Hifair™V Reverse Transcriptase

Product description

Hifair™V Reverse Transcriptase is an updated vesion of Hieff™ M-MLV (H-) Reverse Transcriptase obtained by genetic engineering technology. It has higher cDNA synthesis ability, thermal stability and reaction temperature limit (up to 60°C) than Hieff™ M-MLV (H-) Reverse Transcriptase. The synthesized cDNA product is up to 10 kb. Hifair™ V 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.

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Specifications

Cat.No.	11300ES92 / 11300ES93 / 11300ES98
Size	10,000 U / 5×10,000 U/ 200,000 U
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	11300ES92	11300ES93	11300ES98
11300-A	Hifair™ V Reverse Transcriptase (200 U/μL)	50 μL	5×50 μL	1 mL
11300-B	5×Hifair™V Buffer	250 μL	1.25 mL	5 mL

Storage

This product should be stored at -25~-15°C for 2 years.

Instructions

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 μmol/L)	1
or Random Primers (50 μmol/L)	or 1
or Gene Specific Primers (2 μmol/L)	or 1
RNA template	X *

[Note]: *: Total RNA: 1-5 μg or mRNA: 1-500 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 (μL)
Mixture of previous step	13
5×Hifair™ V Buffer	4
dNTP Mix (10 mmol/L)	1
Hifair™ V 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
42°C**	15~30 min
85°C***	5 min

[Note]:

*: Incubating at 25°C for 5 min is required only for using the random hexamers. Please skip this step when using Oligo (dT)18 or Gene Specific Primer.

**: The recommended reverse transcription temperature is 42°C. For templates with complicated secondary structures or high GC content, it is recommended to raise the reaction temperature to 50~55°C.

***: Heating at 85°C for 5 min to inactivate reverse transcriptase.

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

The product is suitable for one-step RT-qPCR, it is recommended to add 10-20 U reverse transcriptase for every 25 μL reaction system, or gradually increase the amount of reverse transcriptase according to the actual situation.

Notes

1. Please keep the experimental area clean; Clean gloves and masks should be worn during operation. All the consumables used in the experiment should be RNase free to prevent RNase contamination.
2. All procedures should be performed on ice to prevent RNA degradation.
3. High quality RNA samples are recommended to ensure high efficiency of reverse transcription.
4. This product is for research use only.
5. Please operate with lab coats and disposable gloves，for your safety.