HB220429

Hifair™ III Reverse Transcriptase

Product Information

Product Name	Cat#	Size
Hifair™ III Reverse Transcriptase	11111ES92	10000 U
	11111ES93	5×10000 U

Product Description

Hifair™ III Reverse Transcriptase is an updated version of Hifair™ II Reverse Transcriptase obtained through genetic engineering technology. It has higher cDNA synthesis ability and speed, thermal stability and reaction temperature limit (up to 60°C) than Hifair™ II Reverse Transcriptase. The synthesized cDNA product is up to 19.8 kb. Hifair™ III Reverse Transcriptase enhances the affinity of the templates and is suitable for reverse transcription of RNA templates with complex secondary structure or low copy genes.

Package Information

Component Number	Components	Cat#/Size	
		11111ES92 (10000 U)	11111ES93 (5×10000 U)
11111-A	5×Hifair™ III Buffer	250 μL	1250 μL
11111-B	Hifair™ III Reverse Transcriptase (200 U/μL)	50 μL	250 μL

Application

Full-length cDNA library construction; End-point PCR; Real-time PCR

Unit Definition

One unit is defined as the amount of enzyme required for incorporating 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using Oligo(dT) as primers.

Shipping and Storage

The product is shipped with ice packs and can be stored at -20°C for 1 year.

Product Notes

1. Keep the experimental area clean and use RNase-free supplies.
2. All operations should be carried out on ice to prevent RNA degradation.
3. High quality RNA samples are recommended for efficient reverse transcription.
4. For your safety and health, please wear lab coats and disposable gloves for operation.
5. This product is for research use **ONLY**!

Protocol for first strand cDNA Synthesis reaction

1. Denaturation of RNA template (This step is optional, denaturation of RNA template helps to open the secondary structures, which will improve the yield of the first strand cDNA.)

Components	Volume
RNase free ddH_2O	Up to 13 μL
Oligo $(dT)_{18}$ (50 μmol/L)	1 μL
or Random Primers (50 ng/μL)	1 μL
or Gene Specific Primers (2 μmol/L)	1 μL
RNA template	Total RNA: 10 pg -5 μg or mRNA:10 pg-500 ng

Incubating at 65°C for 5 minutes, then transferring on ice immediately to chill for 2 minutes. Brief centrifugation to collect reaction liquid, add the reverse transcription reaction solution as shown in the following table. Gently pipette to mix.

2. Preparation of the reaction mixture (20 μL volume)

Components	Volume
Mixture of previous step	13 μL
5×Hifair™ III Buffer	4 μL
dNTP Mix (10 mM)	1 μL
Hifair™ III Reverse Transcriptase (200 U/μL)	1 μL
RNase inhibitor (40 U/μL)	1 μL

3. Perform the reaction under the following conditions

Temperature	Duration
25°C	5 min
55°C	15-30 min
85°C	5 min

[Note]: 1) When using Random Primers, incubate at 25°C for 5 min; if using Oligo $(dT)_{18}$ or Gene Specific Primers, this step can be omitted.

2) The recommended reverse transcription temperature is 55°C. For templates with complicated secondary structures or high GC content, it is recommended to raise the reaction temperature to 60°C.

3) The reverse transcription time can be extended to 45-60 min, which helps to increase the yield.

4) Heat at 85°C for 5 min to inactivate the reverse transcriptase.

※ The product can be directly used in PCR or qPCR reactions, or stored at -20°C for a short-term storage. It is recommended to aliquot the products and store at -80°C for long-term storage. Avoid repeated freezing and thawing.