Tinzyme Co., Limited

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All Script RT Master Mix

Product Number: PCM71

Shipping and Storage

Store at -20℃.

Components

Component	PCM71 10rxns	PCM71 100rxns
5×All Script RT Master Mix	40μl	400μl
RNase-Free Water	1ml	2×1ml

Description

All Script RT Master Mix is a Real Time PCR reverse transcription reaction kit that adds the function of removing genomic DNA (gDNA), using our company's new high-efficiency reverse transcriptase. The total RNA purified by common RNA extraction methods often contains trace amounts of gDNA. When reverse transcribing the cDNA obtained through Real Time PCR for gene expression analysis, if there are pseudogenes detected in the target gene or primers cannot be designed across introns, the mixed gDNA will be used as a template for amplification, affecting the accuracy of amplification results. This product is pre mixed with a thermosensitive DNase with strong DNA decomposition activity. This DNase is effective, efficient, and easily inactivated. With just one step, cDNA without gDNA can be easily obtained.

The cDNA synthesized using this product is suitable for qPCR analysis using both dye and probe methods. According to the experimental purpose, quantitative PCR reagents such as Universal Super SYBR Master Mix (PCM60) can be used in combination.

Feature

1. Can easily and quickly remove genomic DNA and synthesize cDNA
   All gDNA removal reaction reagents and reverse transcription reaction reagents have been pre mixed, and gDNA removal and cDNA synthesis can be achieved in about 15 minutes through a single tube reaction.
2. High thermal stability
   Perform reverse transcription reaction at 50 ℃.
3. Can perform uniform reverse transcription reactions on the entire region of RNA
   The reaction buffer most suitable for cDNA synthesis in RealTime PCR and Primermix (Oligo dT and Random Primer) mixed in the optimal ratio were used to achieve uniform and efficient reverse transcription of the entire region of RNA.
4. High adaptability to Real Time PCR reagents
   The components that have the least impact on the reaction system of Real Time PCR were used, and even when a maximum of 20% reverse transcription reaction solution was introduced into the PCR reaction solution, it still showed good linearity. Therefore, this kit is suitable for the detection of low abundance mRNA.

Note

1. This product uses a thermosensitive DNase, please make sure to place the 5 x All Script RT Master Mix on ice.
2. Before using the 5×All Script RT Master Mix, please briefly centrifuge and collect it to the bottom of the tube, and gently blow with a pipette to mix thoroughly before accurately aspirating.
3. During the operation, RNase contamination should be avoided. RNase Free Water used for reverse transcription and PCR reactions should be stored separately from RNase Free Water used for other experiments to avoid sharing.

Protocol

RNA denaturation (optional steps)*

After denaturing RNA at 65°C for 5 minutes, immediately cool it on ice.

Note: After the above steps of processing, the reverse transcription efficiency containing secondary structure RNA can be improved. It is recommended to explore the conditions during the initial experiment.

Remove genomic DNA reactions and reverse transcription reactions

1. Please prepare the following reaction solution on ice.

Component	20μL system	Final Conc.
5×All Script RT Master Mix	4μL	1×
RNA template	XμL	10pg-1μg
RNase-Free Water	Up to 20μL	

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

2. After gently stirring the reaction solution evenly, proceed with the reaction at the following temperature.

Temperature	Time	Reaction
50℃	15min	DegDNA+reverse transcription reaction
85℃	5sec	Enzyme inactivation reaction
4℃	∞	Hold

3. After the reaction is complete, please store at 4°C or -20°C (long-term) conditions, and cDNA should avoid repeated freeze-thaw. When using Real Time PCR, it can be added directly or diluted as a template.

Note:Please do not exceed 20% of the reverse transcription reaction solution added to the Real Time PCR reaction solution. Excessive addition can lead to low efficiency of Real Time PCR reaction and inaccurate quantification.