ABScript II One Step RT-qPCR Probe Kit

Catalog: RK20407
Size: 20 RXN / 100 RXN (50 μL/RXN)

2X One Step RT-qPCR Probe Buffer	RM21462
One Step Probe HS *Taq*	RM21463
ABScript II RT Enzyme Mix	RM21464
50X ROX Dye I	RM21465
50X ROX Dye II	RM21466
Nuclease-free H_2O	RM20214

Product Description

ABScript II One Step RT-qPCR Probe Kit 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 ABScript II 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 ABScript II 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 II One Step RT-qPCR Probe Kit is an ideal product for high-speed analyses of low input RNA sample.

Product Components

Component	20 RXN (50 μL / RXN)	100 RXN (50 μL / RXN)
2X One Step RT-qPCR Probe Buffer *	500 μL	1.25 mL X 2
One Step Probe HS *Taq*	20 μL	100 μL
ABScript II RT Enzyme Mix**	20 μL	100 μL
50X ROX Dye I ***	20 μL	100 μL
50X ROX Dye II ***	20 μL	100 μL
RNase-free ddH_2O	500 μL	1.25 mL X 2

** Containing dNTPs, Mg^{2+}, etc.*

*** Containing ABScript II Reverse Transcriptase, RNase Inhibitor*

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

Storage

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

Compatible Instruments

50X ROX Reference Dye I

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

50X ROX Reference Dye II

Applied Biosystems 7500/ViiA7TM, QuantStudio™, Stratagene Real-time PCR Systems, Rotor-gene™ 3000

NO ROX Reference Dye

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

Additional Material

Required but not Supplied

1. RNA templates, primers and probes
2. Optical-grade qPCR tubes, plates, sealing films, and aerosol-resistant pipette tips

Precautions

1. Fully thaw the 2X One Step RT–qPCR Probe Buffer before use. Mix the buffer well and avoid directly sunlight.
2. Determine the total number of reactions required and prepare master mix. Triple replicates for each reaction are recommended.
3. The ABScript II RT Enzyme Mix and One Step Probe HS *Taq* 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.
4. If applicable, use aerosol–resistant pipette tips and microtubes to minimize contamination.
5. High quality RNA templates are recommended for optimal results.
6. Only gene specific primers are recommended. Random primers and Oligo dT primers are NOT recommended in the reverse transcription reaction
7. The optimal length of amplicon is between 70 and 200 bp for general cycling condition.

Protocol

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

Set up One Step RT–qPCR experiment

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	10 μL	25 μL
One Step Probe HS *Taq*	0.4 μL	1 μL
ABScript II RT Enzyme Mix	0.4 μL	1 μL
Forward Primer (10 μM) *	0.4 μLeach	1 μL each
Reverse Primer (10 μM) *	0.4 μLeach	1 μL each
TaqMan Probe (10 μM) ***	0.4 μLeach	1μL each
50X ROX Dye (As require by instrument guideline)	0.4 μL	1 μL
Total RNA **	Up to 2 μL	Up to 5 μL
RNase–free H_2O	Up to 20 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 – 200 bp.*

*** Use 15 pg~150 ng of RNA template in a 20 μL reaction.*

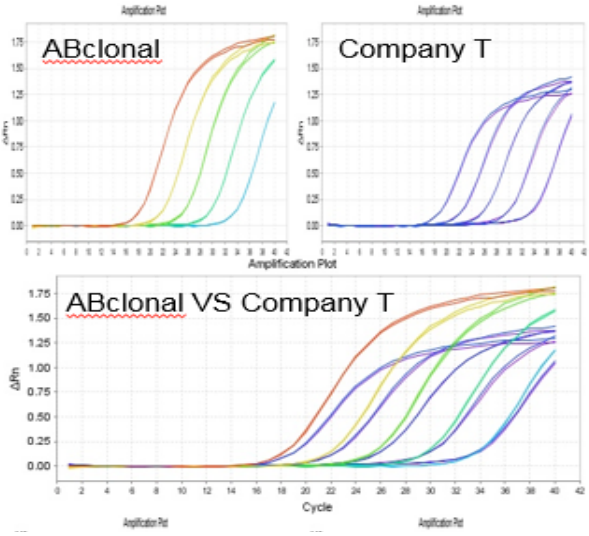
**** A Probe concentration of 50–250 nM is recommended.*

Optimized One Step RT–qPCR program:

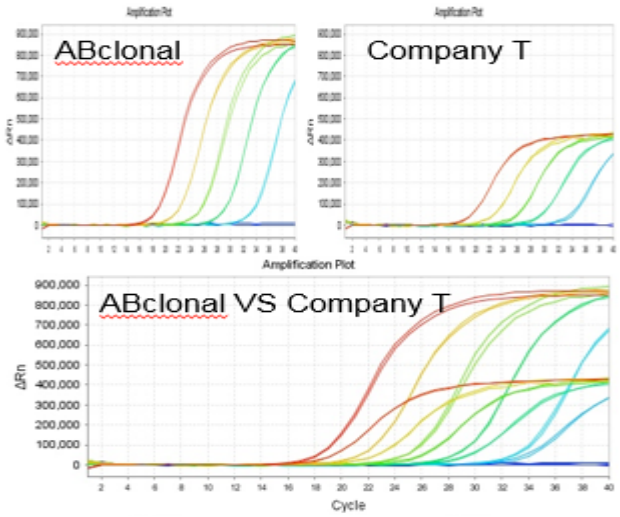
Step	Temperature	Time	Cycles
Reverse Transcription	42℃	5 min	1
Polymerase Activation	95℃	3 min	1
Denaturation, Annealing and Extension	95℃	5–15 s	40
	60℃	30–34 s*	

**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 StepOnePlus™, 31 s for Applied Biosystems 7300, and 34 s for Applied Biosystems 7500)*

2.Data Analysis



Pic 1: Amplification plots comparing ABScript II One Step RT–qPCR Probe Kit and one–step RT–qPCR kit from Company T. Rat total RNA in ten–fold dilution from 150 ng to 15 pg was used as template. Identical RT–qPCR programs were run with Applied Biosystems StepOnePlus™ to detect VIC fluorogenic probe. The ABclonal kit outperformed the kit from competitor T with higher detection sensitivity.



Pic 2: Amplification plots comparing ABScript II One Step RT-qPCR Probe Kit and one-step RT-qPCR kit from Company T. Rat total RNA in ten-fold dilution from 150 ng to 15 pg was used as template. Identical RT-qPCR programs were run with Applied Biosystems StepOnePlus™ to detect FAM fluorogenic probe. The ABclonal kit outperformed the kit from competitor T with higher detection sensitivity.