FISHER MOLECULAR BIOLOGY

FS-RT-1022 cDNA Synthesis Kit (plus gDNA Eraser)

Catalog No.	Specification	Storage/Shelf life
FS-RT-1022	50 rxn	-20°C /three year
FS-RT-1023	200 rxn	-20°C/three year

Introduction

First Strand cDNA Synthesis Kit is a complete system for the efficient synthesis of first-strand cDNA from different types of RNA that can synthesize cDNA up to 13 kb. This product contains **gDNA Eraser**, which can quickly and completely remove genomic contamination.
The reverse transcription primer mix provided along with **First Strand cDNA Synthesis Kit** is the mixture of pd(N)9 and oligo dT(18).Reverse transcription of various cDNAs in the sample. Suitable for reverse transcription of various RNAs such as mRNA, lncRNA and circRNA.
The kit can also be used for gene-specific reverse transcription, such as miRNAreverse transcription.

Kit Components

Components	FS-RT-1022 50 RXNS	FS-RT-1023 200 RXNS
gDNA Eraser	50 µL	200 µL
RNase Inhibitor (40U/uL)	2 x 1,000 units	8 x 1,000 units
5XgDNA Eraser Buffer	100 µL	400 µL
Reverse Transcriptase (200 U/ul)	10,000U	40,000U
5 X Reverse Transcriptase Buffer	0.5 mL	1.0 mL
RNase-Free ddH2O	1.5 mL	1.5 mL
Reverse Transcription Primer Mix* *(it includes Oligo dT and Random Primer)*	100 µL	400 µL
dNTPs (10mM each)	50 µL	200 µL

Advantages

Can efficiently synthesize full-length first-strand cDNA up to 13kb

1. Can withstand reaction temperatures up to 55 ° C
2. Fully provide all the components needed for the Reverse Transcription reaction

Kit application

1. cDNA library construction.
2. RT-qPCR reaction and RT-PCR reaction.
3. Primer extension.
4. RNA sequencing.

Active unit

The Reverse Transcriptase concentration is 200U/µL.
A unit of activity (U) is defined as: Poly (A) as the template and Oligo (dT) as the primer, Reaction at 37°C for 10 minutes can mix 1 nmole of dTTP into the amount of enzyme required for acid-insoluble substances.

Purity: The purity is >90% by Coomassie blue staining SDS-PAGE. The product is free from endonuclease, exonuclease and RNase contamination.
All components of the kit must be store at -20°C. Keep control RNA at -70°C for longer stoker

Required Material Reagents but not supplied

1. RNase-free 200μL microcentrifuge tube
2. Pipettes and tips (to avoid RNase contamination, RNase-free pipette tips with filter cartridges must be used)
3. Disposable gloves, masks and other protective equipment
4. Constant temperature water bath
5. Wear latex gloves and a mask during the whole process of RNA extraction.

Operation steps

1. **Removal of genomic DNA response from RNA preparations (on ice):**
   a) Use a RNase free 200 ul tubes to make the reaction:

Reagents to add	Volume
5X gDNA Eraser Buffer	2.0 μL
gDNA Eraser	1.0 μL
Template RNA*	**0.5-5 μg**
RNase-Free Water	To 10.0 μL

*Incubate at 42 ° C for 2 min (or room temperature for 5 min)Store at 4 ° C
Use the prepared RNA as a template for the Reverse Transcription reaction:

2. **Reverse transcription reaction** (on ice)

Reagent	Volume
Reaction solution (obtained from the first step)	10.0 μL
Reverse Transcription Primer Mix[1] or Gene Specific Primers (10μM)	2.0 μL or 1.0 μL
dNTPs(10mM each)	1.0 μL
RNase-Free ddH2O	to 15.0 μL

*[1]The Reverse Transcription Primer Mix is a mixture of oligo dT and pd(N)9.

3. **First Strand cDNA Synthesis**
   After heating the mixture at 70 ° C for 5 min, it was quickly placed on ice for cooling.
   Make a brief centrifugation, add the following components:

Reagents	Volume
Step 1 reaction solution	
5XReverse Transcriptase Buffer	4.0 μL
Reverse Transcriptase*[2]	1.0 μL
RNase Inhibitor*[3]	1.0 μL

*[2] The amount of Reverse Transcriptase should be reduced to 0.05-0.5 μl when less than 0.5 μg of Total RNA (such as reverse transcription of viral RNA). Otherwise, subsequentPCR amplification may result in non-specific amplification products.

*[3] When adding less than 0.5 μg of Total RNA, it is recommended to add 1 μl of RNase Inhibitor **(cat.# FS-RT-1152)**

4. Mix gently and keep at 37°C for 60 minutes*.

* When using Gene Specific Primers, it is recommended that the reverseTranscription reaction conditions be set to 42°C for 60 min.

5. Heat at 95 °C for 5 minutes, then cool on ice or store at -20 °C for use*.

* Reverse transcription products can be used immediately for subsequent PCR or qPCR reactions, or can 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 dispensing to avoid repeated freezing and thawing.