ABScript II RT Master Mix for qPCR

Catalog: RK20402
Size: 10 RXN / 100 RXN (20 μL / RXN)
Concentration: 5×**Components:**

5×ABScript II RT Mix	RM21452
5×No RT Mix	RM21453
Nuclease-free H_2O	RM20214

5×ABScript II RT Mix contains ABScript II Reverse Transcriptase, RNase Inhibitor, RandomPrimer, Oligo dT Primer, dNTP Mixture and reaction buffer(containing Mg^{2+}).

In addition to ABScript II Reverse Transcriptase, the 5×No RT Mix contains the same components as 5×ABScript II RT Mix, which is used as the negative control of No RT.

Storage：-20℃

Introduction

ABScript II Reverse Transcriptase is an M-Mulv Reverse Transcriptase modified by genetic engineering, which reduces the activity of RNase H, improves the thermal stability and cDNA synthesis efficiency, and ensures the authenticity and repeatability of qPCR results. ABScript II RT Master Mix for qPCR is a reverse transcription detection reagent suitable for two-step RT-qPCR. 5X ABScript II RT Mix contains all the reagents needed for reverse transcription reaction (ABScript II Reverse Transcriptase, RNase Inhibitor, RandomPrimer, Oligo dT Primer, dNTP and reaction buffer), and a reaction can be started simply by adding template RNA and Nuclease-free H_2O. The operation is simple. The volume of RNA templates can be added up to 80% of the total volume, which is very suitable for the reverse transcription reaction of low-concentration RNA templates. 5 x ABScript II RT Mix will not freeze in - 20 ° C, easy access.

This product is specially optimized for qPCR. Oligo $(dT)_{23}VN$ has a stronger ability to anchor mRNA containing Poly(A) than Oligo (dT)18, making reverse transcription more efficient. Proportional optimized Random Primers/Oligo $(dT)_{23}VN$ Primer Mix enables cDNA synthesis to start from each region of RNA transcript and has the same reverse transcription efficiency, which ensures the authenticity and repeatability of qPCR results to the greatest extent. Reverse transcription products are compatible with SYBR Green and probe qPCR, and can be used in combination with corresponding reagents according to experimental purposes for high-performance gene expression analysis.

List of Components:

Component	10RXN	100RXN
5 × ABScript II RT Mix	40 μL	400 μL
5 × No RT Mix	20 μL	40 μL
Nuclease-free H_2O	1 mL	2x 1 mL

Precautions for Use

- 5 × ABScript II RT Mix and 5 × No RT Mix have high viscosity. Before use, briefly centrifuge the reagent to the bottom of the tube, pipette to mix slowly and carefuly, and try to avoid producing bubbles.
- Random Primers and Oligo $(dT)_{23}VN$ Primer have been added to this product, if Specific Primer is used for reaction, this product cannot be used. It is recommended to use ABScript II cDNA first-strand Synthesis Kit (ABclonal RK20400).
- The reverse transcription product (cDNA product) obtained from this reagent product is only suitable for qPCR reaction, and not suitable for long fragment PCR amplification in downstream experiments such as cloning. If necessary, you can use ABScript II cDNA First-Strand Synthesis Kit (ABclonal RK20400) to conduct experiments.
- When dividing up the reagents, please be sure to use new disposable tips to avoid contamination among reagents.

Protocol

Preparation of Experiment :

- Materiales and Equipments: 0.2 mL RNase-free microtubes, 1.5 mL microtubes, Micropipettes and RNase-free tips, PCR instrument and qPCR instrument, ice or ice box.
- RNA： Complete and high quality RNA. (Please check whether the RNA is degraded or contaminated before the experiment.) If RNA contains a complex secondary structure or a high GC content, it can be incubated at 65 ℃ for 5 minutes (and immediately on ice) before reverse transcription.
- qPCR Reagent Selection Guide: The cDNA product (RT

reaction liquid) can be directly added to the reaction system as a template for qPCR reaction. It is suggested that the volume of the template cannot exceed the 1/10 volume of the qPCR reaction, or the Nuclease-free H_2O is used to dilute the cDNA product (RT reaction liquid) and then add to the reaction system. You can choose 2 × SYBR Green Fast qPCR Mix (ABclonal RK21200/21201 /21202) for the following experiment.

Process of Experiment :

1. Reverse transcription

1.1 Reverse transcription reaction system

Add the following reagents in the RNase-free PCR tube on the ice, mix gently, and centrifuge briefly.

Component	Volume
5 × ABScript II RT Mix	4 μL
Total RNA	1 pg -1 μg *
Nuclease-free H_2O	To 20 μL

** According to the requirements of the experiment, the appropriate amount of RNA is added. When the volume of RNA template is large, make sure that RNA is dissolved in water instead of TE, because TE inhibits the reverse transcription reaction.*

No RT Control reaction（optional）*

No RT Control refers to the reverse transcription negative control reaction without reverse transcriptase, which is used to test whether there is genomic DNA residue in the RNA template.

Make up the following mixture, gently mix, instantaneously centrifuge:

Components	Volume
Nuclease-free H_2O	to 20 μL
5X No RT Mix	4 μL
Total RNA	1 pg -1 μg*

1.2 Reverse transcriptional reaction procedure

The reverse transcription reaction was performed on the PCR instrument, according to the follow-up procedure.

Temperature	Time
25℃	5 min
42℃	15min
85℃	5 sec
4℃	Hold

** Product can be applied immediately to the subsequent qPCR reaction, or in - 20℃ storage, product should avoid repeated freezing and thawing*

2. The following is after used this product for reverse transcription, select 2 × SYBR Green Fast qPCR Mix (ABclonal RM21201) reagent to carry out qPCR reaction in *StepOnePlus Real-Time PCR System*.

** Please read the instrument operation manual before the experiment.*

2.1 qPCR reaction system (Take 20 μL as an example)

Component	Volume
2 × SYBR Green Fast qPCR Mix	10 μL
cDNA product（RT reaction liquid）	x μL*
Forward Primer（10 μM）	0.4 μL
Reverse Primer（10 μM）	0.4 μL
Nuclease-free H_2O	to 20 μL

** It is suggested that the volume of the template does not exceed the 1/10 volume of the qPCR reaction, or the Nuclease-free H_2O is used to dilute the cDNA product (RT reaction liquid) and then add to the reaction system.*

2.2 qPCR reaction procedure (two-step)

Step	Temperature	Time	Cycles
Stage1	95°C	1 min	1 cycle
Stage2	95°C	5 sec	40 cycles
	60°C	30 sec	
Melt Curve（automatic instrument setting）			

Analysis of result :

The amplification curve and melting curve of qPCR were confirmed after the reaction, and then the standard curve was made for quantitative analysis. The method of analysis is referred to the manual of the instrument operation.

Quality control:

- All components were free from exonuclease, endonuclease and RNase residues.
- Function test: Gene expression was detected by qRT-PCR using 1 pg~1 g total RNA as template. The standard curve was made for the Ct value with the template values of 5 orders of magnitude and the amplification efficiency was calculated, R^2>0.990. The PCR amplification efficiency is in the range of 0.95 to 1.05.