NZYTrace qPCR Green Master Mix 2x (with UDG)

Catalogue number	Presentation
MB51501	2 x 1 mL (200 rxns of 20 µL)
MB51502	5 x 1 mL (500 rxns of 20 µL)
MB51503	20 x 1 mL (2000 rxns of 20 µL)
MB51504	1 x 50 mL (5000 rxns of 20 µL)

FEATURES

- **Dual-color monitoring control: BLUE master mix** and **YELLOW Template Buffer** combine to generate a **GREEN reaction mix**, enabling immediate tracing of correct pipetting and plate setup.
- **Built-in carryover contamination control (UDG + dUTP):** The master mix incorporates dUTP and a thermolabile UDG to eliminate uracil-containing carryover amplicons from previous runs.
- **Fast qPCR results:** NZYTaq II DNA Polymerase and optimized buffer enable fast qPCR (~25 min) through instant antibody hot-start activation and rapid kinetics.
- **High specificity and sensitivity**: High specificity and sensitivity with <10 copies detected and a single melting peak across targets varying in size, GC content, and sequence complexity.
- **Compatible with standard green-dye detection channels:** Optimized for green dye detection on a wide range of real-time PCR instruments, supporting fast and flexible qPCR protocol design.

Description

NZYTrace qPCR Green Master Mix 2x (with UDG) is an optimized, highly efficient reaction mixture for real-time PCR using a DNA-intercalating green dye. It combines a high-performance hot-start-like Taq DNA polymerase with advanced buffer chemistry and PCR enhancers to deliver ultra-sensitive, highly reproducible amplification in fast qPCR protocols. The master mix features a distinct blue color and includes a powerful 40x concentrated NZY Yellow Template Buffer for superior results. When the yellow buffer is pre-mixed with the DNA (or cDNA) template and subsequently added to the blue master mix, it produces an immediate green reaction. This provides a visual confirmation of pipetting accuracy and plate layout, which is especially important for high-throughput or low-copy-number assays. NZYTrace qPCR Green Master Mix incorporates dUTP together with a thermolabile uracil-DNA glycosylase (UDG) to efficiently remove carryover contamination from uracil-containing amplicons generated in previous runs, helping to prevent false positives in demanding routine workflows. NZYTrace qPCR Green Master Mix (with UDG) is a ready-to-use 2X reaction mix containing a green DNA intercalating dye, a balanced blend of dNTPs including dUTP and dTTP, optimized stabilizers and PCR enhancers. The mix requires only the addition of primers, DNA template and nuclease-free water. The optional NZY Yellow Template Buffer 40x may be used for visual verification of correct pipetting without affecting qPCR performance. NZYTrace qPCR Green Master Mix 2x (with UDG) is ideal for gene expression studies, pathogen and biomarker detection, and the reliable amplification of low-copy or otherwise challenging DNA targets. Its dual-color tracking and built-in UDG carryover control make it especially suitable for high-throughput and routine screening workflows. Ideal for assay development and use across different real-time PCR instruments, while fully compatible with post-run melt curve analysis to confirm amplicon specificity.

Shipping & Storage Conditions

The product can be shipped at temperatures ranging from dry ice to blue ice. Upon arrival, all components should be stored at -85 °C to -15 °C in a constant-temperature freezer to ensure maximum shelf life. Minimize the number of freeze-thaw cycles by storing it in working aliquots. The green dye is light sensitive; as such, the master mix should be protected from light whenever possible. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	MB51501 (200 rxns)		MB51502 (500 rxns)		MB51503 (2000 rxns)		MB51504 (5000 rxns)	
	TUBES	VOLUME	TUBES	VOLUME	TUBES	VOLUME	BOTTLE/ TUBES	VOLUME
NZYTrace qPCR Green Master Mix 2x (with UDG)	2	1 mL	5	1 mL	20	1 mL	1	50 mL
NZY Yellow Template Buffer 40x	1	0.55 mL	1	0.55 mL	2	0.55 mL	5	0.55 mL

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

Specifications

Compatibility with real-time PCR instruments

NZYTrace qPCR Green Master Mix 2x (with UDG) is compatible with both fast and standard cycling modes. In addition, it can be used on instruments that do not require a passive reference signal or have ROX disabled, providing flexibility across different real-time PCR platforms. It has been designed for use with the following real-time PCR instruments:

Applied Biosystems: QuantStudio™ 3 or 5 Real-Time PCR System; QuantStudio™ 6 or QuantStudio™ 7 Pro Real-Time PCR System, 7500 Fast System and 7500 System.

Bio-Rad®: CFX96™; CFX384™; Opticon™; Opticon™ 2.

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

Roche: Lightcycler® 96, 480 & Nano.

Standard Protocol

Recommendations before starting

- Nucleic acid handling and carryover control: To minimize the risk of carryover contamination, manipulate nucleic acids using DNase-free plasticware and reagents in a dedicated clean area. Nucleases & Nucleic Acid Cleaner (Cat. No. MB48301) or DNA & RNA Cleaner (Cat. No. MB46201) can be used to remove nucleases and contaminant nucleic acid from work surfaces and materials. NZYTrace qPCR Green Master Mix 2x (with UDG) contains dUTP and a thermolabile UDG to reduce carryover from uracil-containing amplicons; however, these features complement but do not replace good laboratory practice.
- Workflow separation and handling instructions: Use physically separated areas for reaction setup, qPCR amplification and any post-amplification analysis. Tubes containing amplified products must not be opened in the qPCR setup area. Always use sterile, filtered tips and keep tubes and plates closed whenever possible to minimize aerosol formation and contamination.
- Controls: Always include a no-template control (NTC) to confirm the absence of contamination and a positive control to verify the performance of the assay and detection system. The positive control should display the expected amplification profile and fluorescence signal. When unwanted fluorescence is suspected, a No-Amplification Control (sample plus all reaction components except the polymerase) can be included; higher fluorescence in this control than in the NTC may indicate fluorescent contaminants in the sample or the thermal cycler.
- Template amount and Replicates: For a standard 20 µL reaction, use 10 ng-0.1 pg of cDNA or 100 ng-1 pg of genomic DNA per reaction. Depending on target abundance and sample complexity, template amounts may be further optimized empirically. It is strongly recommended to run technical replicates for each sample and control. Performing at least three, and preferably four, replicates per condition improves the reliability of quantification and facilitates the identification of outliers.

Procedure

The following protocol is a general guideline and a starting point for qPCR using NZYTrace qPCR Green Master Mix 2x (with UDG). Reaction conditions (*e.g.*, cycling times, annealing temperature and template input) may require optimization for individual assays. As a general recommendation, use 10 ng-0.1 pg of cDNA or 100 ng-1 pg of genomic DNA per 20 µL reaction. When working with small genomes, adjust the template input over an approximate range of 10^6 to 1 copy. At limiting dilution (single-copy level), reactions will contain zero, one, or multiple copies as described by Poisson statistics.

1. Thaw NZYTrace qPCR Green Master Mix 2x (with UDG) and the NZY Yellow Template Buffer 40x at room temperature or on ice. Mix thoroughly but gently by pipetting or inverting the tube. Collect liquid at the bottom of the tube by brief centrifugation. Ensure that the master mix is completely thawed and homogeneous before use.
2. Thaw DNA samples and primers on ice. Vortex lightly to mix. Centrifuge briefly to collect contents at the bottom of the tubes.
3. Optional use of NZY Yellow Template Buffer 40x for pipetting control of the DNA template. The use of the yellow dye is optional and does not affect qPCR performance. It provides visual confirmation of template addition by changing the master mix color from blue to green upon proper mixing. For adding NZY Yellow Template Buffer 40x to the template, proceed as follows:
    i. The NZY Yellow Template Buffer is supplied at 40x and must be added only to the DNA template.
    ii. The final concentration of the NZY Yellow Template Buffer in the PCR must be 1x, corresponding to 0.5 µL of dye in a 20 µL reaction.
    iii. It is recommended that the DNA template accounts for 10-40% of the final reaction volume (typically 2-8 µL in a 20 µL reaction).
    iv. Do not exceed 8.5 µL of "template + yellow dye" per 20 µL reaction.

v. Example (20 µL reaction):
   If using a 2 µL template, mix 2 µL DNA + 0.5 µL yellow dye, then add 2.5 µL of this mixture to the reaction.
   If using 8 µL template, mix 8 µL DNA + 0.5 µL yellow dye, then add 8.5 µL of this mixture to the reaction.
   Scale up or down, accordingly.

When the Yellow Template mixture is added to the blue master mix, the reaction turns green, providing a visual confirmation of correct template addition and plate layout.

4. In a clean reaction setup area, prepare the qPCR reaction mixture according to the table below (please note that the given volumes are based on a standard 20 µL final reaction mix and can be scaled and adjusted):
Note 1: *When preparing multiple reactions, prepare a reaction mixture with at least 10% extra volume to compensate for pipetting losses, and then aliquot into wells.*
Note 2: *Always include at least one no-template control (NTC) that contains all components except the template.*
Note 3: *To evaluate background fluorescence from samples, an optional No-Amplification Control (NAC) can be prepared with template (± yellow dye) and nuclease-free water instead of NZYTrace master mix.*
Note 4: *Add the template (or template + NZY Yellow Template Buffer mixture) last, ideally in a separate area, to reduce contamination risk.*
Note 5: *Perform at least three technical replicates for each condition.*

	VOLUME (1 REACTION)	**FINAL CONCENTRATION**
NZYTrace qPCR Green Master Mix 2x (with UDG) (1)	10 µL	1x
Forward primer, 10 µM	0.5 µL	250 nM (2)
Reverse primer, 10 µM	0.5 µL	250 nM (2)
Template ± yellow dye mixture (see Section 3, above) (3)	up to 8.5 µL	-
Nuclease-free water	to 20 µL	-
FINAL VOLUME =	20 µL	-

(1) NZYTrace qPCR Green Master Mix 2x (with UDG) already contains dNTPs (including dUTP), UDG, green intercalating dye, cofactors, stabilizers and enhancers.
(2) Refer to the section of “Technical Notes” below for more details about the primers' final concentrations in the reaction. A final primer concentration of 250 nM is a good starting point; concentrations between 150 – 400 nM may be tested for optimization. Primer specificity must be validated in reactions with an intercalating dye to ensure result accuracy and data integrity.
(3) When using the NZY Yellow Template Buffer, the combined volume of template plus dye must not exceed 8.5 µL in a 20 µL reaction. This corresponds to adding exactly 0.5 µL of NZY Yellow Template Buffer 40x per 20 µL reaction, to achieve a final 1× yellow dye concentration.

5. Gently mix and centrifuge briefly to spin down the contents.
6. Transfer the appropriate volume of each reaction to each well of an optical plate and seal with proper optical adhesive film or caps 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 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

NZYTrace qPCR Green Master Mix 2x (with UDG) has been optimized for amplification of DNA fragments of approximately 60-200 bp using fast real-time PCR protocols. Conditions may be adjusted slightly (within sensible limits) for specific assays or instruments. Select the green intercalating dye/SYBR channel for detection. Set ROX to “OFF”. Suggested cycling conditions (20 µL reaction):

CYCLES	**TEMP.**	**TIME**	**STAGE**
1	95 °C (*)	2 min (*)	Polymerase activation
40	95 °C	3 sec	Denaturation
	60-65 °C	15 sec (**)	Annealing/Extension (endpoint acquisition)

(*) Use 2 min for cDNA; but for complex genomic DNA targets, an activation step of up to 3–5 min may be used if necessary.
(**) For longer amplicons or less efficient assays, the annealing/extension step may be increased to 30 s.

Melting curve analysis: At the end of amplification, perform a melting curve analysis according to the real-time PCR instrument instructions. For intercalating dye-based qPCR, a single sharp melt peak indicates a specific product, whereas multiple peaks or shoulders suggest non-specific products or primer-dimers.

Testing and Ct values

When comparing NZYTrace qPCR Green Master Mix 2x (with UDG) to another supplier’s mix, we strongly recommend using a 10-fold template dilution series. The most reliable indicator of sensitivity is the lowest template concentration that still yields consistent, specific amplification within the expected Ct range. An earlier Ct value at high template input does not necessarily reflect superior sensitivity; it may simply indicate faster reaction kinetics or differences in chemistry and instrument settings. True performance should be evaluated based on the limit of

detection, the linearity of the standard curve, and the efficiency of amplification. Additionally, specific melt curves and the absence of non-specific products are important factors to consider. The NZYTrace qPCR Green Master Mix 2x (with UDG) comes with an optimized concentration of Mg^{2+}, so further adjustments are generally unnecessary and not recommended.

Technical Notes

Primers: The specificity, yield and efficiency of green-dye qPCR depend critically on primer design, primer concentration and amplicon length. When designing and optimizing primers, consider the following:

- Primers should have a melting temperature (T_m) of approximately 58-62 °C;
- The fragment length should be between 70-200 bp; fragments above 250-300 bp generally reduce efficiency and sensitivity in green-dye assays;
- Start with a final primer concentration of 250 nM for both primers. If needed, titrate each primer in the 150 - 400 nM range while keeping forward and reverse primers equimolar;
- When amplifying from cDNA, design intron-spanning or exon–exon junction primers whenever possible to minimise amplification from contaminating genomic DNA.
- Avoid long homopolymer runs, pronounced secondary structures and predicted primer–dimers (especially 3′ complementarity).

Validated primer design software and an *in-silico* specificity check (e.g., against the relevant genome) are strongly recommended before experimental use.

Template: The DNA template must be purified and devoid of contamination by PCR inhibitors (*e.g.* EDTA). The DNA template must be purified and concentrated according to conventional nucleic acid clean-up procedures (NZYGelpure, MB011). 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:** Use up to 1 µg of genomic DNA in a single PCR. We recommend using the NZY Tissue, Blood & Cells gDNA Isolation Kit (Cat. No. MB517) 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; we suggest using up to 100 ng cDNA per reaction. However, this amount may be adjusted to a more appropriate concentration. We suggest using the NZY First-Strand cDNA Synthesis Kit (Cat. No. MB125), or Supreme NZY RT SuperMix 5x (MB499), for reverse transcription of purified RNA. To obtain a high yield of highly purified RNA, we suggest using the NZY Total RNA Isolation Kit (Cat. No. MB134).

As mentioned in the above sections, higher template amounts (up to ~100 ng cDNA or ~1 µg gDNA) may be tolerated for some targets but may reduce efficiency and specificity in green-dye assays.

$MgCl_2$: NZYTrace qPCR Green Master Mix 2x (with UDG) contains $MgCl_2$ at an optimized final concentration of ~3.5 mM in the 1× reaction. This level has been finely tuned together with buffer composition, enhancers and the hot-start–like polymerase to support high efficiency and robust amplification across a broad range of targets.

UDG/dUTP carryover prevention: NZYTrace qPCR Green Master Mix incorporates dUTP and a thermolabile UDG to reduce carryover from uracil-containing amplicons generated in previous runs. UDG remains active at lower temperatures and removes uracil from contaminating U-containing DNA before amplification begins, thereby preventing carryover contamination. The amount of UDG included in the mix is sufficient to remove carryover contamination from uracil-containing amplicons. The subsequent high-temperature denaturation inactivates UDG, allowing normal amplification of newly synthesized U-containing amplicons in the current reaction. For the carryover system to be fully effective, qPCR workflows in the same environment should consistently use dUTP-containing amplification mixes. Good laboratory practice (physical separation of pre- and post-PCR areas, use of filtered tips, closed-tube systems) remains essential and is not replaced by the UDG/dUTP system.

Green intercalating dye: NZYTrace qPCR Green Master Mix 2x (with UDG) contains a non-sequence-specific double-stranded DNA–binding dye. Upon binding to dsDNA, it emits a green fluorescence signal (~520 nm), while the formulation has been optimized to minimize inhibition of PCR. Because the dye binds all dsDNA in the reaction (specific amplicons, non-specific products and primer–dimers):

- Always perform melting curve analysis at the end of the run. A single sharp peak indicates a specific product; multiple peaks or shoulders usually indicate non-specific amplification.
- Inspect amplification curves together with melt curves when validating new assays.
- For publication- or validation-quality assays, confirm that efficiency is typically 90–110% (slope ~–3.1 to –3.6) with $R^2 \geq 0.99$ from a serial dilution, and that a single melt peak is obtained.

Select the appropriate SYBR/green channel on the instrument for data acquisition.

Data

The performance of NZYTrace qPCR Green Master Mix 2x (with UDG) is clearly shown in the figures below, demonstrating high sensitivity, efficient amplification, and effective prevention of carry-over contamination.

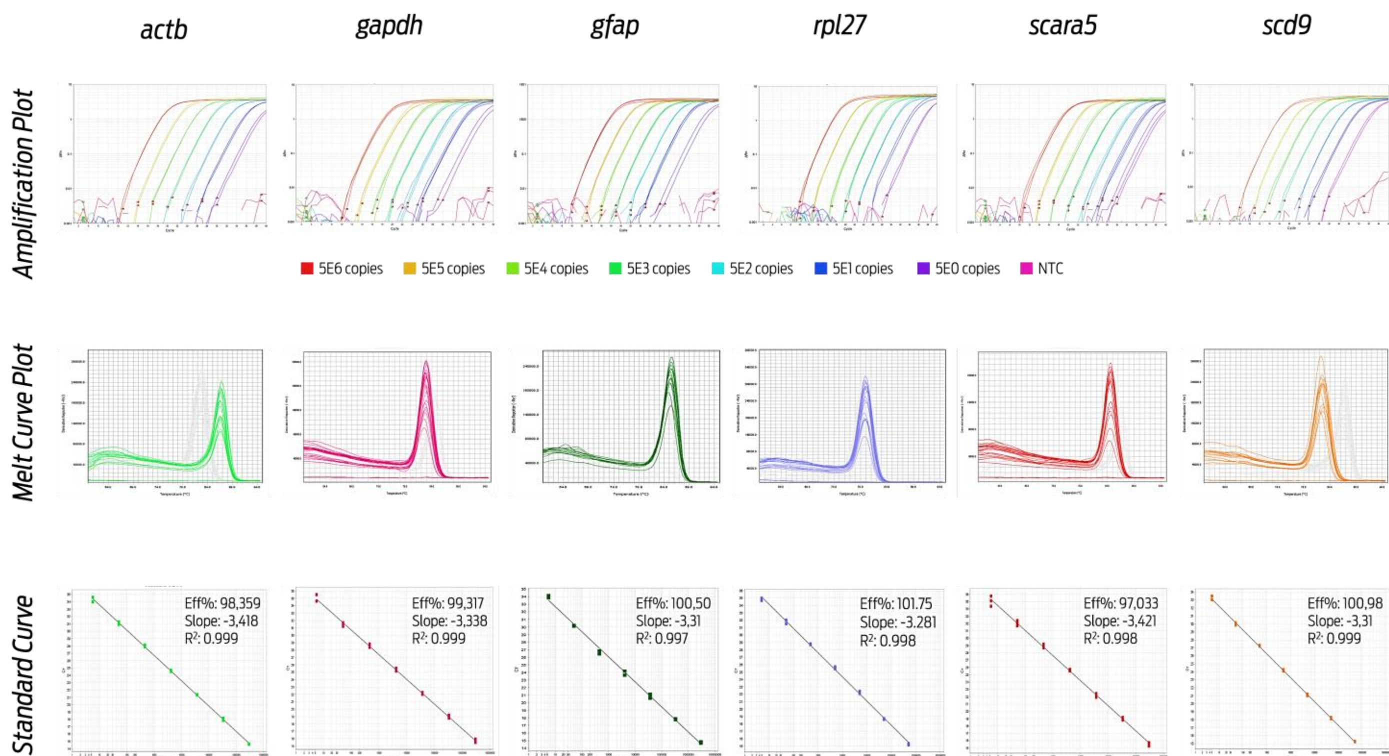

Figure 1 - Performance of NZYTrace qPCR green Master Mix 2x (with UDG). Amplification Plot: The NZYTrace qPCR Green Master Mix 2× (with UDG) provides highly efficient and linear amplification across a wide dynamic range, from 5 × 10^6 to 5 template copies. Reactions targeting six genes (*actb*, *gapdh*, *gfap*, *rpl*27, *scd*9 and *scara*5) were performed using a 10-fold serial dilution series on the QuantStudio™ 5 system (60 °C annealing). The mix consistently demonstrated PCR efficiencies ≈ of 100% and excellent reproducibility across replicates. **Melt Curve Plot:** Melt curve analysis confirmed high specificity, with a single melting peak in 100% of reactions (6 of 24 primer sets tested) and no primer–dimer or non-specific amplification products. Verifying primer specificity in SYBR Green reactions is critical for data quality. NZYTrace qPCR Green Master Mix 2x (with UDG) provides high specificity, minimizing primer redesign and optimization efforts, to ensure reliable results. **Standard Curve:** Standard curves generated from Ct values versus template copy numbers showed high linearity (R^2 >0.998) and a broad quantification range from 5 x10^6 to 5 copies. All primer sets (250 nM each) exhibited consistent performance and robust quantification across the dilution series. The overlay text shows the standard curve data.

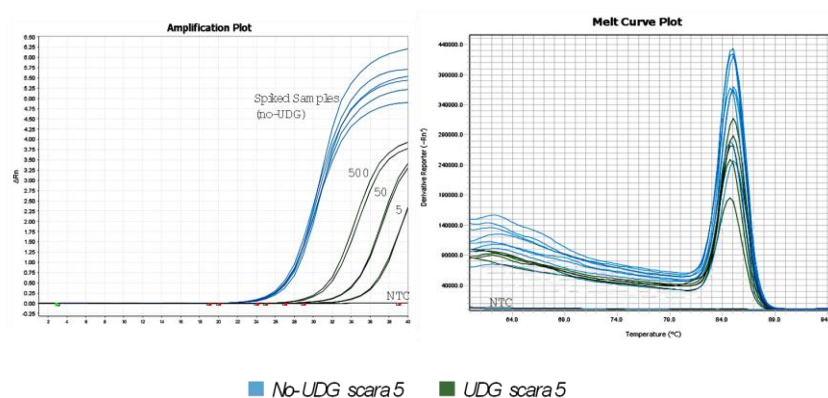

Figure 2. Evaluation of UDG-Mediated Carryover Prevention in qPCR - The effectiveness of UDG in preventing carryover contamination was assessed using three *scara5* template samples (500, 50, and 5 copies/reaction), each spiked with 4,000 copies of dUTP-containing template to simulate carryover contamination. Without UDG (blue curves), all samples showed identical amplification, dominated by the spike and masking the true differences in template copy number. In contrast, reactions performed with NZYTrace qPCR Green Master Mix 2x (with UDG) (green curves) efficiently degraded the spike template, allowing each sample to amplify according to the actual template copy number. NTC indicates No-Template Control. These results demonstrate that UDG efficiently removes contaminating DNA, enabling accurate detection of true samples and preventing false positives in low-copy qPCR assays.

These data demonstrate that NZYTrace qPCR Green Master Mix 2x (with UDG) combines high sensitivity and efficiency while providing visual workflow control and effective prevention of carryover contamination, thereby supporting reliable real-time PCR performance across a range of assays.

Quality control assays

Genomic DNA contamination

The product must comply with internal specifications for DNA contamination, determined by real-time PCR analysis.

Nuclease assays

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA is incubated with the master mix for 14-16 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

NZYTrace qPCR Green Master Mix 2x (with UDG) is extensively tested for activity, processivity, efficiency, sensitivity and heat activation.

Troubleshooting

NO AMPLIFICATION OR VERY LATE CT IN ALL SAMPLES
• **Incorrect reaction setup (missing primers, template or master mix)**
Verify the pipetting scheme and repeat the assay.
• **Wrong cycling conditions or detection channel**
Confirm that the SYBR/green channel is selected and that the recommended protocol is used.
• **Poor template quality or presence of inhibitors (e.g., salts, EDTA, ethanol)**
Repurify the template and repeat
• **Target copy number below the limit of detection**
Increase template input within the recommended range or pre-enrich the target.
AMPLIFICATION IN THE NO-TEMPLATE CONTROL (NTC)
• **Carryover contamination with amplicons or plasmid DNA**
Prepare new reactions in a clean area using fresh aliquots and filtered tips.
• **Non-specific primer–dimer amplification**
Re-evaluate primer design and, if necessary, increase annealing temperature or reduce primer concentration
Ensure that all qPCR runs in the same environment use dUTP-containing mixes so that the UDG/dUTP system can efficiently prevent carryover.
MULTIPLE PEAKS OR SHOULDERS IN THE MELT CURVE
• **Non-specific amplification**
Increase annealing temperature, shorten extension time or redesign primers to improve specificity
• **Excessive template or primer concentration**
Reduce template input or lower primer concentration within the recommended range.
Check primer design for secondary structures and unintended binding sites, and redesign if needed.
PRIMER–DIMER PEAK ONLY (NO SPECIFIC PRODUCT)
• **Primers prone to dimerisation**
Redesign primers to avoid strong 3’ complementarity.
• **Annealing temperature too low**
Increase the annealing temperature by 2–3 °C and reassess.
Reduce primer concentration towards the lower end of the recommended range.
HIGH VARIABILITY BETWEEN TECHNICAL REPLICATES
• **Inaccurate pipetting**
Use calibrated pipettes and low retention, filtered tips, and prepare a common master mix for all replicates.
• **Inconsistent template addition**
Use the yellow dye to visually confirm template addition and plate layout.
• **Edge effects or uneven sealing of the plate**
Ensure proper sealing and brief centrifugation to remove bubbles.

This master mix is manufactured under stringent quality standards and complies with ISO 9001.

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