primaQUANT 1STEP CYBR

2x 1-Step RT-qPCR SYBRGreen Master Mix - no ROX

Article	Content
SL-9551-smp	1 ml, 100 rxn × 20 µL
SL-9551-5ML	5 x 1 ml, 500 rxn × 20 µL
SL-9551-10ML	10 x 1 ml, 1000 rxn × 20 µL
SL-9551-20ML	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 1STEP CYBR 2 x RT-qPCR Master Mix** is an optimized ready-to-use mixture for SYBRGreen RT-qPCR. 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 CYBR 2x Master Mix** contains all components - you just need to add primers and template RNA.

The Master Mix is intended for one-step RT-qPCR and requires no additional cDNA synthesis.

DID YOU KNOW?

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

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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
primaQuant 1STEP Master Mix	2x	10 µl	5 µl	1x
Forward Primer	8 mM	1 µl	0.5 µl	400 nm (100 - 600 nM recommended)
Reverse Primer	8 mM	1 µl	0.5 µl	400 nm (100 - 600 nM recommended)
Probe	8 mM	1 µl	0.5 µl	400 nm (100 - 600 nM recommended)
Template RNA	10 ng/µl	2 µl	1 µl	0.1 - 100 ng / Reaction
Steriles Wasser	–	5 µl	2.5 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: **calculator.steinbrenner-laborsysteme.de**.

qPCR KnowledgeCenter

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Standard Protocol

NOTE

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

ONE-STEP QPCR PROTOCOL WITH ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		10 minutes	50°C
Initial Denaturation		3 minutes	92°C - 95°C
25 - 45 cycles	Denaturation	5 seconds	92°C - 95°C
	Annealing	5 seconds	60°C depending on primer
	Extension	5 - 10 seconds	72°C

ONE-STEP QPCR PROTOCOL WITHOUT ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		10 minutes	50°C
Initial Denaturation		3 minutes	92°C - 95°C
25 - 45 cycles	Denaturation	5 seconds	92°C - 95°C
	Annealing / Extension combined	10 seconds	60°C depending on primer

Ultra-fast Protocol

NOTE

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

ONE-STEP QPCR PROTOCOL WITH ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		5 minutes	50°C
Initial Denaturation		60 seconds	92°C - 95°C
25 - 45 cycles	Denaturation	1 second	92°C - 95°C
	Annealing	1 second	60°C depending on primer
	Extension	1 - 5 seconds	72°C

ONE-STEP QPCR PROTOCOL WITHOUT ADDITIONAL ANNEALING

		Time	Temperature
Reverse Transcription		5 minutes	50°C
Initial Denaturation		60 seconds	92°C - 95°C
25 - 45 cycles	Denaturation	1 second	92°C - 95°C
	Annealing / Extension combined	1 - 5 seconds	60°C depending on primer

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Applications

Dye-based quantitative PCR

RNA and miRNA Expression

High-Resolution Melting Curve Analysis

Transcript Variant Analysis

QUALITY CONTROL

Our **primaQUANT 1STEP CYBR 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: **www.steinbrenner.de**

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Further Products

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