Removal of gDNA and reverse transcription

1. Prepare the reaction system on ice according to the table below.

Reagent	20 μL System	Final Concentration
5 ×HiFiScript All-in-one qRT MasterMix	4 μL	1×
RNA Template	X μL	10 pg-1 μg
RNase-Free Water	to 20 μL	

Note: This product is a premixed reagent containing reverse primers (Random Primer and Oligo dT Primer) and cannot use gene specific primers.

2. Gently mix the reaction solution, and set the program as below.

Temperature	Time	Reaction
50 °C	15 min	Removal of gDNA + reverse transcription
85 °C	5 sec	Inactivation of enzyme
4 °C	∞	Hold

3. At the end of the reaction, please store at 4 °C or -20 °C (for long-term storage). Repeated freeze-thaw should be avoided. When performing Real Time PCR, the product can be added directly or after dilution as template.

Note: The reverse transcription reaction solution added to the Real Time PCR reaction solution should not exceed 20% at most. Excessive addition can result in inefficient Real Time PCR reactions that cannot be quantified accurately.

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HiFiScript All-in-one RT MasterMix for qPCR

Cat. No. : CW3371S (10 rxns)
CW3371M（100 rxns）
Storage Condition: -20 °C

Components

Component	CW3371S 10 rxns	CW3371M 100 rxns
5×HiFiScript All-in-one qRT MasterMix	40 μL	400 μL
RNase-Free Water	1 mL	2×1 mL

Introduction

HiFiScript All-in-one RT Master Mix for qPCR is a Real Time PCR reverse transcription reaction kit with genomic DNA (gDNA) removal function, using our new high-efficiency reverse transcriptase.

A trace amount of gDNA is often mixed into total RNA purified by common RNA extraction methods. When the cDNA obtained after reverse transcription is used for gene expression analysis by Real Time PCR, the mixed gDNA will be amplified as a template and affect the accuracy of amplification results if there is a pseudogene in the detected target gene, or primers cannot be designed across the intron. This product is premixed with a heat-sensitive DNase with strong DNA decomposition activity. The DNase is rapid, efficient and easy to be inactivated, and gDNA-free cDNA can be obtained easily in one step.

The cDNA synthesized using this product is suitable for qPCR analysis by SYBR method and probe method. It can be combined with quantitative PCR reagents such as SuperStar Universal SYBR Master Mix (CWBIO#CW3360) according to the experimental purpose.

Features

1. Genomic DNA and cDNA synthesis can be removed easily and quickly
   All gDNA removal reagents and reverse transcription reaction reagents are premixed, and gDNA removal and cDNA synthesis can be achieved through a single tube reaction in about 15 minutes.
2. High thermal stability
   Reaction at 50 °C.
3. A uniform reverse transcriptional reaction can be performed on RNA
   Using the most suitable reaction buffer and Primermix (Oligo dT and Random Primer) in the optimal ratio for synthesis of cDNA for Real Time PCR. Uniform and efficient reverse transcription of the entire region of RNA can be performed.

4. High adaptability with Real Time PCR reagents
   Using the components that have the least effect on the reaction system of Real Time PCR. Even if the reverse transcription reaction solution is introduced into the PCR reaction solution up to 20% of the liquid volume, it can show good linearity. Therefore, the kit is suitable for the detection of low abundance mRNA.

Precautions

1. This product contains heat-sensitive DNase. 5×HiFiScript All-in-one qRT Master Mix should be put on ice.
2. 5×HiFiScript All-in-one qRT Master Mix should be briefly centrifuged before use, and pipette gently to mix with a pipettor.
3. RNase contamination should be avoided during operation. It is recommended to separate the RNase-Free Water for reverse transcription reaction and PCR reaction from the RNase-Free Water for other experiments.

Procedure

Denaturation of RNA (optional) *

After the RNA is thermally denatured at 65 °C for 5 minutes, immediately put it on ice to cool.

* After the treatment of the above steps, the reverse transcription efficiency of RNA containing secondary structure can be improved. It is recommended to explore the conditions in the initial experiment.