ABScript III One Step RT-qPCR Probe Kit with UDG V5

Catalog : RK20412

Size : 50 RXN / 250 RXN (20 μL / RXN)

2X One Step RT-qPCR Probe Buffer IV	BM0053
One Step Probe Enzyme Mix IV	BM0060
50X ROX Dye I	RM21465
50X ROX Dye II	RM21466
Nuclease-free H_2O	RM20214

Product Description

ABScript III One Step RT-qPCR Probe Kit with UDG V5 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 onestep format significantly improves sensitivity and effectively prevent contamination. The heat-liable UDG in this product could degrade U-contained contamination in room temperature, and inactivated in 50℃, which could prevent false positive results without affect the efficiency and sensitivity. The ABScript III 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℃ and the ABScript III 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 ABScript III One Step RT-qPCR Probe Kit is an ideal product for high-speed analyses of low input RNA sample

Product Components

Prodect Components	50 RXN	250 RXN
2X One Step RT-qPCR Probe Buffer IV *	500 μL	1.25 mL X 2
One Step Probe Enzyme Mix IV**	100 μL	500 μL
50X ROX Dye I ***	20 μL	100 μL
50X ROX Dye II ***	20 μL	100 μL
Nuclease-free ddH_2O	500 μL	1.25 mL 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*

Storage: Upon receipt, store all components at -20℃.

Compatible Instruments:

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

NO ROX Dye

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

Precautions

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.

Protocal ：

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.:

Component	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
Nuclease-free H_2O	to 20 μL	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.*

2. Optimized One Step RT-qPCR program:

Step	Temperature	Time	Cycles
UDG Reaction	25℃	5 min	1
Reverse Transcription	50℃	5 min	1
Polymerase Activation	95℃	3 min	1
Denaturation, Annealing and Extension	95℃	5-15 s	45
	60℃	30~34 s	

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 StepOnePlusTM, 31 s for Applied Biosystems 7300, and 34 s for Applied Biosystems 7500)