Ver. HB221111

Hifair™ II 1st Strand cDNA Synthesis Kit (gDNA Digester Plus)

Product description

Hifair™ II 1st Strand cDNA Synthesis Kit (gDNA Digest plus) is a cDNA first strand synthesis kit containing gDNA removal steps. This kit is based on Hifair™ II Reverse Transcriptase. The thermostability of this enzyme has been greatly improved, and it can withstand the reaction temperature up to 50°C. It is suitable for reverse transcription of RNA templates with complex secondary structures. At the same time, the enzyme enhances the affinity with the template, and is suitable for reverse transcription of a small number of templates and low copy genes. Hifair™ II The ability of Reverse Transcriptase to synthesize full-length cDNA has also been improved, which can expand to 10 kb of cDNA. The kit contains gDNA digester, which can remove the genomic DNA contamination left in the RNA template and ensure more reliable follow-up results. The kit provides two kinds of cDNA synthesis primers: Random Primers N6 and oligo (dT) 18. Users can select Random Primers N6, Oligo (dT) 18 or Gene Specific Primers as reverse transcription primers as required. The synthesized single strand cDNA products can be directly used for subsequent PCR or qPCR reactions.

Components

Components No.	Name	11121ES60 (100T)
11121-A	RNase-free H_2O	2×1 mL
11121-B	5×gDNA digester Buffer	200 μL
11121-C	gDNA digester	100 μL
11121-D	5×Hifair™ II Buffer plus	400 μL
11121-E	Hifair™ II Enzyme Mix	200 μL
11121-F	Oligo $(dT)_{18}$ (50 μM)	100 μL
11121-G	Random Primers N6 (50μM)	100 μL

Specifications

Final product type	cDNA (First-Strand)
PCR method	RT-PCR
Reaction Format	Isolated fraction
Sample type	RNA

Optimal reaction temperature	42°C~50°C
Reverse transcriptase	MMLV

Shipping and Storage

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

Instructions

1. Selection of Reverse Transcription Primers

1.1 If the template is of eukaryotic origin, it is recommended to select Oligo (dT) 18 and pair it with the 3'Poly A tail of eukaryotic mRNA to obtain the highest yield of full-length cDNA.

1.2 For reverse transcription of prokaryotic RNA, please select Random Primers N6 or gene specific primers.

1.3 Random Primers N6 is widely applicable to such templates as mRNA, rRNA, tRNA, small RNA and LncRNA.

1.4 When using Random primers N6 for cDNA synthesis below 2 kb, the usage of Random primers N6 is 1-2 μL. When more than 2 kb of cDNA is synthesized, the usage of Random primers N6 is 0.4-1 μL.

2. First-strand cDNA Synthesis Procedure

2.1 If the experiment requires the removal of residual genomic DNA

2.1.1 Prepare the following mixture in a RNase-free centrifuge tube, gently pipetting the mixture well. Incubate at 42°C for 2 min.

Components	Volume
RNase-free H_2O	To 10 μL
5× gDNA digester Buffer	2 μL
gDNA digester	1 μL
Total RNA	1 ng-5 μg*
or mRNA	1 ng-500 ng*

[Note]: * If the follow-up experiment is qPCR, the Total RNA input is 1 ng - 1 μg; the input amount of mRNA is 1 ng-100 ng. If the expression abundance of the gene is low, up to 5 ug Total RNA or 500 ng mRNA can be committed.

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

Components	Volume
Reaction fluid from the previous step	10 μL
5× Hifair™ II Buffer plus	2 μL*
Hifair™ II Enzyme Mix	2 μL
Random Primers N6 (50 μmol/L)	1 μL
or Oligo $(dT)_{18}$ (50 μmol/L)	or 1 μL
or Gene Specific Primers (2 μmol/L)	or 1 μL
RNase-free H_2O	up to 20 μL

[Note]: a) Reverse transcription primers: Random Primers N6 mixed 1:1 with Oligo (dT) 18 is recommended for fluorescence quantification experiments.

b) 5 × Hifair ™ II Buffer plus added (*): Due to the effect of gDNA digester buffer, only 2 μL was required in this system.

c) It is recommended to add reverse transcription primers after mixing with 5 × Hifair ™ II Buffer plus to ensure complete inhibition of gDNA digester activity.

2.1.3 Reverse transcription program settings

Temperature	Time
25°C	5 min
42°C	30 min
85°C	5 min

[Note]: Reverse transcription temperature: 42°C is recommended. For high GC content templates or complex templates, the reverse transcription temperature can be increased to 50°C.

2.1.4 Reverse transcription products can be stored at -20°C for a short time, if long-term storage is required, it is recommended to store at -80°C after packing to avoid repeated freeze-thaw.

2.2 If the experiment does not need to remove genomic DNA

2.2.1 Preparation of reverse transcription reaction system (20 μL volume).

Components	Volume
Total RNA	1 ng -5 μg
or mRNA	1 ng-500 ng
5× Hifair™ II Buffer plus	4 μL*
Hifair™ II Enzyme Mix	2 μL
Random Primers N6 (50 μmol/L)	1 μL
or Oligo $(dT)_{18}$ (50 μmol/L)	or 1 μL
or Gene Specific Primers (2 μmol/L)	or 1 μL
RNase-free H_2O	up to 20 μL

[Note]: a) Reverse transcription primers: Random Primers N6 mixed 1:1 with Oligo (dT)18 is recommended for fluorescence quantification experiments.

b) 5 × Hifair ™ II Buffer plus added (*): Because there was no effect of gDNA digester buffer, 4 μL needed to be added in this system.

[Optional Steps]: For complex templates, RNA, H_2O, reverse transcription primers are recommended to be incubated at 65 ° C for 5 min and quickly cooled on ice. Reverse transcriptase and Buffer were then added.

2.2.2 The reverse transcription program is set up according to the reverse transcription program after genome removal described above.

Notes

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