Description

1Step Magic SYBR Kit is a convenient one-step solution for the quantitative analysis of RNA. The 2X qPCR mastermix contains all components necessary for a qPCR experiment: Hotstart DNA polymerase, dNTPs (with a blend of dTTP and dUTP to allow for the digestion with UNG), SYBR® Green and a passive reference dye, all supplied in a reaction buffer optimized for selective amplification of target sequences. For the conversion of RNA to cDNA before the amplification, VitaScript™ Reverse Transcriptase is provided as a 50X solution.
Due the one-step formulation, handling steps and experiment time are reduced; furthermore, reliability and reproducibility are increased.

Product	Size	SKU
1Step Magic SYBR Kit	250 rxn/20 µl	PCCSKU1201
	500 rxn / 20 µl	PCCSKU1202

Additional Materials Required

- Nuclease free PCR tubes or plates
- Optical plate sealing films / caps
- Real-time PCR cycler
- PCR Primer
- Template RNA and control RNA standards
- Filter pipette tips
- Sterile, nuclease-free, DNA-free tubes
- *(Optional) Uracil-N Glycosylase (UNG)*

Storage

Store all components at -20°C and avoid repeated freeze and thaw cycles as they may damage the enzyme. Protect 2X qPCR Mix from light.

Template

For use with VitaScript™ One-Step qPCR Kit, 50 fg – 100 ng of RNA template is recommended. In general, the volume of template RNA should not exceed 10% of the reaction volume (e.g. 2 µl in a 20 µl reaction). If genes with a high copy number are expected, we recommend a dilution series of the template to increase accuracy of your results.

PCR Reaction Setup

Before starting the reaction setup, thaw the 2X qPCR Mix and mix thoroughly but gently to ensure even distribution of components.
(Optional) Perform an UNG digestion before your PCR according to manufacturer's guidelines.

Recommended qPCR Protocol

Step	Cycles	Temperature	Time
Reverse Transcription	1	42°C	15 minutes
Inactivation VitaScript™ / Hot Start Activation	1	95°C	10 minutes
Amplification	40	95°C	10 seconds
		60°C/ T_m -5°C	30 seconds
		72°C	30 seconds
Melt Curve	1	60-95°C	/

Dilute your positive control standards and experimental samples with nuclease-free water to the desired concentrations and add them to their designated wells in the multi-well plate. Prepare ≥ 3 replicates each. For negative control, add nuclease-free water instead of template RNA.
Prepare your mastermix + 10% to compensate for pipetting inaccuracies. Keep the plate on ice until further use.

Component	Volume	Final Concentration
2X qPCR Mix	10 µl	1X
VitaScript™ 50X	0.4 µl	1X
Template RNA	X µl	max. 2 µl, 50 fg-100 ng
Forward primer (10 µM)	0.4 µl	0.05 – 0,9 µM each
Reverse primer (10 µM)	0.4 µl	0.05 – 0,9 µM each
Nuclease-free dH_2O	to 20 µl	

Gently mix all components, distribute to your wells and seal the plate. Spin the plate quickly to collect all liquid at the bottom. Place into your device and immediately start the experiment.

General Considerations

Add a control without addition of VitaScript™ to check for genomic DNA contamination.

Design primers to span an intron in your gene of interest to make sure that only cDNA but no genomic DNA is amplified.

Check your melt curve for specific amplification: multiple peaks indicate more than one product or primer dimerization

If your water control produces amplification signal, check for primer dimerization. Prepare fresh reagents for the next experiment as it may also indicate a DNA contamination.

If no fluorescence can be detected, check the instrument settings if SYBR Green is set for detection and that data collection is enabled during amplification.

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