

Product components

Components	catalog	Size-1	Size-2
		20 RXN	100 RXN
5X ABScript III RT Mix *	RM21478	80 µL	400 µL
20X gDNA Remover Mix	RM21479	20 µL	100 µL
Nuclease-free H ₂ O	RM20214	1.25 mL	2 × 1.25 mL

* 5X ABScript III RT Mix contains ABScript III Reverse Transcriptase, RNase Inhibitor, dNTPs, Random Primers/Oligo (dT)₂₀VN Primer Mix, etc.

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

Product Description

ABScript III RT Master Mix for qPCR with gDNA Remover is developed based on ABScript III Reverse Transcriptase and suitable for two-step RT-qPCR detection. The 5X ABScript III RT Mix in this product contains all the reagents required for the reverse transcription reaction. The reaction protocol is simple and can be carried out quickly by adding the RNA template and H₂O. The gDNA Remover Mix in this product can completely remove the genomic DNA remaining in the RNA template and make the qPCR results more accurate. The dsDNase is heat-sensitive and can be quickly and irreversibly inactivated under high temperature conditions. Therefore, it only needs one sample to be used to remove genomic DNA contamination and reverse transcription reactions in the same tube.

This product is specially optimized for qPCR. The proportionally optimized Random Primers/Oligo (dT)₂₀VN 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°C

Precautions for Use

1. Please briefly centrifuge them to the bottom of the tube before use, and gently pipette to mix prior to use.
2. Random Primer and Oligo (dT)₂₀VN Primer have been added to this product, thus gene-specific primers cannot be used.
3. The reverse transcription product (cDNA) obtained by using this product is only suitable for qPCR reaction and is 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.

Protocol

Preparation of Experiment

1. Materials 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°C 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
20X gDNA Remover Mix	1 µL
Total RNA	10 pg -1 µg *
Nuclease-free H ₂ O	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
37 °C	2 min
55 °C	15 min
85 °C	5 min
4 °C	Hold

* Product can be applied immediately to the subsequent qPCR reaction, or in -20°C 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 ₂ O	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 H₂O 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 °C	3 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.