HB220429

Hifair™ III Reverse Transcriptase, Glycerol-free

Product Information

Product Name	Cat#	Size
Hifair™ III Reverse Transcriptase, Glycerol-free	11297ES09	6 KU
	11297ES12	12 KU
	11297ES75	300 KU

Product Description

Hifair™ III Reverse Transcriptase is an updated version 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 19.8 kb. Hifair™ III Reverse Transcriptase enhances the affinity of the templates and is suitable for reverse transcription of RNA templates with complex structure or low copy genes.

Package Information

Component Number	Components	Cat#/Size		
		11297ES09 (6 KU)	11297ES12 (12 KU)	11297ES75 (300 KU)
11297	Hifair™ III Reverse Transcriptase (600 U/μL)	10 μL	20 μL	500uL

Application

One-step RT-qPCR; Gene Cloning; 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 Poly(A). Oligo(dT) as template-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 lab coats and disposable gloves for operation.

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)	or 1 μL
or Gene Specific Primers (2 μmol/L)	or 1 μL
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
Mixture of previous step	13 μL
5×Hifair™ V Buffer	4 μL
dNTP Mix (10 mmol/L)	1 μL
Hifair™ III Reverse Transcriptase (600 U/μL)	200 U
RNase inhibitor (40 U/μL)	1 μL
RNase-free ddH_2O	Up to 20 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]:

a) 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.

b) 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.

c) To obtain higher yield, the RT (reverse transcription) incubation time can be prolonged to 45-60 min.

d) 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.