HB220429

Hifair™ III 1st Strand cDNA Synthesis Kit (gDNA digester plus)

Product Information

Product name	Cat#	Size
HifairTM III 1st Strand cDNA Synthesis Kit (gDNA digester plus)	11139ES60	100 T

Product Description

Hifair™ III 1st Strand cDNA Synthesis Kit (gDNA digester plus) is a first-strand synthesis kit of cDNA that contains gDNA removal steps. The kit was developed based on Hifair™ III Reverse Transcriptase. The enzyme cDNA synthesis speed is fast, the thermal stability is greatly improved, can withstand reaction temperatures up to 60°C, suitable for reverse transcription of RNA templates with complex secondary structure. At the same time, the enzyme enhances the affinity with the template, suitable for a small number of templates and reverse transcription of low-copy genes. Hifair™ III Reverse Transcriptase's ability to synthesize full-length cDNA has also been improved, with a synthesis of up to 19.8 kb cDNA.

The kit contains gDNA digester Mix, which digests residual genomic DNA contamination from the RNA template, ensuring more reliable subsequent results. The kit offers two cDNA synthesis primers: Random Primers N6 and Oligo $(dT)_{18}$, with the user's choice of Random as needed Primers N6, Oligo $(dT)_{18}$ or Gene Specific Primers as reverse transcription primers, synthesized single-stranded cDNA The product can be used directly for subsequent PCR or qPCR reactions.

Product Components

Component Number	Components	Cat#/Size 11139ES60 (100 T)
11139-A	RNase-free H_2O	2×1 mL
11139-B	5×gDNA Digester Mix	300 μL
11139-C	10×Hifair™ III Super Buffer	200 μL
11139-D	Hifair™ III RT Enzyme Mix	100 μL
11139-E	Random Primers N6 (50 μmol/L)	100 μL
11139-F	Oligo $(dT)_{18}$ (50 μmol/L)	100 μL

[Note]: 10× Hifair™ III Super Buffer contains gDNA digester inhibitors and dNTPs. Hifair™ III RT Enzyme Mix include RNase inhibitor.

Shipping and Storage

The product is shipped with ice packs and can be stored at -20℃ for 18 months.

Cautions

1. The preparation of the reaction solution should be completed on ice, and the operation process should avoid RNase contamination.
2. It is recommended that the RNA is soluble in water rather than TE, as TE interferes with gDNA removal as well as reverse transcription.
3. 5×gDNA digester Mix、10×Hifair™ III Super Buffer、Hifair™ III RT Enzyme Mix gently upside down and carefully centrifuge to the bottom before use. Use a pipette with the right range when aspirating liquid, and do not insert the tip too deep under the liquid level.
4. qPCR experiments recommend genomic removal steps to ensure that the results are more realistic and reliable.
5. This product is for research use **ONLY**!

On the selection of reverse transcription primers

1. If the template is of eukaryotic origin, it is recommended to choose Oligo $(dT)_{18}$, paired with the 3' PolyA tail of eukaryotic mRNA, to obtain the maximum yield of full length cDNA.

2. Reverse transcription of prokaryote RNA should use Random Primers N6 or gene-specific primers.

3. Random Primers N6 is widely applicable to mRNA, rRNA, tRNA, small RNA and LncRNA plates.

First-strand cDNA synthesis procedure

1. If the experiment requires the removal of residual genomic DNA

1.1 gDNA digestion

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 15 μL
5×gDNA Digester Mix	3 μL
Total RNA	10 pg -5 μg*
or mRNA	10 pg-500 of*

[Note**]:** * If the follow-up experiment is qPCR, the Total RNA input is 10 pg - 1 μg; the input amount of mRNA is 10 pg-100 ng. If the expression abundance of the gene is low, up to 5 ug Total RNA or 500 ng mRNA can be committed.

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

Components	Volume
Reaction fluid from the previous step	15 μL
10×Hifair™III Super Buffer	2 μL
Hifair™III RT Enzyme Mix	1 μL
Random Primers N6 (50 μmol/L)	1 μL
or Oligo $(dT)_{18}$ (50 μmol/L)	or 1 μL
or Gene Specific Primer (2 μmol/L)	or 1 μL
RNase-free H_2O	up to 20 μL

[Note**]:** Reverse transcription primers: For subsequent qPCR, Random Primers N6 and Oligo $(dT)_{18}$ are recommended 1:1 mixed. It is recommended to add 10× Hifair™ III Super Buffer mixing before adding reverse transcription primers to ensure complete inhibition of gDNA digester activity. After the system is prepared, please gently blow and mix well with a pipette.

1.3 Reverse transcription program settings

Temperature	Time
25°C	5 min
55°C*	15 min*
85°C	5 min

[Note**]:** *Reverse transcription temperature: 55°C is recommended. For high GC content templates or complex templates, the reverse transcription temperature can be increased to 60°C. Reverse transcription time: 15 min is recommended for subsequent qPCR, and 30 min-60 min for subsequent PCR.

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. If the experiment does not need to remove genomic DNA

2.1 RNA template denaturation

Prepare the following mixture in a RNase-free centrifuge tube, gently pipetting the mixture well. Incubate at 65°C for 5 min, place the tube quickly on ice and incubate for 3 mins.

Components	Volume
RNase-free H_2O	To 17 μL
Total RNA	10 pg -5 μg
or mRNA	10 pg-500 of
Random Primers N6 (50 μmol/L)	1 μL
or Oligo $(dT)_{18}$ (50 μmol/L)	1 μL
or Gene Specific Primer (2 μmol/L)	or 1 μL

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

Components	Volume
Reaction fluid from the previous step	17 μL
10×Hifair™ III Super Buffer	2 μL
Hifair™ III RT Enzyme Mix	1 μL

2.3 Follow up with the above procedure for follow-up experiments.