Steinbrenner Laborsysteme GmbH

primaQUANT PROBE

# 2x Blue qPCR Probe Master Mix - no ROX

| Article | Content |
|---|---|
| SL-9802B-smp | 1 ml, 100 rxn × 20 µl |
| SL-9802B-5ML | 5 x 1 ml, 500 rxn × 20 µl |
| SL-9802B-10ML | 10 x 1 ml, 1000 rxn × 20 µl |
| SL-9802B-20ML | 20 x 1 ml, 2000 rxn × 20 µl |

**Long-Term Storage**
at -20°C in the dark

**Short-Term Storage**
at 4°C in the dark

## DESCRIPTION

Our **primaQUANT PROBE Blue 2x qPCR Master Mix** is an optimized ready-to use mixture for probe-based assays such as Taqman®, Beacons and MGBs. It contains a modified HotStart DNA Polymerase, as well as dNTPS and $MgCl_2$. The sophisticated buffer system provides fast kinetics and target amplification even for difficult templates.

The **primaQUANT 2x Master Mix** contains all components - you just need to add primers and template DNA/cDNA. The **blue color does not interfere** with the qPCR reaction but helps you to track wells already filled during pipetting steps.

The Master Mix can be used not only for expression analysis but also for genotyping, copynumber analysis and all sorts of probe-based quantitative PCR.

## DID YOU KNOW?

Some qPCR cyclers require ROX as reference dye. Please choose between **primaQUANT PROBE** with low or high concentrations of ROX.



VERSION **1.0 – 06 / 2022**

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# Recommended Reaction Mixture per Well

## BEFORE YOU START

- After thawing, please **invert the Master Mix tube 6-8 times**.
- **Do not vortex** the Master Mix to avoid damaging the enzyme.

| Component | Stock Concentration | 20 µl Reaction | 10 µl Reaction | Final Concentration |
|---|---|---|---|---|
| 2x primaQUANT Master Mix | 2x | 10 µl | 5 µl | 1x |
| Forward Primer | 4 µM | 1 µl | 0.5 µl | 200 nM (100 - 400 nM recommended) |
| Reverse Primer | 4 µM | 1 µl | 0.5 µl | 200 nM (100 - 400 nM recommended) |
| Probe | 8 µM | 1 µl | 0.5 µl | 400 nM (200 - 600 nM recommended) |
| Template DNA | - | variable | variable | 0.1 - 10 ng per reaction |
| Sterile Water | – | adjust to 20 µl | adjust to 10 µl | – |

## NOTE

For maximum efficiency and specificity, adjustments of annealing temperature as well as extension time, primer/probe concentration and template concentration may be needed.

## CALCULATOR TOOL

Please feel free to download our Excel sheet calculator to calculate the necessary volumes: **calculator.steinbrenner-laborsysteme.de**.

qPCR KnowledgeCenter

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# Standard Protocol

**NOTE**

- For the majority of qPCR assays, standard cycling conditions can be applied for the majority of qPCR assays out-of-the box.
- However, cycling conditions strongly depend on the primer, probe, amplicon and input material and thus some of these factors might need adjustments.

## 3-STEP PROTOCOL

| Step | Time | Temperature | |
|---|---|---|---|
| Initial Denaturation | 1 - 3 minutes | 92°C - 95°C | |
| Denaturation | 5 - 10 seconds | 92°C - 95°C | 25 - 40 cycles |
| Annealing | 1 - 5 seconds | 60°C depending on primer | |
| Extension | 10 - 20 seconds | 72°C | |

## 2-STEP PROTOCOL

| Step | Time | Temperature | |
|---|---|---|---|
| Initial Denaturation | 1 - 3 minutes | 92°C - 95°C | |
| Denaturation | 5 - 10 seconds | 92°C - 95°C | 25 - 40 cycles |
| Annealing / Extension combined | 10 - 20 seconds | 60°C depending on primer | |

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# Ultra-fast Protocol

**NOTE**

- Ultra-fast cycling conditions highly depend on the ramping rate of your qPCR cycler, primer, probe, amplicon and input material and thus might need adjustments.
- Ultra-fast cycling conditions can be applied for the majority of qPCR assays out-of-the box, provided that your primer/probe sets do not show unspecific binding.

## 3-STEP PROTOCOL

| Step | Time | Temperature | |
|---|---|---|---|
| Initial Denaturation | 1 minute | 92°C - 95°C | |
| Denaturation | 1 - 5 seconds | 92°C - 95°C | 25 - 40 cycles |
| Annealing | 1 - 5 seconds | 60°C depending on primer | |
| Extension | 1 second | 72°C | |

## 2-STEP PROTOCOL

| Step | Time | Temperature | |
|---|---|---|---|
| Initial Denaturation | 1 minute | 92°C - 95°C | |
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# Applications

Probe-based quantitative PCR

- TaqMan® Probes
- Any kind of Dual-Labeled Hydrolysis Probe
- Molecular Beacons
- Scorpion Probes

DNA Genotyping

DNA SNP Analysis

RNA and miRNA Expression

Multiplexing (up to 4 colors)

Transcript Variant Analysis

## QUALITY CONTROL PROCEDURE

Our **primaQUANT PROBE 2x qPCR Master Mix** undergoes stringent quality controls. Each lot is tested in a probe-based qPCR with cDNA and lambda DNA input.

Enzyme purity and homogenity of > 98 % is validated using a Bioanalyzer SDS protein electrophoresis.

All **primaQuant** Master Mixes are free of detectable endonuclease- & exo-nuclease activity:

- Incubation of 1 µg of plasmid DNA with 5 U for 4h at 37°C and 72°C
- Incubation of 1 µg of a DNA size standard with 5 U for 4h at 37°C and 72°C

For more information, please visit our website: **www.steinbrenner.de**

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# Further Products

Products that may also interest you

## REVERSE TRANSCRIPTION KIT

primaREVERSE RT-KIT

For the efficient cDNA synthesis out of total RNA extractions try the primaREVERSE RT-Kit with article number SL-9540