

**Description**

Script-III One Step RT-qPCR Taqman Probe Kit with UDG a ready-to-use kit allowing reverse transcription and subsequent probe-based qPCR in a single tube. It contains all components for RT-qPCR except primers, probes and RNA templates. The one-step format significantly improves sensitivity and effectively prevent contamination. The heat-labile UDG in this product could degrade U-contained contamination in room temperature, and inactivated in 50°C, which could prevent false positive results without affect the efficiency and sensitivity. The Script Reverse Transcriptase in the kit provides reliable reverse transcription to a wide range of RNA template amount. After reverse transcription, the Hot-start version of Taq polymerase is activated at 95 ° C and the Script Reverse Transcriptase is inactivated simultaneously. In the sequential PCR reaction, the 5'-3' exonuclease activity of Taq polymerase cleaves the hybridized probe, separating the reporter from the quencher and releasing fluorescent signal. The Script-III One Step RT-qPCR Probe Kit is an ideal product for high-speed

**Recommended Protocol**

1. Fully thaw the 2X One Step RT-qPCR Probe Buffer IV before use. Mix the buffer well and avoid directly sunlight. Determine the total number of reactions required and prepare master mix. Triple replicates for each reaction are recommended.
2. The One Step Probe Enzyme Mix IV contain high concentration of glycerin. Mix gently before use without generating air bubbles. Spin briefly to collect all the contents at the bottom. After use, return it to -20°C immediately.
3. If applicable, use aerosol-resistant pipette tips and microtubes to minimize contamination.
4. High quality RNA templates are recommended for optimal results
5. Only gene specific primers are recommended. Random primers and Oligo dT primers are NOT recommended in the reverse transcription reaction.
6. The optimal length of amplicon is between 70 and 200 bp for general cycling condition.

**Prepare materials before reaction setup:**

Pipette, aerosol-resistant pipette tip, cold blocks and ice. Gene expression primers and probes. RNA templates.

1.5 mL RNase-free EP tubes, Real-time PCR tubes and plates.

1. Prepare the reaction mix :

Set up the reaction on ice by adding the following components for the number of reactions required. :

**Reaction Conditions**

Component	20 µL reaction	25 µL reaction	50 µL reaction
2xOne Step RT-qPCR Probe Buffer IV	10 µl	12,50µl	25µl
One Step Probe Enzyme Mix IV	2 µl	2,5 µl	5µl
10uM Forward Primer*	0.4 µl	0.5-0.6 µl	1µl
10uM Reverse Primer*	0.4 µl	0.5-0.6 µl	1µl
TaqMan Probe (10µM)***	0.4 µl	0.5-0.6 µl	1µl
50X Rox Dye (optional)	0.4 µl	0.5-0.6 µl	1µl
Total RNA **	2 µL	2,5 µL	5µl
Water, RNase-Free	Up to 20µl	up to 25 µl	up to 50 µl

\* A final primer concentration of 0.2 µM is recommended for most reactions. However, to optimize individual reaction, a primer titration from 0.1 µM to 1.0 µM can be performed. The length of amplified PCR products should ideally be in the range of 70 - 200bp.

\*\* Use 10 pg~100 ng of RNA template in a 20 µL reaction.

\*\*\* A Probe concentration of 50-250 nM is recommended.

**Optimized One Step RT-qPCR Conditions**

Step	Temp (°C)	Time	Cycle
UDG Reaction	25°C	5 min.	1
Reverse Transcription	50°C	5 min.	1
Polymerase Activation	95°C	3 min.	1
Denaturation Annealing, and Extension	95° C	5-15 sec.	45
	60° C	30-34 sec.	

The extension time should be adjusted to the minimum time required for data acquisition according to qPCR instrument guidelines used. (30 s for Applied Biosystems StepOnePlus™, 31 s for Applied Biosystems 7300, and 34 s for Applied Biosystems 7500)

**For Research Use Only**

**Kit Contents**

Contents	Cat.#	Size
2xOne Step RT-qPCR Probe Buffer IV*	FS-RT-21402	1.25ml x 2
One Step Probe Enzyme Mix IV**		500µl
50X ROX Dye I (High Rox)***		100µl
50X ROX Dye II (Low Rox)***		100µl
Nuclease Free Water H <sub>2</sub> O		1.25ml x 2

\* Containing dNTP/dUTP Mix, prevent false positive caused by cross contamination with UDG.

\*\* the Taq polymerase is blocked by antibody, containing RNase Inhibitor, Heat-labile UDG

\*\*\* Passive reference dye to normalize the fluorescence signals

**Applications**

- Real-time PCR
- Detection and quantification of DNA and cDNA targets
- Gene expression profiling
- Microbial detection
- Viral load determination
- Array validation
- SNP genotyping

**Storage Conditions**

Upon receipt, store all components at -20°C.

**Use of the ROX Reference Dye:**

**-50x Rox Dye I (High Rox)**

Applied Biosystems 7000/7300/7700/7900, Applied Biosystems StepOne™/StepOnePlus™.

**-50x ROX Dye II (Low Rox)**

Applied Biosystems 7500/ViiA7™, QuantStudio™, Stratagene Real-time PCR Systems, Rotor-gene™ 3000

**-NO ROX Dye**

Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers®, QIAGEN/Corbett Systems, Eppendor Mastercyclers