang-ended (predominantly) and blunt-ended PCR products, being suitable for cloning with NZYtech's TA PCR cloning kits (NZYtech, Cat. No. MB053 or 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
Supreme NZYLong 2x Green Master Mix	**MB33301**	2	1.25 mL
	MB33302	4	1.25 mL
	MB33303	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 template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune primer concentrations, test the recommended variations provided in brackets in the table below.

1. Gently mix and briefly centrifuge the master mix after thawing.
2. Set up the PCR reaction at room temperature or on ice. 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:

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	1 REACTION
Forward and Reverse Primers	0.35 (0.25-0.5) µM each
Supreme NZYLong 2× Green Master Mix	25 µL
DNA Template	1 ng-0.5 µg
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 following cycling parameters:

CYCLES	TEMP.	TIME	STAGE
1	94 °C	5 min	Initial Denaturation
25-35	94 °C	20 sec	Denaturation
	*	30 sec	Annealing
	68 °C	1 min/kb	Extension
1	68 °C	1.5 min/kb	Final Extension

(*) The annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm - 5 °C.

5. Analyse the PCR products by agarose gel electrophoresis (0.6-0.8%, w/v) and visualise with GreenSafe Premium (NZYtech, Cat. No. MB132) or any other mean.

Technical Notes

Primers: Optimal primer design is critical for long-range amplifications. PCR primers should be designed to have 18–35 bases in length and a GC content of 45-60%. Pay special attention to avoid sequences that might produce internal secondary structures. The 3´-ends of the primers should not be complementary to avoid the production of primer-dimers, and it is recommended to have at least 2 Cs or Gs. Ideally, both primers should have nearly identical melting temperatures (Tm), allowing their annealing with the denatured template DNA at roughly the same temperature. For long PCRs avoid using primers that have been previously subjected to multiple freezing-thawing cycles. Note that primer annealing and DNA extension can be combined into one step if primers are designed to have a Tm ≥ 70 °C.

Template: The amplification of long PCR products requires high quality gDNA retaining long DNA fragments (it is not possible to amplify a 20 kb product from damaged gDNA with an average fragment size of 5 kb, for example). 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 1 ng of human gDNA, for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-50 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Try to add the DNA as the latest component to the PCR reaction and avoid pipetting after this. To retain DNA integrity, avoid multiple freeze-thawing cycles stock DNA solutions and keep working DNA at small aliquots.

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.

Cycling conditions: It is highly recommended to use incubation and extension temperatures as high as required by the experiment. An extension performed at 68 °C favours the accumulation of long PCR products without compromising enzyme performance.

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 Supreme NZYLong 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.

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Functional assay

Supreme NZYLong 2× Green Master Mix is tested for performance in a polymerase chain reaction (PCR) for the amplification of different-sized DNA fragments (until 15kb) from human genomic DNA. The resulting PCR products are visualized 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 of 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.

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