ABScript II One Step SYBR Green RT-qPCR Kit

Catalog: RK20404
Size: 10 RXN / 100 RXN (50 μLsetup / RXN)
Components：

2X One Step SYBR Green RT-qPCR Buffer	RM21454
ABScript II One Step Enzyme Mix	RM21455
50X ROX Dye I	RM21465
50X ROX Dye II	RM21466
Nuclease-free H_2O	RM20214

Introduction

ABScript II One Step SYBR Green RT-qPCR Kit is a special kit for one-step RT-qPCR reaction by using SYBR Green I chimeric fluorescence. The kit takes RNA as the template, uses gene-specific primers, and reverse transcription and PCR reaction can be carried out continuously in the same tube without additional pipe opening and pipetting operations, greatly improving the detection flux and effectively preventing contamination. This reaction system can detect the amplification products in real time, greatly improving the detection sensitivity, and omitting the electrophoresis step after PCR reaction, which is very suitable for the detection of RNA virus and other trace RNA. This product integrates the superiority of ABScript II Reverse Transcriptase and *Taq* DNA Polymerase, cooperate with optimized buffer system, with high amplification efficiency and high amplification specificity, one-step RT - qPCR reaction can be stable. In addition, all the enzymes used in the reaction are made into enzyme Mix, which is easier and more convenient to operate.

List of Components:

Components	10 RXN (50 μLsetup / RXN)	100 RXN (50 μL setup / RXN)
2X One Step SYBR Green RT-qPCR Buffer *	250 μL	1.25 mL X 2
ABScript II One Step Enzyme Mix **	20 μL	200 μL
50X ROX Dye I ***	10 μL	100 μL
50X ROX Dye II ***	10 μL	100 μL
RNase-free ddH_2O	1.25 mL	1.25 mL X 2

* *contain dNTPs, Mg^{2+}, SYBR Green,* etc.
** *contain ABScript II Reverse Transcriptase , RNase Inhibitor ,Taq DNA Polymerase.*
*** *Use to correct the error of fluorescence signal between holes.*

Storage：-20℃ protected from light

ROX Dye suitable model：

ROX	qPCR model
No ROX	Bio-Rad iCycler series, Roche Light Cycler series, Qiagen/Corbett series, Eppendorf etc.
ROX Dye I (High Rox)	ABI 7000/7300/7700/7900, ABI StepOne/StepOnePlus etc.
ROX Dye II (Low Rox)	ABI 7500, ABI ViiA™7, ABI QuantaStudio series, Stratagene series, Corbett Rotor Gene 3000 etc.

Usage Notes

1. When using 2X One Step SYBR Green RT-qPCR Buffer, please fully melt it, mix it well and then use it. Avoid direct sunlight and keep it away from light. If multiple One Step RT-qPCR reactions need to be prepared at the same time, it is recommended to allocate all groups except primers and templates to make premix, and then separate it into each reaction tube to reduce the loss of reagents.
2. The ABScript II One Step Enzyme Mix in the kit contains high concentration of glycerol, before using the Enzyme Mix, please mix gently and avoid foaming; Please use immediately after centrifugal. After usage please put it back - 20℃ refrigerator.
3. The configuration and split charging of the reaction liquid must use non-polluting spear and Microtube to avoid contamination as far as possible.
4. To ensure the success of the reaction, it is recommended to use high-quality RNA templates.
5. The kit can only use specific primers, and can't use random primers or Oligo dT primers for reverse transcription reaction.
6. When the one-step RT-qPCR experiment is designed for amplification of primers, the recommended product length of 100-200 bp has the best effect.

Protocol

Provision of Experiment：

★ 1.5 mL RNase-free EP tubes、RNase-free PCR tubes、pipettors and spears、ice or ice box.

★ PCR specific primers and templates.

★ Real-time PCR special tubes or plates.

Method of Experiment：

Prepare reagents：RNA templates、primers. Please follow the instructions of different brands of fluorescence quantitative PCR instrument for experimental operation

1. Preparation of One Step RT-qPCR reaction system：

Prepare the following reaction system on the ice.Take the 20 μL reaction system as an example：

Components	Volume	Volume
2X One Step SYBR Green RT-qPCR Buffer	10 μL	25 μL
ABScript II One Step Enzyme Mix	0.8 μL	2 μL
Forward primer (10 μM) *	0.4 μL	1 μL
Reverse primer (10 μM) *	0.4 μL	1 μL
ROX Dye I (50X)	0.4 μL	1 μL
Total RNA **	2 μL	5 μL
RNase-free H_2O	to 20 μL	To 50 μL

** The final concentration of primer is usually 0.2 μM, which can get better results. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1-1.0 μM. The length of the amplification product is recommended to be within the range of 70-200 bp*

*** It is recommended to input 30 pg~300 ng Total RNA as template in 20 μl system.*

2. One Step RT-qPCR reaction procedure：

Step	Temperature	Time	Cycles
Reverse Transcription	42℃	5 min	1
Pre-denaturation	95℃	1 min	1
Circular reaction	95℃	5 s	40
	60℃	30~34 s	
Melt Curve		automatic instrument setting	

** Please adjust the extension time according to the minimum time limit for data collection required by your Real Time PCR device: set it to 30 s when using StepOnePlus; Please set it to 31 s when using 7300; Set it to 34 s when using 7500.*

Confirm the amplification curve and melting curve after the reaction, make the standard curve.

qPCR Response Validity Criteria

1） **Linear relationship and amplification efficiency confirmation：** Standard curve correlation coefficient（R^2）should be more than 0.98；the slope of curve should be in the range of -3 to -3.5.

The PCR amplification efficiency（E^2）is in the range of 0.9 to 1.2.

2） **Repetitive confirmation**

The standard deviation (STD) of Ct values should be less than 0.2

3） **Specific confirmation**

Melting curve of amplification product is not obvious non-specific amplification product or primer dimer complex peak (if necessary, please conduct agaros electrophoresis for confirmation)

Common problems and solutions

1) Melt Curve Show Multiple Peaks

a. Primer design is not optimized：Redesign primers according to primers design principles

b. Primer concentration too High：lower down the concentration of primers appropriately

c. RNA templates carry genomic contamination: template genome contamination is removed.

2) Unusual Amplification Curves

a. Individual Amplification Curves suddenly Plunged：

There are bubbles in the reaction tube, which are caused by the sudden decrease of fluorescence value detected by the instrument due to the bubble breaking after the temperature rising. Please pay attention to centrifugation during sample processing, and avoid sucking out bubbles during the sample adding process

b. Inconsistent Amplification Curve：The template concentration is higher, and the baseline endpoint value is greater than the Ct value. Reduce baseline endpoint and reanalyze data.

3) No Amplification Curves after Reaction

a. Not Enough PCR Cycles：the PCR cycle number is usually set to be 40. It should be noted a higher cycle number may increase the background signal.

b. Primer Degradation：Use electrophoresis to confirm the Integrity of primers.

c. Confirm the Signal Collection Step：the signal collection step are usually set to be after the annealing-extending step for two-step qPCR and after extending step for three-step qCPR.

d. Template Input Too Low：Increase template concentration or add extra repetition.
e. Template Degradation：Use freshly prepared template(Use electrophoresis to confirm Integrity of template)

4) Ct Value Too Late

a. Low Amplification Efficiency：Optimize reaction condition or change primer.
b. Template Input Too Low：Increase template concentration or add extra repeat
c. Template Degradation：Use freshly prepared template(Use electrophoresis to confirm Integrity of template)
d. Too Long PCR Products：The length of amplification products is usually in the range of 100 bp-200 bp.
e. Reaction Inhibitors：Increase the dilution of the template or prepare a new template.

5） NTC Shows Amplification

a. Contamination：Use sterile water to conduct experiment and the all operation is suggested to be done in clean room to avoid aerosol contamination.
b. Non-Specific PCR Products: analyze with melt curve.

6） Inconsistent Results

a. Inconsistent Sample Added：Use proper pipetting techniques
b. Inconsistent Temperature in qPCR Machine：ensure periodic machine calibration.
c. Template Concentration Too Low：the lower template input, the poorer qPCR result is. Increase the template concentration.