Article	Content
SL-9550-smp	1 mL, 100 rxn á 20µL
SL-9550-5ML	5x 1 mL, 500 rxn á 20µL
SL-9550-10ML	10x 1 mL, 1000 rxn á 20µL
SL-9550-20ML	20x 1 mL, 2000 rxn á 20µL

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

primaQUANT 1STEP *MULTIPLEX*

2x *1-Step* RT-qPCR Probe Master Mix - no ROX

DESCRIPTION

Our **primaQUANT 1STEP** *MULTIPLEX* **2x RT-qPCR Master Mix** is an optimized ready-to-use mixture for probe-based (multiplex) assays such as Taqman®, Beacons and MGBs. It contains all enzymes for both Reverse Transcription and qPCR, 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 1STEP** *MULTIPLEX* **2x Master Mix** contains all components - you just need to add primers, probes and template RNA.

The Master Mix can be used for one-step RT-qPCR, so there is no need for additional reverse transcription.

DID YOU KNOW?

Some qPCR cyclers require ROX - **primaQUANT 1STEP** ***MULTIPLEX*** is also available with low or high concentrations of ROX.

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 qPCR Protocol

Step	Time	Temperature
Reverse Transcription	**10-15 minutes**	50 °C
Initial Denaturation	1-3 minutes	92 °C - 95 °C
25- 40 cycles		
Denaturation	5 - 10 seconds	92 °C - 95 °C
Annealing	5 seconds	60 °C - **depending on primer**
Extension	**20 - 30 seconds**	72 °C

2-Step qPCR Protocol

Step	Time	Temperature
Reverse Transcription	**10-15 minutes**	50 °C
Initial Denaturation	1-3 minutes	92 °C - 95 °C
25- 40 cycles		
Denaturation	5 seconds	92 °C - 95 °C
Annealing/Extension Combined	**20 - 40 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 qPCR Protocol

Step	Time	Temperature
Reverse Transcription	**10-15 minutes**	50 °C
Initial Denaturation	60 seconds	92 °C - 95 °C
25- 40 cycles		
Denaturation	1-5 seconds	92 °C - 95 °C
Annealing	1-5 seconds	60 °C - **depending on primer**
Extension	**5 seconds**	72 °C

2-Step qPCR Protocol

Step	Time	Temperature
Reverse Transcription	**10-15 minutes**	50 °C
Initial Denaturation	60 seconds	92 °C - 95 °C
25- 40 cycles		
Denaturation	1 second	92 °C - 95 °C
Annealing/Extension Combined	**5-10 seconds**	60 °C - **depending on primer**

RECOMMENDED REACTION MIXTURE PER WELL

Components	20 µL Reaction	10 µL Reaction	Final Concentration
2x primaQUANT 1STEP Master Mix	10 µL	5 µL	1x
Forward Primer	variable (e.g. 2 µL)	variable (e.g. 1 µL)	100 - 600 nM
Reverse Primer	variable (e.g. 2 µL)	variable (e.g. 1 µL)	100 - 600 nM
Probe	variable (e.g. 2 µL)	variable (e.g. 1 µL)	100 - 600 nM
Template RNA	**variable**	**variable**	**0.1 - 100 ng/reaction**
Sterile Water	**adjust to 20 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 KNOWLEDGE CENTER

http://www.qpcr-guide.com

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

RNA Dilution Series

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

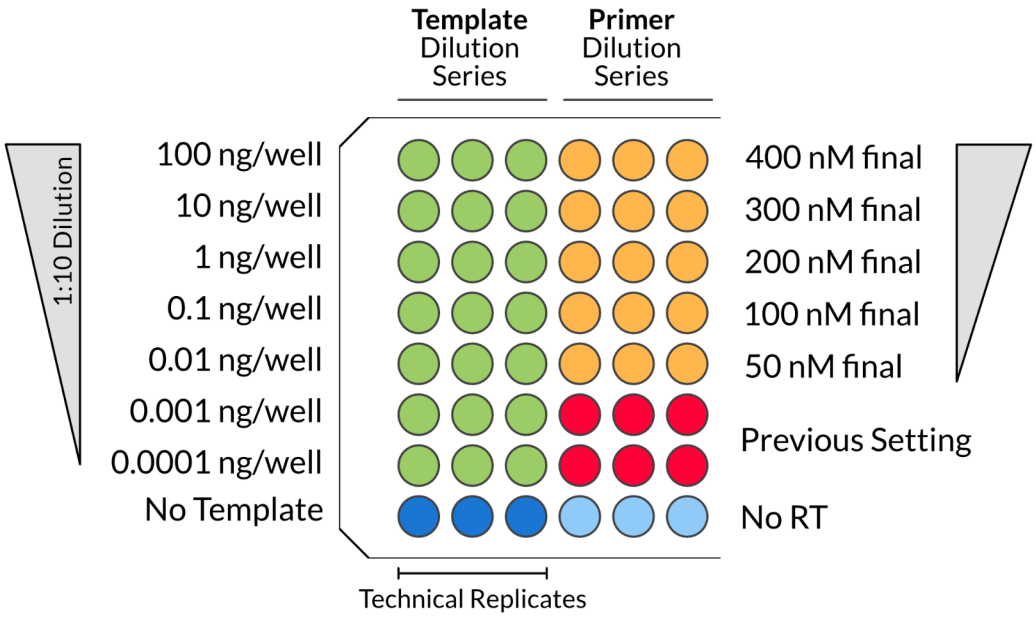
Primer Dilution Series

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.

Recommended Validation Layout



APPLICATIONS

- Probe-based quantitative PCR
  - TaqMan® Probes
  - Any Dual-Labeled Hydrolysis Probe
  - Molecular Beacons
  - Scorpion Probes
- RNA and miRNA Expression
- Multiplexing (up to 4 colors)
- Transcript Variant Analysis

QUALITY CONTROL

Our **primaQUANT 1STEP *MULTIPLEX* 2x RT-qPCR Master Mix** undergoes stringent quality controls.
Each lot is tested in a probe-based qPCR with RNA and MS2 Phage RNA 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 KNOWLEDGE CENTER
http://www.qpcr-guide.com

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