Ver.EN20230621

Hifair™ III Reverse Transcriptase

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.

Specifications

Cat.No.	11111ES92/11111ES93/11111ES08
Size	10,000 U/50,000 U/ 5 mL
Unit Definition	One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C using Oligo(dT) as primers.

Components

Components No.	Name	11111ES92	11111ES93	11111ES08
11111-A	5×Hifair™ III Buffer	250 µL	1,250 µL	25 mL
11111-B	Hifair™ III Reverse Transcriptase (200 U/µL)	50 µL	250 µL	5 mL

Storage

This product should be stored at -25~-15°C for 18 months.

Instructions

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 (µL)
RNase free ddH_2O	to 13
Oligo (dT)18 (50 µM) or Random Primers (50 µM) or Gene Specific Primers (2 µM)	1 or 1 or 1
RNA template	Total RNA: 10 pg -5 µg or mRNA:10 pg-500 ng

Incubate at 65°C for 5 minutes, place on ice rapidly and let rest for 2 minutes. Brief centrifugation to collect reaction liquid, add the reverse transcription reaction solution in the table below, and gently pipette to mix.

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

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

3. Perform the reaction under the following conditions

Temperature	Time
25°C*	5 min
55°C**	15-30 min***
85°C****	5 min

* 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.

** 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.

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

**** Heat at 85°C for 5 min, which is order to inactivate the reverse transcriptase.

4. 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 them at -80°C for a long-term storage. Avoid repeated freezing and thawing.

Notes

1. Keep the experimental area clean; Wear clean gloves and masks during operation; all supplies used in the experiment must be RNase free to prevent RNase.
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. This product is for research use only.
5. Please operate with lab coats and disposable gloves，for your safety.