Steinbrenner Laborsysteme GmbH

primaQUANT PROBE NEW ADVANCED

ADVANCED qPCR Probe Master Mix

Article	Content
SL-9803-smp	1 ml, 100 rxn × 20 µl
SL-9803-5ML	5 x 1 ml, 500 rxn × 20 µl
SL-9803-10ML	10 x 1 ml, 1000 rxn × 20 µl
SL-9803-20ML	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 ADVANCED 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 and multiplexing applications.

The **ADVANCED qPCR Master Mix** contains all components - you just need to add primers and template DNA/cDNA. This Master Mix contains no ROX - please make sure to choose the right setting on your qPCR cycler.

DID YOU KNOW?

- For qPCR cyclers requiring other concentrations of ROX, **primaQUANT PROBE** is also available with ROX.

VERSION **1.0 – 08 / 2023**

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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 seconds	92°C - 95°C	25 - 40 cycles
Annealing	5 seconds	60°C depending on primer	
Extension	5 - 10 seconds	72°C	

2-STEP PROTOCOL

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

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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 ADVANCED 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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