

# **qNTQX® Universal Superfast qPCR Mix (One Tube)**

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**Cat. no. LM602016**

**Address:** SHT technology GmbH, Niederhöchstädter Str. 64, 61476 Kronberg im  
Taunus, Germany

**Tel.:** +49 176 34989414

**Website:** <https://sht-medino.de/>

# qNTQX® Universal Superfast qPCR Mix (One Tube)

Cat. no. LM602016

## Product Information

| Cat. No. | Product name                                   | Pack size |
|----------|------------------------------------------------|-----------|
| LM602016 | qNTQX® Universal Superfast qPCR Mix (One Tube) | 100 T     |
|          |                                                | 1,000 T   |
|          |                                                | 10,000 T  |

One test (T) corresponds to a 25 µL reaction. Please state the required pack size when ordering.

## Kit Contents

| Contents              | 100 T   | 1,000 T | 10,000 T |
|-----------------------|---------|---------|----------|
| 2× Superfast qPCR Mix | 1.25 mL | 12.5 mL | 125 mL   |

## Storage

All components of the kit can be stored for up to 12 months at –25 °C to –15 °C.

## Introduction

qNTQX® Universal Superfast qPCR Mix (One Tube) is a fluorescence quantitative PCR (qPCR) premix built on a new-generation antibody-mediated hot-start Taq DNA polymerase. The premix offers excellent reagent universality and supports both fast cycling protocols and a fully pre-mixed, single-tube (one-tube) reagent format, simplifying reaction set-up.

The reagent has been validated with primer/probe sets against a broad range of bacterial, fungal and viral targets, delivering excellent amplification performance across assay types. Primers and probes may be pre-mixed with the reagent in advance: the fully pre-mixed formulation retains unchanged amplification performance after accelerated ageing for 7 days at 37 °C. A dUTP/UDG carry-over prevention system is included, in which dUTP replaces dTTP, effectively preventing aerosol contamination and safeguarding the accuracy of results.

## Features

1. Excellent reagent universality — validated with primer/probe sets against a wide range of bacterial, fungal and viral targets, all showing excellent amplification performance.
2. Full pre-mix stability — primers and probes may be pre-mixed with the reagent in advance; amplification performance is unchanged after accelerated ageing for 7 days at 37 °C.
3. Fast-protocol compatible — supports rapid cycling programmes with a detection time of ≤ 20 min.
4. One-tube, fully pre-mixed format — a single 2× premix tube simplifies reaction set-up and reduces pipetting steps.
5. Carry-over contamination prevention — the dUTP/UDG system replaces dTTP with dUTP, effectively preventing aerosol contamination and safeguarding result accuracy.
6. Broad instrument compatibility — validated on Bio-Rad CFX96, Applied Biosystems QuantStudio 5 (Q5) and 7500, SLAN 48S and many further qPCR platforms.

**Notes** Please be sure to read this precaution before using the kit.

1. This product is for research use only. Not for use in diagnostic procedures.
2. For your safety and health, wear a laboratory coat and disposable gloves when handling this product.
3. Mix thoroughly before use; avoid vigorous vortexing, which can generate excess bubbles.
4. Primer concentration: Primer Mix contains multiple primer pairs; the final concentration of each primer is typically adjusted between 0.1 and 1.0  $\mu\text{M}$  as appropriate.
5. Probe concentration: Probe Mix contains multiple probes with different fluorescent signals; the concentration of each probe can be adjusted between 50 and 300 nM as appropriate.
6. Template dilution: qPCR is extremely sensitive — dilute the template so that Ct values fall within the optimal range of 20–35.
7. Reaction volume: a 25  $\mu\text{L}$  or 50  $\mu\text{L}$  reaction volume is recommended to ensure effective and reproducible target amplification.
8. Reagent set-up: prepare reactions in a clean bench using nuclease-free tips and reaction tubes; filter tips are recommended to avoid cross- and aerosol contamination.

## Protocol

### A. Reaction Set-up (25 $\mu\text{L}$ system)

| Component                      | Volume ( $\mu\text{L}$ ) | Final concentration     |
|--------------------------------|--------------------------|-------------------------|
| 2 $\times$ Superfast qPCR Mix  | 12.5                     | 1 $\times$              |
| Primer mix (10 $\mu\text{M}$ ) | X                        | 0.1–0.5 $\mu\text{M}$   |
| Probe mix (10 $\mu\text{M}$ )  | X                        | 0.05–0.25 $\mu\text{M}$ |
| Template                       | 1–10                     | —                       |
| ddH <sub>2</sub> O             | up to 25                 | —                       |

Mix thoroughly before use; avoid vigorous vortexing, which can generate excess bubbles. Primers and probes may be pre-mixed with the reagent in advance.

### B. Compatible Instruments

**Applied Biosystems:** 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOnePlus™, 7500, 7500 Fast, ViiA™ 7, QuantStudio™ 3 and 5, QuantStudio™ 6, 7 and 12K Flex.

**Bio-Rad:** CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4.

**Eppendorf:** Mastercycler ep realplex, realplex 2s.

**Qiagen:** Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000.

**Roche Applied Science:** LightCycler 480, LightCycler 2.0, LightCycler 96.

**Stratagene:** MX3000P™, MX3005P™, MX4000P™.

**Thermo Scientific:** PikoReal Cyclor.

**Others:** Cepheid SmartCycler; Illumina Eco qPCR; SLAN 96S, SLAN 96P, SLAN 48S.

### C. Recommended Cycling Programmes

#### 1. Standard programme

| Step                    | Temperature | Time  | Cycles |
|-------------------------|-------------|-------|--------|
| Carry-over digestion*   | 37 °C       | 5 min | 1      |
| Initial denaturation    | 95 °C       | 5 min | 1      |
| Denaturation            | 95 °C       | 15 s  | 45     |
| Annealing / extension** | 60 °C       | 30 s  |        |

## 2. Fast programme (e.g. SLAN 48S)

| Step                    | Temperature | Time | Cycles |
|-------------------------|-------------|------|--------|
| Initial denaturation    | 95 °C       | 15 s | 1      |
| Denaturation            | 95 °C       | 2 s  | 45     |
| Annealing / extension** | 60 °C       | 8 s  |        |

\* This step is optional — digestion of uracil-containing template is already completed during reaction set-up, so no additional run time is required.

\*\* The annealing / extension temperature may be adjusted as required by the assay.

## Application Cases

### 1. Fast Amplification with Superior Performance

|                           |                                                                                                                                                     |                        |                                                                          |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|--------------------------------------------------------------------------|
| <b>Instrument</b>         | SLAN                                                                                                                                                | Reaction volume        | 25 µL total, including 5 µL template                                     |
| <b>Template</b>           | Bacterial plasmid templates (Tube B, Tube P)                                                                                                        | Template concentration | Diluted to near the limit of detection for the Ct 30–35 sensitivity test |
| <b>Cycling programmes</b> | 96S / 48S standard: 95 °C 5 min; 40 cycles (95 °C 15 s; 60 °C 30 s) — ~50 min.<br>48S fast: 95 °C 15 s; 40 cycles (95 °C 2 s; 60 °C 8 s) — ~20 min. |                        |                                                                          |

**Table 1. Sensitivity evaluation of Cat. No. 16924 across cycling programmes**

| Programme                     | Tube B       |              |              | Tube P        |                |              |                |
|-------------------------------|--------------|--------------|--------------|---------------|----------------|--------------|----------------|
|                               | FAM          | VIC          | ROX          | FAM           | VIC            | CY5          | ROX            |
| 96S standard — Ct (mean)      | 36.16        | 36.13        | 33.23        | 36.61         | 38.81          | NA           | 36.03          |
| 96S standard — Detection rate | 100% (16/16) | 100% (16/16) | 100% (16/16) | 75% (12/16)   | 81.25% (13/16) | 0            | 93.75% (15/16) |
| 48S fast — Ct (mean)          | 36.54        | 36.31        | 33.54        | 36.46         | 39.14          | 36.87        | 36.31          |
| 48S fast — Detection rate     | 100% (16/16) | 100% (16/16) | 100% (16/16) | 87.5% (12/16) | 68.75% (11/16) | 12.5% (2/16) | 81.25% (13/16) |
| 48S standard — Ct (mean)      | 36.3         | 36.34        | 33.77        | 36.54         | 38.73          | 39.41        | 35.58          |
| 48S standard — Detection rate | 100% (16/16) | 100% (16/16) | 100% (16/16) | 87.5% (14/16) | 56.25% (9/16)  | 6.25% (1/16) | 87.5% (14/16)  |

Ct values and detection rates are based on n = 16 replicates per fluorescence channel.

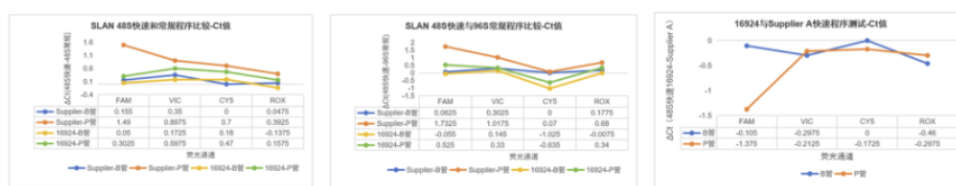


Figure 1.  $\Delta C_t$  comparison between SLAN 48S fast and standard programmes, between 48S fast and 96S standard programmes, and between Cat. No. LM602016 and a competing supplier's reagent under the fast programme.

## 2. Strong Full-Premix Stability

|                          |                                                                                                                                                                         |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Instrument</b>        | ABI 7500, SLAN 96S                                                                                                                                                      |
| <b>Reaction volume</b>   | 20 $\mu$ L total, including 5 $\mu$ L template                                                                                                                          |
| <b>Template</b>          | Customer-provided plasmids (Group 1: 4-plex; Group 2: 3-plex); customer-provided nucleic acid from a real African swine fever virus (ASFV) sample (Group 3: singleplex) |
| <b>Control</b>           | Reagent stored at $-20^{\circ}\text{C}$ , freshly prepared                                                                                                              |
| <b>Test group</b>        | Cat. No. LM602016 pre-mixed with the two primer/probe sets and stored at $37^{\circ}\text{C}$ for 7 days                                                                |
| <b>Cycling programme</b> | $95^{\circ}\text{C}$ 90 s; 45 cycles ( $95^{\circ}\text{C}$ 5 s; $55^{\circ}\text{C}$ 30 s)                                                                             |

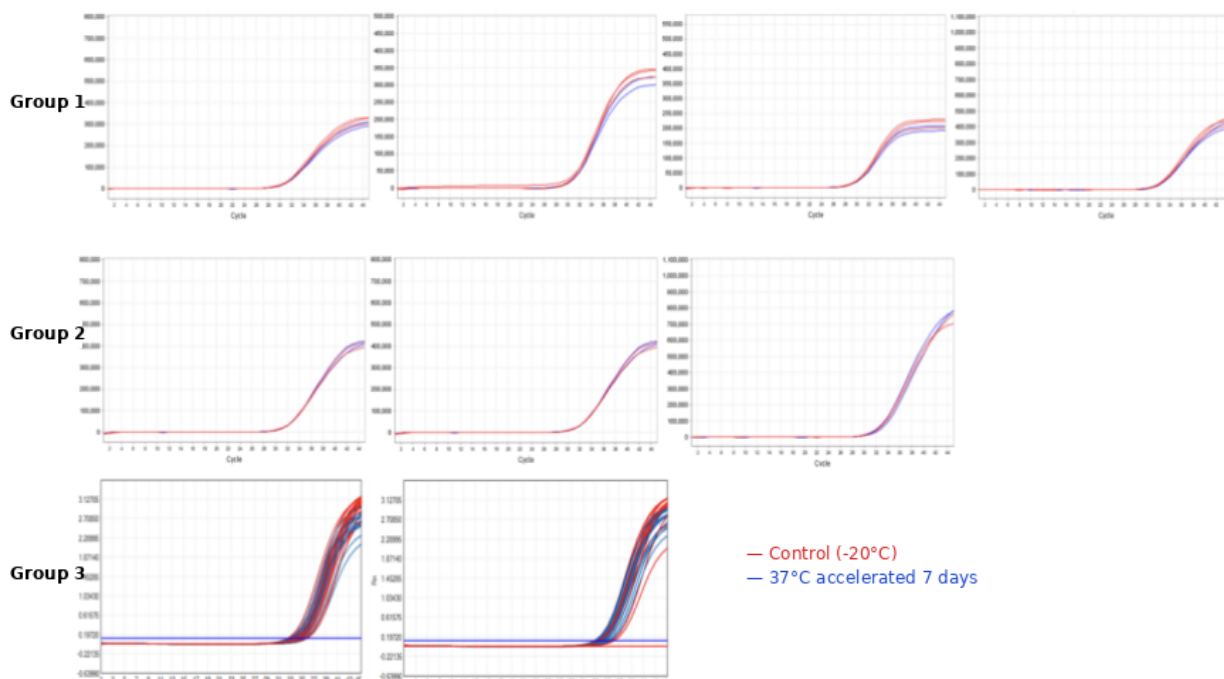


Figure 2. Full-premix stability across different targets (Group 1/2/3). Amplification curves of the  $37^{\circ}\text{C}$  / 7-day accelerated-ageing test group (blue) overlap closely with the  $-20^{\circ}\text{C}$  freshly-prepared control (red) across all three template groups and fluorescence channels, indicating no measurable loss of performance after pre-mixing and accelerated storage.

### 3. Broad Compatibility with Template Dilution Buffers

|                          |                                                 |                        |                                      |
|--------------------------|-------------------------------------------------|------------------------|--------------------------------------|
| <b>Instrument</b>        | SLAN 96S                                        | Reaction volume        | 25 µL total, including 5 µL template |
| <b>Template</b>          | Bacterial plasmid template                      | Template concentration | Ct 32–38                             |
| <b>Cycling programme</b> | 95 °C 5 min; 45 cycles (95 °C 15 s; 60 °C 30 s) |                        |                                      |

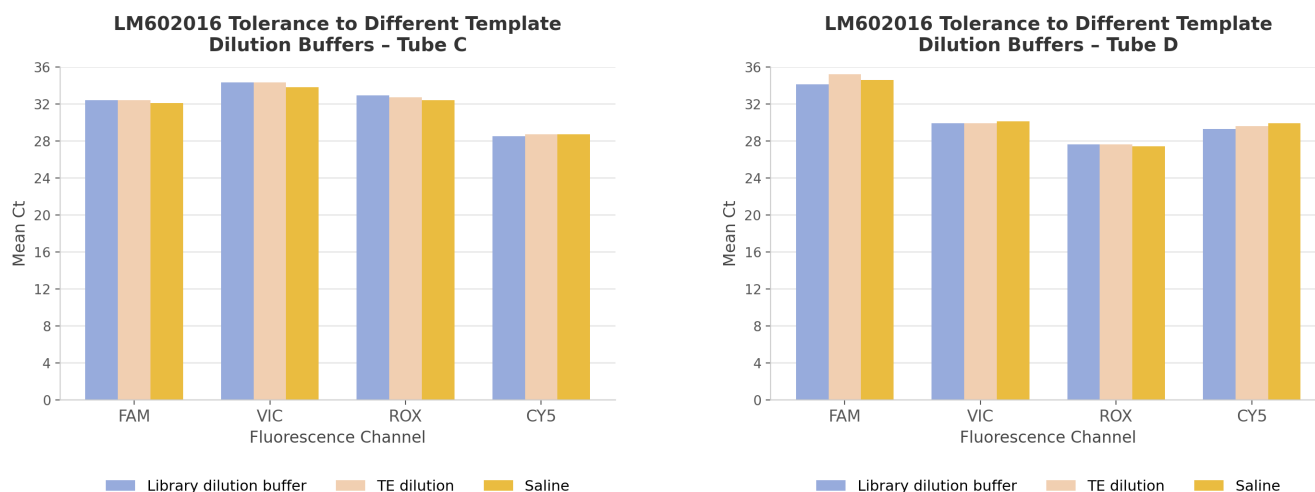


Figure 3. Mean Ct values for a 4-plex plasmid template diluted in library dilution buffer, TE buffer, or physiological saline, detected in two independent reaction sets (Tube C and Tube D) across the FAM/VIC/ROX/CY5 channels. Ct values are consistent across all three diluents, indicating that the reagent's performance is not affected by the choice of template diluent.

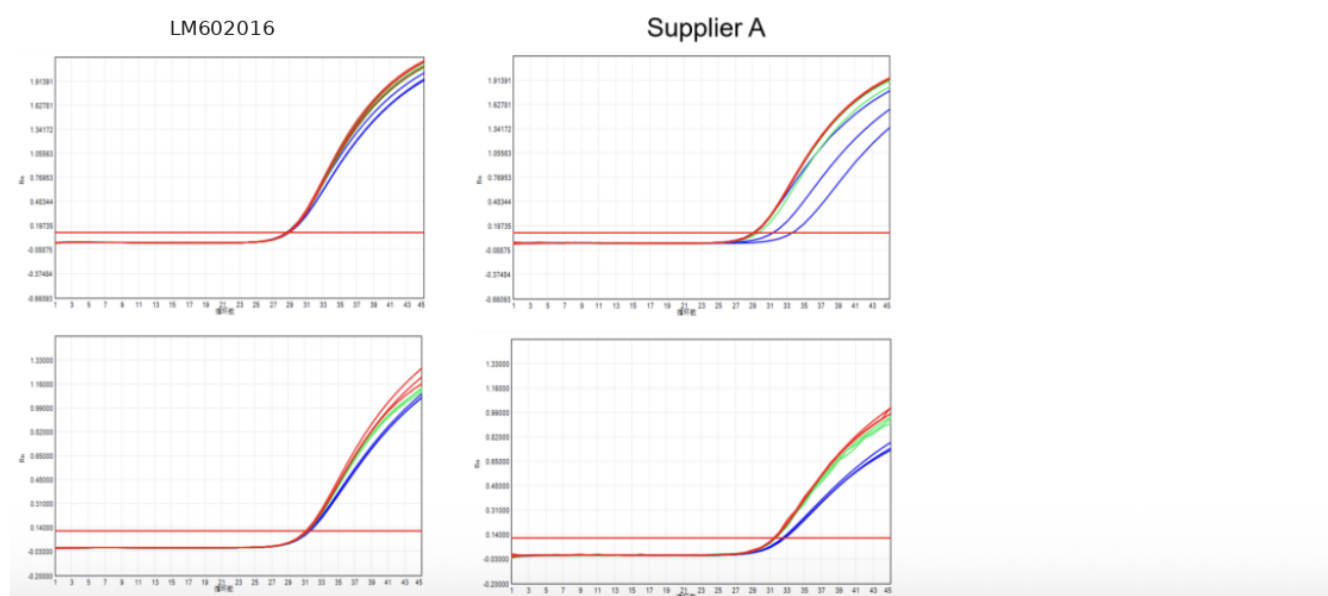


Figure 4. Amplification curves for Cat. No. LM602016 versus a competing supplier's reagent, using the same diluted 4-plex plasmid template (Tube C and Tube D, Cy5 channel). Cat. No. LM602016 produces steeper, more tightly clustered amplification curves across dilution conditions, indicating stronger tolerance to template dilution buffer composition.