

Hifair™ IV Reverse Transcriptase

Product Information

Product Name	Cat#	Specification
Hifair™ IV Reverse Transcriptase	11112ES92	10000 U
	11112ES93	5×10000 U

Product Description

Hifair™ IV Reverse Transcriptase is a new reverse transcriptase based on Hieff™ M-MLV (H-) Reverse Transcriptase. The enzyme possesses high thermal stability (withstand 60°C), which is suitable for reverse transcription of RNA templates with complex secondary structures compared with Hieff™ M-MLV (H-) Reverse Transcriptase. Hifair™ IV Reverse Transcriptase enhances the affinity with the template, which is suitable for reverse transcription and mRNA library preparation of low-copy genes. The ability of Hifair™ IV Reverse Transcriptase to synthesize full-length cDNA has also been improved which enable the enzyme to amplify cDNA up to 10 kb.

Product Components

Components	11112ES92 (10000 U)	11112ES93 (5×10000 U)
11112-A 5×Hifair™ IV Buffer	250 µL	1200 µL
11112-B Hifair™ IV Reverse Transcriptase (200 U/µL)	50 µL	250 µL

Applications

preparation of full-length cDNA library; end-point PCR; real-time quantitative PCR, etc.

Shipping and Storage

All the components are shipped with ice packs and can be stored at -20°C for one year.

Cautions

- 1) Please keep the experimental area clean; wear clean gloves and masks during operation; all consumables used in the experiment must be RNase free to prevent RNase contamination;
- 2) All operations should be performed on ice to prevent RNA degradation;
- 3) To ensure the success of reverse transcription, it is recommended to use high-quality RNA samples;
- 4) For your safety and health, please wear a lab coat and disposable gloves for operation.
- 5) For research use only!

First-strand cDNA synthesis steps

1. RNA denaturation (optional step, RNA denaturation helps to open the secondary structure, which can greatly improve the yield of first-strand cDNA.)

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

Heated at 65°C for 5 min, then quickly cooled on ice for 2 min. After a brief centrifugation to collect the reaction solution, add the reverse transcription reaction solution in the table below, and mix by gently pipetting.

2. Preparation of reverse transcription reaction solution

Components	Volume
RNA denaturation product	13 μ L
5 \times Hifair™ IV Buffer	4 μ L
dNTP Mix(10 mM)	1 μ L
Hifair™ IV Reverse Transcriptase (200 U/ μ L)	1 μ L
RNase Inhibitor (40 U/ μ L)	1 μ L

3. Reverse transcription program setup

Temperature	Duration
25°C	5 min
50°C	15 min
85°C	5 min

[Notes]

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) Reverse transcription temperature: 50°C is recommended. For templates with high GC content or complex templates, the reverse transcription temperature can be increased to 55°C -60°C ;

3) Heating at 85°C for 5 min, the purpose is to inactivate reverse transcriptase.

※ The reverse transcription product can be used immediately for subsequent PCR or qPCR reaction, and can also be stored at -20°C for a short period of time. If long-term storage is required, it is recommended to store at -80°C after aliquoting to avoid repeated freezing and thawing.