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Hifair™ III 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA Digester Plus)

Product description

Hifair™ III 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA Digester Plus) is a readjust premix developed based on Hifair™III Reverse Transcriptase. Compared with Hifair™ II Reverse Transcriptase, Hifair™III Reverse Transcriptase has significantly higher thermal stability and can withstand reaction temperatures up to 60°C, and is suitable for Reverse transcription of RNA templates with complex secondary structures. At the same time, the enzyme enhances the affinity with templates, which is ideal for reverse transcription of a small number of templates and low-copy genes.

The premix contains 5×gDNA Digester Mix and 4×Hifair™ III SuperMix Plus. 5×gDNA Digester Mix can remove residual genomic DNA contamination in RNA templates to ensure more reliable follow-up results. 4×Hifair™ III SuperMix Plus contains all components required for Reverse Transcriptase (Buffer, dNTP, Hifair™ III Reverse Transcriptase, RNase inhibitor, Random Primers/Oligo (dT)18 Primer Mix), RNA template and Rnase-free H_2O are added to reverse transcription, and gDNA digester is terminated simultaneously to ensure the integrity of cDNA.

The product is suitable for two-step RT-QPCR detection, specially optimized for qPCR, proportionally optimized Random Primers/Oligo (dT)18 Primer Mix, enabling cDNA synthesis to be initiated from all regions of RNA transcript with the same reverse transcription efficiency. The authenticity and repeatability of qPCR results are guaranteed to the greatest extent. The reverse transcription product is compatible with SYBR Green method and TaqMan probe method qPCR, Hieff™ UNICON™ qPCR SYBR Green Master Mix, Hieff™ qPCR SYBR Green Master Mix or Hieff™ qPCR TaqMan Probe Master Mix can be selected according to experimental purposes, is used in combination to perform high-performance gene expression analysis.

Components

Components No.	Name	11141ES10 (10T)	11141ES60 (100T)
11141-A	RNase-free H_2O	1 mL	2×1 mL
11141-B	5×gDNA digester Mix	30 μL	300 μL
11141-C	4×Hifair™ III SuperMix plus	50 μL	500 μL

Specifications

Type of end product	cDNA (First-Strand)
PCR method	RT-PCR
Type of reagent	Reverse Transcription
Type of sample	RNA

Optimal reaction temperature	55°C-60°C
Reverse transcriptase	M-MLV

Shipping and Storage

The product is shipped with dry ice and can be stored at -15°C ~ -25°C for 18 months.

Instructions

1. Residual genomic DNA removal

Prepare the following mixture in an RNase Free centrifuge tube and gently blow the mixture with a pipette. Incubate at 42°C for 2 mins.

Components	Volume (μL)
RNase-free H_2O	to 15 μL
5×gDNA digester Mix	3 μL
Total RNA	10 pg -5 μg*
or mRNA	10 pg-500 ng*

[Note]: *: Total RNA input in 20 μL reverse transcription reaction system should not exceed 1 μg. If the expression abundance of the target gene is low, the maximum amount of total RNA is 5 μg; otherwise, the amount of RNA input is too high and may exceed the linear range of subsequent quantitative PCR.

2. Preparation of reverse transcription reaction system (20 μL system)

Add 4×HifairTM III SuperMix Plus directly to the reaction tube in step 1 and gently blow the mixture with a pipette.

Components	Volume (μL)
The product of step 1	15
4×Hifair™ III SuperMix plus	5

3. Perform the reaction under the following conditions

Temperature	Time (min)
25°C	5
55°C	15
85°C	5

[Note]:This Reverse transcription temperature: 55°C is recommended. For high GC content templates or complex templates, the reverse transcription temperature can be raised to 60°C.

Notes

Please wear the necessary PPE, such lab coat and gloves, to ensure your health and safety!