Article	Reactions
SL-9802-5X10ML	8x 1.25 mL, 2000 rxn á 25µL

Storage Conditions
Long-Term Storage at -20 °C in the dark
Short-Term Storage at 4 °C in the dark

primaQUANT PROBE

5x custom qPCR Probe Master Mix - no ROX

DESCRIPTION

Our **primaQUANT *PROBE* 5x custom 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 $MgCl_2$ - combined in an optimized buffer system that provides fast kinetics and target amplification even for difficult templates.

The **primaQUANT 5x Master Mix** contains all components - you just need to add primers and template DNA/cDNA.

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

DID YOU KNOW?

- **primaQUANT *PROBE*** 5x is compatible with BD MAX™ systems.
- **primaQUANT *PROBE*** 5x is especially designed for multiplexing applications.

STANDARD PROTOCOL

BEFORE YOU START

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

NOTE

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

3-Step Protocol

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

2-Step Protocol

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

ULTRA-FAST PROTOCOL

BEFORE YOU START

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

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	60 seconds	92 °C - 95 °C
25- 40 cycles		
Denaturation	1 seconds	92 °C - 95 °C
Annealing	1 seconds	60 °C - **depending on primer**
Extension	**1 second**	72 °C

2-Step Protocol

Step	Time	Temperature
Initial Denaturation	60 seconds	92 °C - 95 °C
25- 40 cycles		
Denaturation	1 second	92 °C - 95 °C
Annealing/Extension Combined	**1 second**	60 °C - **depending on primer**

RECOMMENDED REACTION MIXTURE PER WELL

Components	25 µL Reaction	10 µL Reaction	Final Concentration
5x primaQUANT Master Mix	5 µL	2 µL	1x
Forward Primer	variable (e.g. 2 µL)	variable (e.g. 1 µL)	100 - 400 nM
Reverse Primer	variable (e.g. 2 µL)	variable (e.g. 1 µL)	100 - 400 nM
Probe	variable (e.g. 2 µL)	variable (e.g. 1 µL)	200 - 600 nM
Template DNA	**variable**	**variable**	**0.1 - 10 ng/reaction**
Sterile Water	**adjust to 25 µL**	**adjust to 10 µL**	

NOTE

For maximum efficiency and specificity annealing temperatures as well as extension time, primer/probe concentration and template concentration need to be optimized.

CALCULATOR TOOL

Please feel free to download our Excel sheet calculator to calculate the necessary volumes:

http://calculator.steinbrenner-laborsysteme.de.

qPCR KnowledgeCenter

https://www.steinbrenner-laborsysteme.de

HOW TO VALIDATE A QPCR SETUP

BEFORE YOU START

You can find additional information on how to validate and set up a qPCR in our **qPCR Knowledge Center**.

Required Controls

DNA Dilution Series

A DNA dilution series is used to validate the dynamic range, find the optimal DNA input amount and estimate the overall PCR efficiency.

Primer Dilution Series

High primer amounts can result in unspecific primer binding that limit the fidelity of your qPCR.

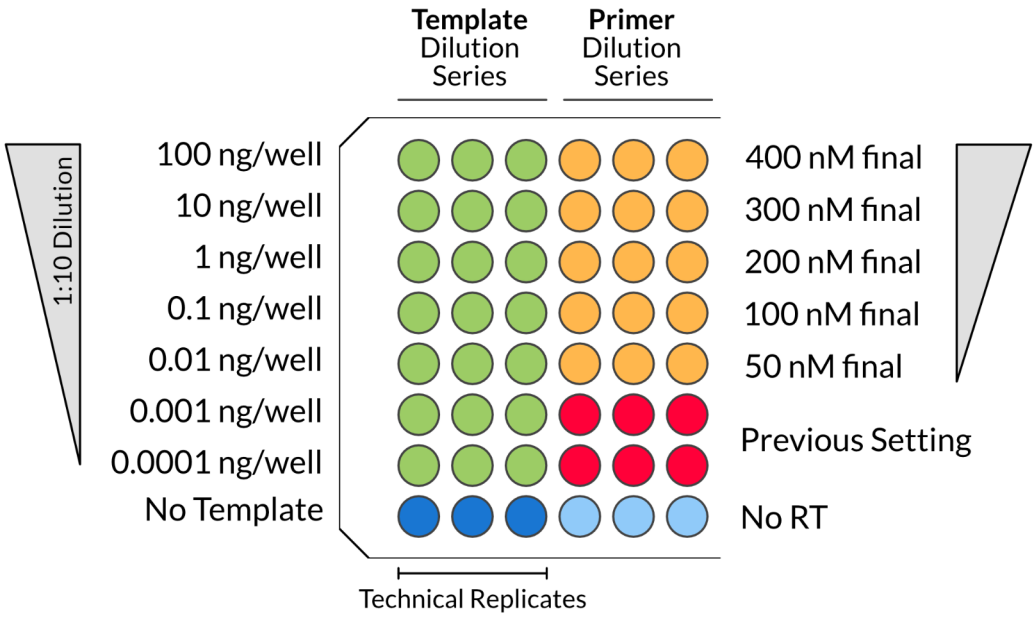
No Template Control (NTC)

A control in which all components except the template are added - this control is used as a negative control and should not show amplification.

No Reverse Transcription (NoRT)

For this control, reverse transcription is performed without the reverse transcriptase. It helps to identify unwanted amplification from left-over gDNA or unwanted templates.

Recommended Validation Layout



APPLICATIONS

- Probe-based quantitative PCR
  - TaqMan® Probes
  - Any 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

Our **primaQUANT** ***PROBE*** **5x 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 Master Mixes are free of detectable endonuclease- & exonuclease 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

FURTHER INFORMATION

For more information, please visit our website

https://www.steinbrenner-laborsysteme.de

qPCR KnowledgeCenter

Steinbrenner Laborsysteme GmbH