MB507_IFU_EN_V2501

NZY Ultra Multiplex qPCR Probe Master Mix 2x

Catalogue number	Presentation
MB50701	2 x 1 mL (200 rxns of 20 µL)
MB50702	5 x 1 mL (500 rxns of 20 µL)
MB50703	20 x 1 mL (2000 rxns of 20 µL)
MB50705	1 x 50 mL (5000 rxns of 20 µL)

Features

- **Fast Performance:** Accelerated qPCR cycling protocols reduce assay time significantly.
- **Ultra Multiplexing Capability:** Amplification and detection of up to 7 targets in a single reaction.
- **Exceptional Sensitivity and Specificity:** Multiple hot-start enzyme control for low background and reliable detection of low-abundance targets.
- **High Inhibitor Tolerance:** Robust performance in complex and inhibitor-rich samples.
- **Enhanced Stability:** Consistent results with minimal variability across workflows.
- **ROX-Free Formulation:** Designed for use with probe-based assays without interference from passive reference dyes.

Description

NZY Ultra Multiplex qPCR Probe Master Mix 2x is a cutting-edge, highly optimized master mix engineered for superior real-time quantitative PCR (qPCR) performance. Designed for high-throughput and multiplex applications, this master mix ensures fast, sensitive, and robust detection of DNA targets with exceptional reproducibility. The advanced formulation of NZY Ultra Multiplex supports the simultaneous amplification and detection of up to 7 targets in a single reaction. With an accelerated qPCR cycling protocol, this mix significantly reduces assay time without compromising sensitivity, specificity, or precision, making it ideal for demanding research and quality control workflows. NZY Ultra Multiplex qPCR Probe Master Mix 2x is formulated as a concentrated solution (2×) containing all necessary components for robust, high-efficiency qPCR amplification, including specially engineered enzymes, dNTPs, stabilizers, and PCR enhancers. The master mix incorporates a multiple hot-start enzyme activation system, which provides optimal specificity, minimizes non-specific amplification, and ensures a low background signal. Its advanced buffer chemistry and proprietary enzyme engineering further enhance stability and ensure highly reproducible results, even in the presence of PCR inhibitors commonly found in clinical, environmental, and complex sample matrices. This master mix is ROX-free and specifically optimized for probe-based qPCR detection technologies, offering flexibility to adapt to various qPCR instruments. For instruments requiring a passive reference dye, the mix accommodates its addition, with recommended ROX concentration ranges outlined in the “Technical Notes” section.

The NZY Ultra Multiplex qPCR Probe Master Mix 2x is ideal for applications requiring unmatched speed, high-throughput multiplexing, and reliable performance, positioning it as the premier solution for real-time qPCR-based research.

Shipping & Storage Conditions

The product can be shipped from dry ice to ambient 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 product is stable for up to 10 freeze-thaw cycles; however, avoid excessive handling to ensure optimal results. Do not expose the master mix to direct sunlight. The product will remain stable until the expiry date if stored as specified. It can be stored at room temperature (15–22°C) for up to 2 weeks.

Components

COMPONENT	SKU	TUBES/BOTTLES	VOLUME
NZY Ultra Multiplex qPCR Probe Master Mix 2x	**MB50701**	2	1 mL
	MB50702	5	1 mL
	MB50703	20	1 mL
	MB50705	1	50 mL

Note: Consider preparing multiple aliquots of the master mix to reduce freeze/thaw cycles and minimize the risk of contamination.

Specifications

Compatibility with real-time PCR instruments

NZY Ultra Multiplex qPCR Probe Master Mix 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™ Touch; CFX OPUS 96™; 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): 7300; 7700; 7900; 7900HT; 7900HT FAST; StepOne™ & StepOnePlus™; 7500; 7500 FAST; QuantStudio™ 5, 6, 7, 12k Flex & ViiA7™; QuantStudio™ 5 DX.

Sensitivity

The NZY Ultra Multiplex qPCR Probe Master Mix 2x demonstrates exceptional sensitivity in nucleic acid detection, as evidenced by its performance in a heptaplex assay. The mix ensures reliable and reproducible detection of low-copy targets across all seven analytes, demonstrating its superior performance in high-sensitivity multiplex assays, particularly in complex testing settings.

Standard Protocol

Recommendations before starting

- Nucleic acid manipulation: The quality and integrity of DNA templates are critical for achieving reliable qPCR results. Use high-quality DNA extraction kits to isolate clean, contaminant-free DNA. To avoid degradation or contamination, handle DNA samples with care and work in designated clean areas. Consider using NZYtech Nucleases & Nucleic Acid Cleaner (Cat. No. MB48301) or DNA & RNA Cleaner (Cat. No. MB46201) to remove nucleases or residual contaminants from surfaces and materials.
- Handling instructions: To prevent carry-over contamination, dedicate separate areas for reaction setup, qPCR amplification, and any post-PCR analysis. Never open tubes containing amplified PCR products in the qPCR setup area. Always use sterile, filtered pipette tips and avoid reusing any consumables. Excessive exposure to light can degrade fluorescent probes. Protect assay components from light until use.
- Controls: Proper controls must be included to validate qPCR assay performance. Use a No-Template Control (NTC) to confirm the absence of contamination. Include a Positive Control to ensure the correct functioning of the qPCR reaction and detection system; the positive control should generate the expected amplification curve and fluorescence signal. To rule out interference from unwanted fluorescent substances, consider running a No-Amplification Control (NAC), which includes the sample but excludes the enzyme master mix. Elevated fluorescence in the NAC compared to the NTC may indicate fluorescent contaminants in the sample or thermal cycler.
- Template Preparation and Storage: Use high-quality DNA isolation kits to obtain clean and pure DNA samples. Store isolated DNA at -20 °C in the elution buffer provided by the extraction kit or in low EDTA (0.1 mM) TE buffer. If dilution is required, use low EDTA (0.1 mM) TE buffer or Nuclease-Free Water. Avoid using DEPC-treated water, as it may inhibit enzymatic reactions.
- 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 qPCR run.
- Validation of each primer/probe pair: Before performing multiplex qPCR assays, validate the functionality and specificity of each primer/probe pair in singleplex reactions. This step ensures that individual targets are amplified efficiently and specifically. Evaluate each primer/probe set under optimal qPCR conditions before combining them in a multiplex assay to prevent competition between targets and minimize signal interference.

Procedure

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.

Two approaches can be used to set up the experiment: 1) adding the primers and probes individually, or 2) adding primers and probes as pre-prepared mixture solutions. Please select the protocol that is more convenient for your experiments:

Protocol 1 – Set up adding primers and probes individually

1. Thaw the master mix at room temperature or on ice. Mix the master mix gently by flicking the tube and inverting it. Keep the master mix on ice. For automated workflows, the master mix remains stable on the benchtop for extended periods (up to two weeks at room temperature).
   Note: *Please notice that a precipitate in the bottom of the master mix tube may be observed, after multiple freeze/thaw cycles. To ensure optimal performance, please make sure all components are thawed and gently resuspended before use. In this case, do not spin the master mix before pipetting.*
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 µL final reaction mix and can be scale adjusted):

   Note 1: *If setting up more than one reaction, prepare a reaction mixture volume 5 to 10% greater than the total required for the number of reactions to be performed. Include sufficient reactions for the No-Template and positive controls.*
   Note 2: *We recommend performing replicates of all reactions.*

	1 REACTION VOLUME	FINAL CONCENTRATION
NZY Ultra Multiplex qPCR Probe Master Mix 2x (1)(2)	10 µL	1×
10 µM forward primer	0.8 µL	400 nM (3)
10 µM reverse primer	0.8 µL	400 nM (3)
10 µM probe	0.4 µL	200 nM (3)
FINAL VOLUME =	up to 12 µL(4)	-

(1) If a slight precipitate is observed at the bottom of the master mix tube, please refer to the note above (step 1).
(2) The master mix does not contain ROX, but, if required, the 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 the instructions described in the section "Technical Notes" below.
(3) Refer to the section of "Technical Notes" below for more details about primers and probes final concentrations in the reaction.
(4) If using smaller volumes for the primers & probe, supplement the volume up to 12 µL with Nuclease-free Water.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Pipette 12 µL 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 µL of DNA sample/positive control in each respective well. If using less volume than 8 µL, supplement with Nuclease-free Water up to 8 µL. In total, the final volume of the reaction will be 20 µL.
    b. Add up to 8 µL of Nuclease-free Water for the negative control to achieve a final volume of 20 µL.

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.

Protocol 2 – Set up adding primers and probes as pre-prepared mixtures

For easy handling of multiple primers used in multiplex assays, we recommend preparing primer/probe mixes for each target by combining the different sets of primers and probes at required concentrations. For example, for a double gene targeting multiplex qPCR, we recommend preparing two 10x concentrated mixtures of primers and probes (or 20x concentrated mixes) for both targets (Table 1) and proceed as follows:

Table 1. Concentrated assay mixtures of primers and probes with suggested concentrations for a standard qPCR reaction.

MIX (FINAL)	PRIMER FORWARD	PRIMER REVERSE	PROBE
10x	4 µM	4 µM	2 µM
20x	8 µM	8 µM	4 µM

1. Thaw the master mix at room temperature or on ice. Mix the master mix thoroughly by flicking the tube and inverting it. Keep the master mix on ice. For automated workflows, the master mix remains stable on the benchtop for extended periods.
Note: *A precipitate in the bottom of the master mix tube may be observed, in particular after multiple freeze/thaw cycles. To ensure optimal performance, please make sure all mix components are thawed and resuspended/homogenized before use. In this case, do not spin down the master mix before pipetting.*

2. In a clean reaction setup area, prepare the qPCR reaction mixture according to the table below (please notice that the given volumes are based on a standard 20 µL final reaction mix and can be scale adjusted):
Note 1: *If setting up more than one reaction, prepare a reaction mixture volume 5 to 10% greater than the total required for the number of reactions to be performed. Include sufficient reactions for the No-Template and positive controls.*
Note 2: *We recommend performing replicates of all reactions.*

	1 REACTION VOLUME	FINAL CONCENTRATION
NZY Ultra Multiplex qPCR Probe Master Mix 2x (1)(2)	10 µL	1×
10x primer + probe mix – **target 1** (*see Table 1*)(5)	2 µL	Primers: 400 nM (3) Probe: 200 nM (3)
10x primer + probe mix – **target 2** (*see Table 1*)(5)	2 µL	Primers: 400 nM (3) Probe: 200 nM (3)
FINAL VOLUME =	up to 14 µL(4)	-

(1) If a slight precipitate is observed at the bottom of the master mix tube, please refer to the note above (step 1).

(2) The master mix does not contain ROX, but, if required, the 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 the instructions described in the section "Technical Notes" below.
(3) Refer to the section of "Technical Notes" below for more details about primers and probes final concentrations in the reaction.
(4) If using smaller volumes for the primers & probe, supplement the volume up to 14 µL with Nuclease-free Water.
(5) If it is more convenient, alternatively prepare 20x concentrated mixes, and then use 1 µL in the reaction mix.

3. Gently mix and centrifuge briefly to spin down the contents.
4. Pipette 14 µL of the Mix into each well, according to your experimental plate/strip/tube configuration.
5. Pipette template (samples and controls):
   a. Add up to 6 µL of DNA sample/positive control in each respective well. If using less volume than 6 µL, supplement with Nuclease-free Water up to 6 µL. In total, the final volume of the reaction will be 20 µL.
   b. Add up to 6 µL of Nuclease-free Water for the negative control to achieve a final volume of 20 µL.

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

6. Proceed according to Protocol 1 (steps 6 to 8).

Suggested thermal cycling conditions

NZY Ultra Multiplex qPCR Probe Master Mix was optimized for the amplification of DNA fragments up to 200 bp under different RT-qPCR 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 °C	2 min	Polymerase activation
40	95 °C 60 °C	3 sec 15 sec	Denaturation Annealing/Extension

Testing and Ct values

When comparing this qPCR master mix with 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 focus on the design and setup of dual-labeled probes for probe-based qPCR experiments. The efficiency, specificity, and yield of qPCR are critically influenced by primer and probe design, as well as by amplicon length. Primers should be designed with a melting temperature (Tm) of approximately 58–62°C, while the Tm of the probe should be 8–10°C higher to ensure efficient hybridization. The amplicon length should be kept as short as possible, ideally between 50–150 bp, to maximize amplification efficiency and consistent results.

For dual-labeled probes, it is crucial to use spectrally distinct fluorophores in multiplex assays to prevent crosstalk and signal interference. The primer and probe concentrations must also be optimized to balance amplification across targets. A final primer concentration of 400 nM is suitable for most applications; however, this can be titrated within a range of 150–600 nM. Forward and reverse primer concentrations must always be equimolar. For probes, a final concentration of 150–200 nM is generally optimal, ensuring it remains at least two-fold lower than the primer concentration to minimize competition. Probes can also be titrated within the range of 50–300 nM to achieve the best results.

To ensure reliable amplification, validate the specificity and efficiency of each primer/probe pair in singleplex reactions before combining them in multiplex assays. This step is essential to identify potential primer-dimer formation or competition effects between targets.

Guidelines for multiplexing: Multiplex assays allow the simultaneous detection of multiple targets in a single reaction and are widely applicable to both quantitative and qualitative qPCR experiments. For quantitative assays, multiplexing enables the simultaneous detection and comparison of multiple gene targets within the same sample, with normalization achieved using a reference gene, such as a stable housekeeping gene. In qualitative assays, multiplexing is ideal for detecting the presence or absence of specific targets, such as pathogens or genetic markers, in a single reaction. The NZY Ultra Multiplex qPCR Probe Master Mix 2x supports the amplification and detection of up to seven targets in a single reaction. To achieve success in multiplexing, careful optimization is required, including the design and concentration of primers and probes, as well as consideration of target abundance. It is critical to ensure balanced amplification efficiency across all targets, as this minimizes variation in Ct values between singleplex and multiplex reactions. Selecting fluorophores with non-overlapping emission spectra is also essential to avoid signal interference. Before setting up a multiplex assay, validate each primer/probe pair individually under singleplex conditions to confirm specificity and efficiency. Combining validated pairs in a multiplex format ensures the sensitivity and specificity observed in singleplex reactions are retained. The optimized formulation of NZY Ultra Multiplex qPCR Probe Master Mix 2x allows for consistent and reliable results, even in complex samples or for targets of varying abundance.

Template: The DNA template must be of high purity and free of qPCR inhibitors, such as EDTA or residual salts, to ensure optimal performance. Use high-quality DNA isolation methods for clean and intact templates. Store DNA samples at -20°C in the appropriate buffer (e.g., low EDTA (0.1 mM) TE buffer or nuclease-free water). For best results:

- Use 0,01 pg to 5 µg of total DNA per 20 µL reaction, depending on the source and complexity of the template.
- Avoid repeated freeze-thaw cycles to maintain DNA integrity.

For high-quality DNA isolation, we recommend using any of the NZYtech DNA Isolation Kit or other validated nucleic acid extraction methods.

$MgCl_2$: It is not necessary to supplement the reaction mixture with $MgCl_2$ as the NZY Ultra Multiplex qPCR Probe Master Mix already contains an optimized concentration of $MgCl_2$.

qPCR optimization: In some cases, such as when working with multiplex assays targeting more than five target genes, or with longer amplicons exceeding 150 bp, optimization may be necessary. The following adjustments can improve results:

- Extend the annealing/extension step up to 30 seconds to allow for more efficient amplification.
- Ensure primers and probes are fully validated for multiplex conditions before assay execution.

By adhering to these guidelines, the NZY Ultra Multiplex qPCR Probe Master Mix 2x ensures exceptional performance, sensitivity, and reproducibility in both simple and complex qPCR workflows.

ROX reference dye: NZY Ultra Multiplex 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 1 mL OF MASTER MIX 2x (*)
Applied Biosystems: 7300/7700/7900/7900HT/7900HT FAST/ StepOnePlus™	500-725 nM (20-29 µL) (**)
Applied Biosystems: 7500/7500FAST/QuantStudio™ 5 DX, 5, 6, 7, 12k Flex/ViiA7™	50-100 nM (2-4 µL) (**)
Bio-Rad®: CFX96™ Touch/CFX OPUS 96/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.*
***(**)** The volume was calculated considering a ROX reference dye stock at 25 µM.*

Data

NZY Ultra Multiplex qPCR Probe Master Mix 2x high multiplexing capacity is exemplified by its ability to simultaneously detect five DNA targets in a multiplex qPCR assay, demonstrating exceptional sensitivity, specificity, and amplification efficiency. The test highlights the mix's capacity to provide reliable and reproducible results even in complex reactions. The assay was designed to detect *Staphylococcus aureus* targets and an endogenous internal control. The resultant amplification curves, shown in Figure 1, illustrate the robust multiplex detection of these targets using distinct fluorescent channels.

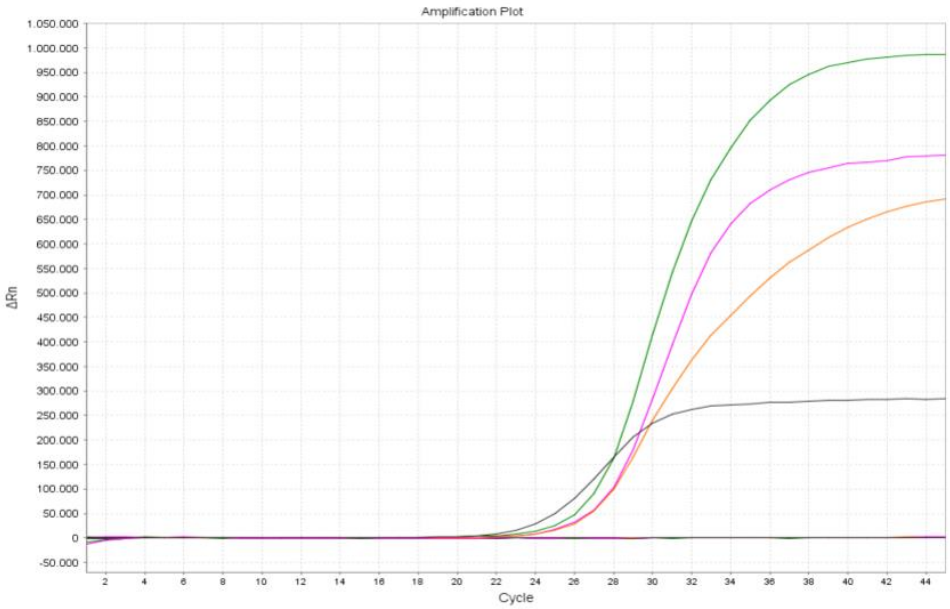

Figure 1. Simultaneous detection of five targets in a pentaplex assay using NZY Ultra Multiplex qPCR Probe Master Mix 2x. The assay was performed on a positive using MRSA Multiplex Real-time PCR Kit, IVD (MD04931). Green curve: detection of mecA/mecC targets through the FAM channel; Pink curve: detection of nuc target through the TexasRed channel; Orange curve: detection of SCCmec/orfX junction target through the VIC/HEX channel, and Grey curve: detection of human RNase P target through the Cy5 channel. This figure demonstrates the ability of the NZY Ultra Multiplex qPCR Probe Master Mix 2x to detect multiple targets simultaneously with high efficiency, enabling robust and precise results in multiplex qPCR assays.

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 AMPLIFICATION
• **Missing or degraded reagents**
Ensure all components are added, not expired, and stored properly. Store primers at -15°C to -30 °C in aliquots to avoid repeated freeze-thaw cycles. Protect the probe from direct sunlight.
• **Poor DNA template quality**
Assess DNA integrity and purity. Run a sample through agarose electrophoresis; use high-quality DNA isolation methods.
• **Incorrect thermal cycling conditions**
Verify the thermal cycling protocol matches the recommended settings. Verify the adequacy of the primers' melting temperature as outlined in the Technical Notes above mentioned.
• **Primer/probe design issues**
Redesign primers/probes, following the recommendations outlined in the Technical Notes; confirm specificity and efficiency.
LATE Ct VALUES
• **Low template concentration**
Reassess DNA concentration using reliable quantification methods. If necessary, optimize DNA extraction to increase yield or start with a higher DNA concentration.
• **Suboptimal primer/probe concentrations.**
Optimize primer/probe concentrations within the recommended ranges provided in the Technical Notes section. Store primers/probe appropriately to prevent degradation; use fresh, high-quality primers/probes.
• **Presence of PCR inhibitors**
Use purified DNA; assess inhibitor impact and dilute the template if needed. Remove inhibitors (e.g., SDS, EDTA, glycerol) from the DNA preparation through ethanol precipitation, followed by washing the pellet with 70% ethanol. Begin with DNA purified using a silica-based method and verify its purity using the A_{260}/A_{280} ratio.
HIGH BACKGROUND SIGNAL
• **Contamination of reagents, workspace or equipment**
Follow strict contamination control practices and use fresh reagents to avoid contamination from previously opened materials. Ensure proper storage of reagents to maintain their integrity. Use filtered tips to minimize cross-contamination and ensure that only molecular-grade water is used. Inspect PCR plates before use to ensure they are free from contamination. If possible, use PCR-grade plates. Run Empty Control Plates: Before running your samples, perform a No Template Control (NTC) or run an empty plate to check for background signals. If background fluorescence is detected in the NTC, it could indicate contamination in the instrument. If necessary, clean the qPCR machine, including the optical system, to eliminate any potential sources of contamination.
• **Non-specific amplification**
Optimize primer/probe design and concentrations; shorten amplicon length. Use appropriate tools to check for potential off-target binding sites. Fine-tune the concentrations of primers and probes within the recommended ranges.
FLUORESCENT SIGNAL INTERFERENCE
• **Spectral overlap between fluorophores**
Confirm fluorophores are spectrally distinct; adjust instrument settings.
• **Issues in the detection step or instrument calibration**
Ensure that fluorescence detection occurs during the extension step of the real-time PCR cycling program. Verify that the correct fluorescent channels are being used. Ensure that the qPCR instrument is calibrated properly.
VARIABLE OR INCONSISTENT RESULTS
• **Poor mixing of reaction components**

Mix the master mix and all reagents thoroughly without vortexing before use.
• **Inconsistent DNA preparation**
Standardize DNA extraction, quantification, and handling procedures.
• **Temperature fluctuations**
Confirm that the qPCR machine is working properly and that the block temperature is uniform.
• **Handling variability**
Minimize operator-dependent variability by following strict protocols for pipetting, mixing, and handling the reagents and templates. Confirm that the pipettes used are calibrated and working properly.

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

NZY Ultra Multiplex qPCR Probe Master Mix 2x is extensively tested for activity in multiplex qPCR assays using DNA templates to confirm amplification efficiency, specificity, and sensitivity. Performance is validated in heptaplex assays to ensure the simultaneous detection of up to seven targets without loss of sensitivity or signal interference. Each lot is tested to guarantee consistent Ct values across replicates and to ensure the detection of low-abundance targets.

This master mix is manufactured under stringent quality standards and complies with ISO 9001 and ISO 13485 certifications for research and diagnostic-grade reagents.