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FS-RT-0432 Script Neo RT Master Mix for qPCR

Components	Size 20 RXN	Size 100 RXN
4X Script Neo RT Master Mix *	100 μL	500 μL
Nuclease-free H_2O	1.25 mL	2 × 1.25 mL

* 4X Script Neo RT Master Mix contains Script III Reverse Transcriptase B, RNase Inhibitor, dNTPs, Random Primers/Oligo $(dT)_{20}$VN Primer Mix.

Product Description

Script Neo RT Master Mix for qPCR is an efficient and fast cDNA first-strand synthesis master mix, suitable for two-step RT-qPCR detection. The 4X Script Neo 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 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. 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 $(dT)_{20}$VN 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 Script II cDNA First-Strand Synthesis Kit to conduct experiments.
4. Replace pipette tips when transferring reagents to avoid cross-contamination.

Requirements

1. 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.
2. RNA: Complete and high-quality RNA is essential for obtaining high quality cDNA.
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.

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Experimental procedure

1. Reverse transcription

(1) Reverse transcription reaction system

Add the components to the RNase-free PCR tube on ice according to the following recommendations, mix well and centrifuge briefly.

Components	Volume
4X Script Neo RT Master Mix	5 μL
Total RNA	10 pg -1 μg *
Nuclease-free H_2O	to 20 μL

* Add the appropriate amount of RNA according to the experimental requirements. When the RNA template is too much, make sure that the RNA is soluble in water and not in TE Buffer, as TE inhibits the reverse transcription reaction.

(2) **Reverse transcription reaction procedure**

Temperature	Time
55 °C	15 min
85 °C	5 min
4 °C	Hold

* Product can be applied immediately to the subsequent qPCR reaction, or stored at - 20°C . 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 reagent to carry out qPCR reaction in Step One Plus 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 °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.

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