

Product components

Components	Component number	Size-1	Size-2
		50 RXN (20 µL/RXN)	250 RXN (20 µL/RXN)
One Step Probe Enzyme Mix IV*	RM21476	100 µL	500 µL
2X One Step RT-qPCR Probe Buffer IV**	RM21475	500 µL	2 × 1.25 mL
50X ROX Dye I***	RM21465	20 µL	100 µL
50X ROX Dye II***	RM21466	20 µL	100 µL
Nuclease-free H ₂ O	RM20214	500 µL	2 × 1.25 mL

* Contains dNTP/dUTP Mix; false positives caused by cross-contamination are prevented by the addition of UDG.

** Utilizes an antibody-based hot-start system, and contains RNase Inhibitor and Heat-labile UDG.

*** Used as a passive reference dye to normalize fluorescence signals between wells.

Product Description

ABScript III One-Step RT-qPCR Probe Kit with UDG V5 is a ready-to-use kit that allows 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 prevents contamination. The heat-labile UDG in this product can degrade uracil-containing contaminants at room temperature and is inactivated at 50°C, thereby preventing false-positive results without affecting efficiency or sensitivity. The ABScript III Reverse Transcriptase in the kit provides reliable reverse transcription for a wide range of RNA template amounts. After reverse transcription, the hot-start Taq polymerase is activated at 95 °C while the ABScript III Reverse Transcriptase is simultaneously inactivated. In the subsequent PCR, the 5' → 3' exonuclease activity of Taq polymerase cleaves the hybridized probe, separating the reporter from the quencher and releasing a fluorescent signal. The ABScript III One-Step RT-qPCR Probe Kit is an ideal choice for high-speed analysis of low-input RNA samples.

Instruments

Rox types	qPCR Machines
No ROX	Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers®, QIAGEN/Corbett Systems, Eppendorf Mastercycler®
50X ROX Dye I	Applied Biosystems 7000/7300/7700/7900, Applied Biosystems StepOne™/StepOnePlus™
50X ROX Dye II	Applied Biosystems 7500/ViiA7™, QuantStudio™, Stratagene Real-time PCR Systems, Rotor-gene™ 3000

Note: Different instruments require different ROX Dyes. Please refer to the instruments above.

Storage

-20°C

Notes

- Before use, completely thaw the 2X One Step RT-qPCR Probe Buffer IV. Mix the buffer thoroughly and protect from direct sunlight. Prepare the master mix according to the total number of reactions required. Three replicates per reaction are recommended.
- The One Step Probe Enzyme Mix IV contains a high concentration of glycerol. Mix gently before use to avoid bubble formation. Briefly centrifuge to collect all liquid at the bottom of the tube. After use, store immediately at -20 °C.
- If applicable, use aerosol-resistant pipette tips and microtubes to minimize contamination.
- For optimal performance, high-quality RNA templates are recommended.

- Only gene-specific primers are recommended for the reverse transcription reaction. The use of random primers or oligo(dT) primers is not recommended.
- Under general cycling conditions, the optimal amplicon length is 70 bp to 200 bp.

Operation Description

Experimental Preparation

- RNase-free 1.5 mL EP tubes, RNase-free PCR tubes, pipettes and tips, icebox or ice.
- PCR probes, primers, and templates.
- Fluorescent quantitative PCR-specific tubes or plates.

Experimental Procedure

- It is recommended to prepare a reaction system mix on ice and quickly transfer the mix to a qPCR instrument.

Components	Volume	Volume
2X One Step RT-qPCR Probe Buffer IV	10 µL	25 µL
One Step Probe Enzyme Mix IV	2 µL	5 µL
Forward Primer(10 µM) *	0.4 µL	1 µL
Reverse Primer(10 µM) *	0.4 µL	1 µL
TaqMan Probe (10 µM) ***	0.4 µL	1 µL
50X ROX Dye (As require by instrument guideline)	0.4 µL	1 µL
Total RNA **	2 µL	5 µL
ddH ₂ O	To 20 µL	To 50 µL

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

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

*** A probe concentration of 50-250 nM is recommended.

- Optimized One Step RT-qPCR Program:

Step	Temp	Time	Cycles
UDG Reaction	25°C	5 min	1
Reverse Transcription	50°C	5 min	1
Pre-denaturation	95°C	3 min	1
Cycle Reaction	95°C	5-15 s	45
	60°C	30-34 s*	

* If not otherwise specified, default to 30 s. The extension time varies according to the instrument used: 30 s for StepOne Plus, 31 s for 7300, and 34 s for 7500. If not otherwise specified, set to 30 s.