HB220429

Hifair™ V Reverse Transcriptase (Glycerol-free)

Product Information

Product Name	Cat#	Size
Hifair™ V Reverse Transcriptase (Glycerol-free)	11301ES06	3 KU
	11301ES12	12 KU
	11301ES62	120 KU
	11301ES75	300 KU

Product Description

Hifair™ V Reverse Transcriptase is an updated version of Hieff™ M-MLV (H^-) Reverse Transcriptase obtained through 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.

Package Information

Component Number	Components	Cat#/Size			
		11301ES06（3 KU）	11301ES12 (12 KU)	11301ES62 (120 KU)	11301ES75 (300 KU)
11301	Hifair™ V Reverse Transcriptase (600 U/μL)	5 μL	20 μL	200 μL	500 μL

Application

One-step RT-qPCR; Gene Clone; Fluorescent Quantitation.

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 4°C for 6 months.

Cautions

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 personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.

Protocol for 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 μmol/L)	1
or Random Primers (50 μmol/L)	1
or Gene Specific Primers (2 μmol/L)	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.

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 (600 U/μL)	200 U
RNase inhibitor (40 U/μL)	1
RNase free ddH_2O	to 20

3. Perform the reaction under the following conditions

Temperature	Time
25°C	5 min
42°C	15-30 min
85°C	5 min

[Note]:

a) This step is required only for using the random hexamers. Please skip when using Oligo $(dT)_{18}$ or Gene Specific Primer.

b) 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.

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.