ABScript III RT Master Mix for qPCR

Cat. No. : RK20428

Product components

Components	catalog	Size-1 20 RXN	Size-2 100 RXN
5X ABScript III RT Mix *	RM21478	80 μL	400 μL
Nuclease-free H_2O	RM20214	1.25 mL	2 × 1.25 mL

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

Product Description

ABScript III RT Master Mix for qPCR is developed based on ABScript II Reverse Transcriptase and suitable for two-step RT-qPCR detection. 5X ABScript III RT Mix contains all the reagents needed for reverse transcription reaction (ABScript III 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.

This product is specially optimized for qPCR. The proportionally optimized Random Primers/Oligo $(dT)_{20}VN$ Primer Mix enables cDNA synthesis to progress from each region of RNA transcription efficiently, 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.

Storage

-20℃

Precautions for Use

1. Before use, briefly centrifuge the reagent to the bottom of the tube, pipette to mix slowly and carefuly, and try to avoid producing bubbles.
2. Random Primers and Oligo $(dT)_{20}VN$ Primer have been added to this product, thus gene-specific primers cannot be used.
3. 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.
4. When dividing up the reagents, please be sure to use new disposable tips to avoid contamination among reagents.

Protocol

Preparation of Experiment

1. 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.
2. RNA: Complete and high quality RNA. (Please check whether the RNA is degraded or contaminated before the experiment.
3. Ensure RNA is not 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.

Process of Experiment

1. Reverse transcription

(1) Reverse transcription reaction system

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

Components	20 μL
5X ABScript III RT Mix	4 μL
Total RNA	10 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 too much, make sure that RNA is dissolved in water instead of TE, because TE inhibits the reverse transcription reaction.**

(2) Reverse transcriptional reaction procedure

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

Temperature	Time
55 ℃	15 min
85 ℃	5 min
4 ℃	Hold

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

2. qPCR

The following is after used this product for reverse transcription, select 2X Universal SYBR Green Fast qPCR Mix (ABclonal RK21203) reagent to carry out qPCR reaction in StepOnePlus Real-Time PCR System.

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

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

Component	Volume
2X Universal 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) qPCR reaction procedure （two-step）

Step	Temperature	Time	Cycles
Stage1	95 ℃	3 min	1 cycle
Stage2	95 ℃	5 sec	40 cycles
	60 ℃	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.