Steinbrenner Laborsysteme GmbH

primaQUANT 1STEP

primaQUANT 1-Step RT-qPCR Master Mix with SYBRGreen

Article	Content
SL-9561_smp	0.5 ml, 50 rxn × 20 µL
SL-9561-1ML	1 ml, 100 rxn × 20 µL
SL-9561-5ML	5 ml, 500 rxn × 20 µL
SL-9561-10ML	10 ml, 1000 rxn × 20 µL
SL-9561-20ML	20 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 1STEP RT-qPCR Master Mix with SYBRGreen** for probes is an optimized ready-to-use Master Mix dye-based assays and can be directly used with both DNA and RNA as a starting material.

It contains a modified and proprietary HotStart DNA Polymerase and Reverse Transcriptase, as well as dNTPS, MgCl2, SYBRGreen and other components in optimized concentrations. It provides fast kinetics, a RNA sensitivity of < 10 fg, target amplification even for difficult templates and multiplexing of more than 6 targets.

The **primaQUANT 1STEP RT-qPCR 2x qPCR Master Mix with SYBRGreen** contains all components - you just need to add primers and template DNA/cDNA or RNA.

DID YOU KNOW?

For qPCR cyclers requiring ROX as a reference dye, **primaQUANT 1STEP Master Mix with SYBRGreen** is also available with ROX (SL-9561R).

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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 prevent damage of the enzyme.

Component	Stock Concentration	20 µl Reaction	10 µl Reaction	Final Concentration
2x primaQUANT 1STEP RT-qPCR Master Mix with SYBRGreen	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)
Template RNA or DNA/cDNA	–	variable	variable	0.1 - 100 ng / 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 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 RT-qPCR assays, standard cycling conditions can be applied.
- However, cycling conditions strongly depend on the primer, amplicon and input material and thus some of these factors might need adjustments.

ONE-STEP RT-QPCR WITH ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		10 minutes	50°C
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	5 - 10 seconds	72°C
Melting Curve (optional)		–	60°C ramping up to 95°C

ONE-STEP RT-QPCR PROTOCOL WITHOUT ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		10 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	5 - 20 seconds	60°C depending on primer
Melting Curve (optional)		–	60°C ramping up to 95°C

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Ultra-fast Protocol

NOTE

- Ultra-fast cycling conditions highly depend on the ramping rate of your qPCR cycler, primer, 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 sets do not show unspecific binding.

ONE-STEP RT-QPCR PROTOCOL WITH ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		5 - 10 minutes	50°C
Initial Denaturation		1 minute	92°C - 95°C
25 - 40 cycles	Denaturation	1 second	92°C - 95°C
	Annealing	1 - 5 seconds	60°C depending on primer
	Extension	1 second	72°C
Melting Curve (optional)		–	60°C ramping up to 95°C

ONE-STEP RT-QPCR PROTOCOL WITHOUT ADDITIONAL ANNEALING

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Melting Curve (optional)		–	60°C ramping up to 95°C

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Applications

Dye-based quantitative PCR with RNA Input

Dye-based quantitative PCR with cDNA/DNA Input

High Resolution Melting Curve

QUALITY CONTROL PROCEDURE

Our **primaQUANT 1STEP PROBE 2x qPCR Master Mix** is subject to strict quality controls. Each lot is tested in a dye-based qPCR with cDNA and DNA input and must conform to our quality control chart.

Enzyme **purity and homogeneity of > 98 %** is validated by Bioanalyzer SDS protein electrophoresis.

The primaQUANT 1STEP PROBE 2x qPCR Master Mix is free of:

- RNA
- DNA
- RNase
- DNAse
- Endo- & Exonuclease activity

FURTHER INFORMATION

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

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Further Products

Products that may also interest you

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