Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x

Catalogue number	Presentation
MB49101	for 3.75 ml (All colours, 150 rxns of 50 µL)
MB49102	for 2.5 ml (Yellow, 100 rxns of 50 µL)
MB49103	for 2.5 ml (Red, 100 rxns of 50 µL)
MB49104	for 2.5 ml (Pink, 100 rxns of 50 µL)
MB49105	for 2.5 ml (Purple, 100 rxns of 50 µL)
MB49106	for 2.5 ml (Blue, 100 rxns of 50 µL)
MB49107	for 2.5 ml (Green, 100 rxns of 50 µL)
MB49108	for 2.5 ml (Colourless, 100 rxns of 50 µL)

Description

Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x represents a revolutionary advancement in end-point PCR, combining outstanding performance with environmental sustainability. Designed for high-speed molecular applications, this master mix delivers reliable and consistent results, with PCR runs completed in under 45 minutes for amplicons up to 1kb. Successful amplification of higher DNA fragments up to 5 kb in size can be reached using a 20 sec/kb extension step. A key feature of this product is its unique offering of colour options and the ready-to-load format for agarose gels, allowing researchers to personalize their experiments. These colour variations add visual screening to routine procedures and aid in sample tracking during electrophoresis and differentiation during reaction and storage, enhancing the functionality and aesthetics of laboratory work. Furthermore, the lyophilized format of Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x underscores our commitment to sustainability. By enabling room temperature shipping without the need for dry ice, this product significantly reduces both carbon footprint and shipping costs, making it an ideal choice for eco-conscious scientific research. Optimized for versatility, Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x is suitable for various end-point PCR applications, ensuring speed, functionality, and specificity across various experimental conditions. Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x is the best choice for faster, more colourful, and environmentally friendly molecular biology.

Shipping & Storage Conditions

This product is shipped at room temperature. Upon receipt, store all components at -85 °C to -15 °C in a constant temperature freezer. These meticulous storage procedures ensure that the Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x delivers consistent and reliable results across its lifespan and usage. All components are formulated to be ready-to-use. The product will remain stable until the expiry date if stored as specified.

Components

All versions comprise a combination of Lyo Speedy Supreme NZY Taq Master Mix 2x and a Reconstitution Buffer:

COMPONENT	MB49101	MB49102	MB49103	MB49104	MB49105	MB49106	MB49107	MB49108
Lyo Speedy Supreme NZY Taq Master Mix 2x	**6 tubes** (for 650 µL)	**4 tubes** (for 650 µL)-	**4 tubes** (for 650 µL)-	**4 tubes** (for 650 µL)-	**4 tubes** (for 650 µL)-	**4 tubes** (for 650 µL)-	**4 tubes** (for 650 µL)	**4 tubes** (for 650 µL)-
Yellow RBuffer (*)	**1 Tube** (1500 µL)	**2 tubes** (1500 µL)	-	-	-	-	-	-
Red RBuffer (*)	**1 Tube** (1500 µL)	-	**2 tubes** (1500 µL)	-	-	-	-	-
Pink RBuffer (*)	**1 Tube** (1500 µL)	-	-	**2 tubes** (1500 µL)	-	-	-	-
Purple RBuffer (*)	**1 Tube** (1500 µL)	-	-	-	**2 tubes** (1500 µL)	-	-	-
Blue RBuffer (*)	**1 Tube** (1500 µL)	-	-	-	-	**2 tubes** (1500 µL)	-	-
Green RBuffer (*)	**1 Tube** (1500 µL)	-	-	-	-	-	**2 tubes** (1500 µL)	-
Colourless RBuffer (*)	-	-	-	-	-	-	-	**2 tubes** (1500 µL)

(*) Reconstitution Buffer for Lyo Speedy Supreme NZY Taq Master Mix 2x.

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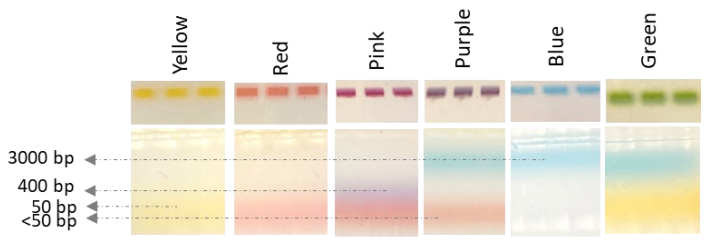
Specifications

Master Mix concentration: 2x

Protocol Time: ≈ 45 minutes (PCR products < 1 Kb). For 5kb fragment ≈ 1h40 minutes.

Optional tracking dye in 6 different colours

- **Dyes migration in agarose gel (TAE) 1% (w/v):**



Master Mix	Migrates similar to DNA (bp) – 1% (w/v) agarose gel
Yellow	50
Red	<50
Pink	400 + <50
Purple	3000 + <50
Blue	3000
Green	3000 + <50

Standard Protocol

The following standard protocol serves as a general guideline for 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 the recommended variations provided in brackets in the table below.

Procedures before starting

- Reconstitute one tube of lyophilized Lyo Speedy Supreme NZY Taq Master Mix 2x by adding 650 µL of the selected Reconstitution Buffer.
- Mix by gently flicking or pipetting up and down, until complete resuspension.
- Allow the mixture to stand for about 1 minute, then flick again.
- Spin down briefly to collect the solution at the bottom of the tube. Do not replace the provided Reconstitution Buffer with water or any other buffer.

Procedure

1. Due to the master mix's hot start technology, reaction setup can occur at room temperature (+18°C to +25 °C). A single reaction mixture of 50 µL should combine the following components: (*Note:* template DNA should be the last component to be added to the reaction mixture)

COMPONENT	1 REACTION VOLUME / AMOUNT
Lyo Speedy Supreme NZY Taq Master Mix 2× (previously resuspended)	25 µL
Primers (Fw + Rev)	0.25 (0.1-0.5) µM
Template DNA	1 pg-0.5 µg
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters:

CYCLES	TEMP.	TIME	CYCLE STEP
1	95 °C	3 min	Initial denaturation
30-35(**)	94 °C	2 sec	Denaturation
	(*)	5 sec	Annealing
	72 °C	5 sec **(¥)**	Extension
1	72 °C	1 min	Final Extension

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(*) *The annealing temperature should be optimized for each primer set, typically set at 5°C below the primer's melting temperature.*

(**) *Total PCR time for 35 cycles is 44 min, using a BioRAD T100 System with a ramp rate of 1.5°C/sec.*

(¥) *For templates >1kb, adjust extension time to 20s/kb.*

Quality control

Genomic DNA Contamination

Lyo Speedy Supreme NZY Taq Master Mix 2x and the different variations of the Reconstitution Buffer must comply with internal standards of DNA contamination as evaluated through polymerase chain reaction (PCR).

Nucleases assay

Reconstituted Lyo Speedy Supreme NZY Taq Master Mix 2x and the different variations of the Reconstitution Buffer are tested for nuclease contamination. To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the samples for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the enzyme 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

Lyo Speedy Supreme NZY Taq Rainbow Master Mix 2x is tested for performance in a polymerase chain reaction (PCR) using human genomic DNA.

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 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+} 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).
• **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.
• **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. Ina addition, if required, re-purify DNA.
PRESENCE OF NON-SPECIFIC BANDS
• **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.

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• **Inadequate extension time**
Increase the extension time during the PCR cycling program. This can help ensure complete amplification of the target sequence.
• **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.

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