ABScript II RT Mix for qPCR with gDNA Remover

Catalog: RK20403

Size: 10 RXN / 100 RXN (20 μL / RXN)

Components:

5X ABScript II RT Mix	RM21452
5X No RT Mix	RM21453
4X gDNA Remover Mix	RM21458
Nuclease-free H_2O	RM20214

List of Components:

Components	10 RXN	100 RXN
5X ABScript II RT Mix*	40 μL	400 μL
4X gDNA Remover Mix	40 μL	400 μL
5X No RT Mix**	20 μL	40 μL
Nuclease-free H_2O	1.25 mL	1.25 mL X 2

** 5X ABScript II RT Mix contains ABScript II Reverse Transcriptase, RNase Inhibitor, dNTPs, Random Primers/Oligo $(dT)_{23}$VN Primer Mix etc.*

*** 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 to reduce the activity of RNase H, to improve thermostability, and to enhance cDNA synthesis efficiency, which ensures the authenticity and repeatability of qPCR results.

ABScript II RT Mix for qPCR with gDNA Remover is developed based on ABScript II Reverse Transcriptase and suitable for two-step RT-qPCR detection. The 5X ABScript II 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 4X gDNA Remover Mix in this product completely removes residual genomic DNA contamination in the RNA template, increasing the accuracy of qualitative results.

4 x gDNA Remover Mix and 5 x ABScript II RT Mix remains unfrozen at - 20 ° C for easy access and use.

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. The proportionally optimized Random Primers/Oligo $(dT)_{23}$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.

Precautions for Use

- In this product, 4X gDNA Remover Mix, 5X ABScript II RT Mix and 5X No RT Mix have high viscosity. Please briefly centrifuge them to the bottom of the tube before use, and gently pipette to mix prior to use.
- Random Primer and Oligo dT Primer have been added to this product, thus gene-specific primers cannot be used.
- 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. Alternatively, the ABScript II cDNA first-strand Synthesis Kit (ABclonal RK20400) can be used when long fragment PCR amplification is desired.
- Replace pipette tips when transferring reagents to avoid cross-contamination.

Requirements

- Materials and Equipment: 1.5 mL RNase-free EP tubes, 200 μL RNase-free PCR tubes、RNase-free Pipette tips, pipettors, PCR instrument (and qPCR instrument), ice or ice box.
- RNA: Complete and high-quality RNA is essential for obtaining high quality cDNA.

- 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.
- qPCR reagent selection guide: cDNA product (RT reaction liquid) can be directly used as the template for qPCR reactions. cDNA volume as the qPCR template cannot exceed 1/10 of the qPCR reaction volume; cDNA can be diluted with RNase Free H_2O. 2X Universal SYBR Green Fast qPCR Mix (ABclonal RK21203) is recommended as a qPCR reagent for follow-up experiments.

Experimental procedure

1. Genomic DNA removal

1.1 Add the following components into the RNase-free reaction tube, mix with pipette and centrifuge briefly:

Components	Volume
4X gDNA Remover Mix	4 μL
Total RNA	1 pg ~1 μg*
Nuclease-free H_2O	to 16 μL

** Add the appropriate amount of RNA according to experimental requirements. When the RNA template is large, ensure that the RNA is dissolved in water instead of TE, because TE inhibits the reaction.*

1.2 Incubate according to the following procedure:

Temperature	Time
37℃	5 min
85℃	5 min
4℃	Hold

2. Reverse transcription response system/negative control

2.1 Add 5X ABScript II RT Mix directly into the reaction tube in step one, mix with pipette and centrifuge briefly:

Components	Volume
5X ABScript II RT Mix	4 μL
The first reaction fluid	16 μL

2.2 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
5X No RT Mix	4 μL
The first reaction fluid	16 μL

** The No RT Control reaction is not required but can be used to verify the gDNA removal efficiency of reverse transcription products, which may not be carried out in normal experimental procedures.*

2.3 Reverse transcription reaction procedure:

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

** Product can be applied immediately to the subsequent qPCR reaction, or stored at - 20℃. Avoid repeated freezing and thawing.*

3. qPCR reaction

Following the reverse transcription of this product, 2X Universal SYBR Green Fast qPCR Mix (ABclonal RK21203) reagents and the StepOnePlus real-time PCR System (Thermofisher) instrument are selected as examples to conduct the follow-up qPCR test (please read the instrument operation manual before the experiment).

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

Components	Volume
2X Universal SYBR Green Fast qPCR Mix	10 μL
cDNA (RT reaction solution)	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 recommended that the volume of cDNA (RT reaction liquid) does not exceed 1/10 of the total volume of the qPCR reaction. If necessary, cDNA should be diluted with Nuclease free H_2O*

3.2 qPCR Response 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)			

4. Results analysis：

Confirm the amplification curves and the melting curve after the qPCR reaction is complete, and compare to the standard curve. The analytical method is shown in the corresponding instrument operation manual.

Quality control

- All components are free from exonuclease, endonuclease and RNase residues.
- Functional test：Gene expression was detected by qRT-PCR using 1 pg~1 g total RNA as template. 100 ng rat genomic DNA was mixed into the total RNA of 1μg rat tissue, after 4X gDNA Remover Mix treatment, qRT-PCR of two pairs of quality control primers was performed. The PCR amplification efficiency is in the range of 0.95 to 1.05. 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., and the Ct value of No RT Control was greater than 35.