

Lyo NZYSupreme qPCR Probe Master Mix (2x)

Catalogue number	Presentation
MB41701	3 x 0.5 mL (150 rxns of 20 µL)

Description

Lyo NZYSupreme qPCR Probe Master Mix (2x) is an optimized and highly efficient freeze-dried reaction mixture developed for real-time PCR. This master mix was engineered with a multiple hot-start enzyme control mechanism to provide the highest detection sensitivity. In addition, the latest developments in PCR enhancers have been incorporated in the Lyo NZYSupreme qPCR Probe Master Mix, including buffer chemistry and incorporation of highly robust engineered enzymes. This master mix does not contain ROX and it was specifically developed for probe-detection technology, including molecular beacons. For qPCR instruments that require this reference dye, we indicate a concentration range for your assay according to the table shown in the section "ROX reference dye" (ROX not provided). Lyo NZYSupreme qPCR Probe Master Mix (2x) is provided as a simple-to-use, stabilized 2x reaction mixture that includes all components for quantitative PCR, except sample DNA, primers, probe and water. It also supports various types of assays, from single to triplex, providing experiment flexibility.

Shipping & Storage Conditions

The product can be shipped in a range of temperatures 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. Once the lyophilized master mix has been resuspended using the buffer provided, it should be stored for short-term at -85 °C to -15 °C. Short-term storage for 1-2 weeks at room temperature will not compromise the performance of the product. Minimize the number of freeze-thaw cycles by storing it in working aliquots. Do not expose the master mix to direct sunlight. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	TUBES	VOLUME
Lyo NZYSupreme qPCR Probe Master Mix (2x)	3	For 525 µL
qPCR master mix reconstitution buffer	1	1.75 mL

Specifications

Compatibility with real-time PCR instruments

Lyo NZYSupreme qPCR Probe Master Mix (2x) is compatible with instruments that do not require a passive reference signal for data normalization. It was optimized to be compatible with the following real-time PCR instruments:

Bio-Rad®: CFX96™; CFX384™; iCycler®; iQ™5; Opticon™; Opticon™ 2

Qiagen (Corbett): Rotor-Gene™ 3000; Rotor-Gene™ 6000 & Rotor-Gene™ Q

Roche: Lightcycler® 96; Lightcycler® 480 & Lightcycler® Nano

Applied Biosystems (with optional ROX addition or ROX OFF): 7000; 7300; 7700; 7900; 7900HT; 7900HT FAST; StepOne™ & StepOne™plus; 7500; 7500 FAST; QuantStudio™ 6, 7, 12k Flex & ViiA7™

Multiplex capacity

Supporting nucleic acid analysis, NZYSupreme qPCR Probe Master Mix (2x), ROX plus, covers a spectrum from single to triplex assays, providing flexibility for diverse experimental requirements.

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. It is essential that any tubes containing amplified PCR product should not be opened in the PCR set-up area. Use sterile filtered tips. Minimize exposure by keeping reaction and components capped whenever possible.

- **Controls:** For verification of the absence of contamination, prepare a mixture sample without a DNA template (negative control). Additionally, include a positive control to serve as a reference for ensuring the correct functioning of the qPCR reaction and detection system. The positive control should exhibit the expected amplification and/or fluorescence signal, confirming the assay's ability to accurately detect the target sequence. To avoid incorrect results caused by unwanted fluorescent substances, consider adding a No-Amplification Control tube. This tube should have the sample but not the enzyme master mix. Elevated fluorescence in the No-Amplification Control compared to the No-Template Control suggests potential fluorescent contaminants in either the sample or the thermal cycler's heat block.

Procedure before starting

- **Reconstitution of Lyo NZYSupreme qPCR Probe Master Mix (2x):** Reconstitute each tube of Lyo NZYSupreme qPCR Probe Master Mix with 525 μ L of the qPCR master mix reconstitution buffer provided. Complete hydration of the mixture might take 2-3 min. Mix gently by flicking the tube, and spin down. Do not replace the reconstitution buffer with water or any other buffer. The master mix is then ready to use as a 2x RT-qPCR master mix. Store the reconstituted master mix at at -85 $^{\circ}$ C to -15 $^{\circ}$ C in a constant temperature freezer or use the master mix directly after reconstitution.

Protocol

The following protocol serves as a general guideline and a starting point for any qPCR procedure. Optimal reaction conditions (e.g. incubation times, temperatures and template concentration) may vary and, in particular conditions, may require further optimization.

1. Thaw the master mix at room temperature or on ice. Mix the master mix thoroughly by flicking the tube and inverting.
2. In a clean reaction setup area, prepare the qPCR reaction mixture (without template) according to the table below (please notice that the given volumes are based on a standard 20 μ L final reaction mix and can be scale adjusted):

Note 1: If setting up more than one reaction, prepare a reaction mixture volume 10% greater than the total required for the number of reactions to be performed. Include sufficient reactions for the negative and positive controls.

Note 2: If necessary, prepare a No-Amplification Control by adding nuclease-free water instead of the qPCR master mix.

Note 3: We recommend performing replicates of all reactions.

	1 REACTION VOLUME	FINAL CONCENTRATION
Lyo NZYSupreme qPCR Probe Master Mix (2x) ⁽¹⁾	10 μ L	1 \times
10 μ M forward primer	0.8 μ L	400 nM ⁽²⁾
10 μ M reverse primer	0.8 μ L	400 nM ⁽²⁾
10 μ M probe	0.4 μ L	200 nM ⁽²⁾
FINAL VOLUME =	up to 12 μ L ⁽³⁾	-

(1) The master mix does not contain ROX, but, if required, addition of this internal passive reference dye can be conducted in a separate step. The final concentration will vary according to the qPCR instrument used. Please follow instructions described in the section of "Technical Notes" below.

(2) Refer to the section of "Technical Notes" below for more details about primers and probes final concentrations in the reaction.

(3) If using smaller volumes for the primers & probe, supplement the volume up to 12 μ L with Nuclease-free Water.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Pipette 12 μ L of the Mix into each well, according to your experimental plate/strip/tube configuration.
5. Pipette template (samples and controls):
 - a. Add up to 8 μ L of DNA sample/positive control into each respective well. If using less volume than 8 μ L, supplement with Nuclease-free Water up to 8 μ L. In total, the final volume of the reaction will be 20 μ L.
 - b. Add up to 8 μ L of Nuclease-free Water for the negative control to achieve a final volume of 20 μ L.

Note: To avoid cross-contamination, we strongly recommend pipetting the template at last, preferably in a work separate area.

6. Cover and seal the plate/strip/tube with appropriate caps or optical adhesive film before proceeding with the real-time PCR detection steps.
7. Centrifuge briefly to spin down the contents and eliminate any air bubbles from the reaction mixtures.
8. Place the reaction plate/strip/tube within the real-time PCR instrument and run the general protocol defined below. These conditions might be adapted to suit your specific needs, within sensible limits.

Suggested thermal cycling conditions

Lyo NZYSupreme qPCR Probe Master Mix (2x) was optimized for the amplification of DNA fragments up to 200 bp under different real-time PCR cycling conditions. The table below displays a standard qPCR setup optimized on several platforms. However, these conditions may be adapted to suit different equipment-specific protocols.

CYCLES	TEMP.	TIME	STAGE
1	95 $^{\circ}$ C	2 - 5 min (*)	Polymerase activation
40	95 $^{\circ}$ C 60 $^{\circ}$ C	5 sec 30 - 60 sec (**)	Denaturation Annealing/Extension

(*) 2 min for cDNA, up to 3 or 5 min for genomic DNA.

(**) Amplicons exceeding 200 base pairs in length can be employed, although optimizing extension times may be necessary. An additional increment in the extension step, up to 60 seconds, may also be required for complex assays.

Testing and Ct values

When comparing Lyo NZYSupreme qPCR Probe Master Mix (2x) a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed.

Technical Notes

Primers and probe: These guidelines refer to the design and set-up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your real-time PCR experiment:

- Primers should have a melting temperature (T_m) of approximately 58-62 °C. The probe T_m should be approximately 10°C higher than that of the primers;
- The fragment to amplify should be between 80-200 bp in length and not superior to 300 bp;
- Final primer concentrations of 400 nM are suitable for most probe-based reactions. However, to determine the optimal concentration we recommend titrating in the range 150-600 nM. Forward and reverse primers concentration should be equimolar;
- A final probe concentration of 100-200 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration; to determine the optimal concentration we recommend titrating in the range 50-300 nM;
- For multiplex RT-qPCR, high probe concentrations can result in cross channel fluorescence. For high-complexity multiplex assays, we highly recommend utilizing the NZYSupreme Multiplex qPCR Probe Master Mix (2x) (Cat. No. MB452) or Lyo NZYSupreme Multiplex qPCR Probe Master Mix (2x) (Cat. No. 453).

Template: The DNA template must be purified and devoid of contamination by PCR inhibitors (e.g. EDTA). The recommended amount of template is dependent upon the source of DNA used. Please consider the following points when selecting genomic DNA or cDNA templates:

- **Genomic DNA:** we recommend using up to 1 µg of genomic DNA in a single PCR. We recommend using the NZY Tissue gDNA Isolation kit (Cat. No. MB135) for high yield and purity from both prokaryotic and eukaryotic sources.
- **cDNA:** the optimal amount of cDNA to use in a single PCR depends upon the copy number of the target gene; as a good starting point for efficient qPCR, we recommend using 1-100 ng of cDNA as template per reaction. However, this amount may be adjusted based on the specific assay requirements, target abundance, template quality, and sample availability. We suggest using the Supreme NZY RT SuperMix 5x (Cat. No. MB499) or one of the NZYtech kits) for reverse transcription of purified RNA. To obtain a high yield of highly purified RNA we suggest using NZY Total RNA Isolation Kit (Cat. No. MB134).

MgCl₂: It is not necessary to supplement the reaction mixture with MgCl₂ as the Lyo NZYSupreme qPCR Probe Master Mix (2x) already contains an optimized concentration of MgCl₂.

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 qPCR design, replacing template DNA/cDNA with nuclease-free PCR-grade water (DEPC-treated Water, Cat. No. MB43701).

ROX reference dye: Lyo NZYSupreme qPCR Probe Master Mix (2x) is compatible with most thermocyclers available in the market and can include ROX passive reference dye (not provided) to normalize non-PCR-related fluctuations in fluorescence. If ROX addition is required for your qPCR platform, an optimal quantity of this dye should be included in your master mix. The recommended amount of ROX for the most common qPCR instruments is stated in the table below:

qPCR INSTRUMENT	VOLUME OF ROX PER 525 µL OF MASTER MIX (2x) (*)
Applied Biosystems: 7000/7300/7700/7900/7900HT/7900HT FAST/StepOne™/StepOne™plus	500-725 nM (10.5-15.2 µL) (**)
Applied Biosystems: 7500/7500FAST/QuantStudio™ 6, 7, 12k Flex/ViiA7™	50-100 nM (1-2.1 µL) (**)
Bio-Rad®: CFX96™/CFX384™/iCycler®/iQ™5/Opticon™/ Opticon™ 2 Qiagen: Rotor-Gene™ 3000/6000/Q Roche: Lightcycler® 96/480/Nano	Not required

(*) For different volumes please scale-up or scale-down the volume of ROX accordingly. Please notice that ROX is not provided by NZYtech.

(**) Volume calculated considering a ROX reference dye stock at 25 µM.

Quality control assays

Genomic DNA contamination

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

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the master mix for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the master mix 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 NZYSupreme qPCR Probe Master Mix (2x) is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

FOR REFERENCE USE ONLY

For life science research only. Not for use in diagnostic procedures.