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rtStar™ First-Strand cDNA Synthesis Kit (3' and 5' adaptors)(12 Reactions)

Cat#: AS-FS-003L

Instruction Manual version 1.0

Product Summary

Product description

The rtStar™ First-Strand cDNA Synthesis Kit (5' and 3' Adaptors) is designed to create cDNA libraries from small RNAs for qPCR detection. The method sequentially ligate 3'-Adaptor with its 5'-end to the 3'-end of the RNAs, and 5'-Adaptor with its 3'-end to the 5'-end of the RNAs. The non-ligation ends of 3' and 5' Adaptors are blocked by modification. 3' Adaptor contains a universal priming site for Reverse Transcription (RT) Primer. The Kit uses a novel protocol to produce higher yields and lower adaptor-dimer formation. The Spike-in RNA can be used for monitoring the cDNA synthesis efficiency and as a quantitative reference.

Kit components

The provided kit components are sufficient for up to 12 reactions. The recommended range of total RNA sample input amount is 100 ng ~ 2.0 μg.

Kit component	Amount	Storage	Shipping
3' Adaptor	12 μL	-20°C	Dry Ice
3' Ligation Enzyme Mix	12 μL	-20°C	Dry Ice
3' Ligation Reaction Buffer	88 μL	-20°C	Dry Ice
5' Adaptor	12 μL	**-80°C**	Dry Ice
5' Ligation Enzyme Mix	12 μL	-20°C	Dry Ice
5' Ligation Reaction Buffer	6 μL	-20°C	Dry Ice
10 mM ATP	30 μL	-20°C	Dry Ice
First-Strand Synthesis Reaction Buffer	96 μL	-20°C	Dry Ice
0.1 M DTT	36 μL	-20°C	Dry Ice
2.5 mM dNTP Mix	24 μL	-20°C	Dry Ice
Reverse Transcription Primer	powder	-20°C	Dry Ice
Reverse Transcriptase	12 μL	-20°C	Dry Ice
RNase Inhibitor	24 μL	-20°C	Dry Ice
RNA Spike-in	powder	-20°C	Dry Ice
RNA spike-in qPCR Primer Mix	powder	-20°C	Dry Ice
Nuclease-free Water	1 mL	-20°C	Dry Ice

Additional required materials

- RNase-free 200 μL PCR tubes
- Microcentrifuge for 200 μL tubes
- Thermal cycler
- Pipettors and tips

Protocol

3´ Adaptor Ligation

Note *To reconstitute RNA Spike-in and Reverse Transcription Primer, add 80 μL and 60 μL Nuclease-free Water to the corresponding tube respectively.*

For smaller amounts of total RNA at 100 ng, reduce the use of 3´Adaptor by diluting 1:2 in Nuclease-free Water.

1. Combine the reagents in a RNase-free 200 μL PCR tube according to the order in the table:

Nuclease-free Water	variable
Input RNA	1-7.3 μL
3' Adaptor	1 μL
RNA Spike-in	0.5 μL
Total volume	8.8 μL

2. Incubate the mix at 70°C for 2 min. Transfer the tube to ice.
3. Ligate 3' Adaptor to the RNA by adding the Components:

3' Ligation Reaction Buffer	7.2 μL
3' Ligation Enzyme Mix	1 μL
RNase Inhibitor	1 μL
Total volume	9.2 μL

4. Incubate at 25°C for 1 hour.

Note *To improve the ligation efficiency for end-methylated RNA such as piRNA, longer incubation at lower temperature (e.g.16°C for 18 hrs) may be used. However, some concatemerization products may occur.*

Hybridization of Reverse Transcription Primer

The excess of the unligated 3' Adaptor is hybridized with Reverse Transcription Primer to prevent adaptor-dimer formation. The double stranded DNA duplexes are not substrates for T4 RNA Ligase 1 and will not ligate to 5' Adaptor in the 5' Adaptor Ligation step.

Note *For smaller amounts of total RNA at 100 ng, reduce the use of Reverse Transcription Primer by diluting 1:2 in Nuclease-free Water.*

5. Add following reagents to the tube from Step 4 and mix well:

Nuclease-free Water	1 µL
Reverse Transcription Primer	1 µL
Total volume	20 µL

6. Hybridize the primers at 75°C for 5 min, 37°C for 15 min and 25°C for 15 min.

5´ Adaptor Ligation

Note *For smaller amounts of total RNA at 100 ng, reduce the use of 5' Adaptor by diluting 1:2 in Nuclease-free Water. Store unused 5' Adaptor at -80°C.*

7. Aliquot 1.1 × number of samples (µL) of 5' Adaptor in a Nuclease-free 200 µL PCR tube.

8. Denature the adaptor at 70°C for 2 min and then immediately chill on ice. Use the denatured 5' Adaptor within 30 minutes.

9. Add the following components to the Reverse Transcription Primer hybridized 3' Adaptor ligation product from Step 6 and mix well:

5´ Adaptor (denatured)	1 µL
10 mM ATP	2.5 µL
5´ Ligation Reaction Buffer	0.5 µL
5´ Ligation Enzyme Mix	1 µL
Total volume	25 µL

10. Incubate at 25°C for 1 hr.

Reverse Transcription

11. Mix the components in a RNase-free 200 µL PCR tube:

Adaptor Ligated RNA	25 µL
First-Strand Synthesis Reaction Buffer	8 µL
0.1 M DTT	3 µL
2.5 mM dNTP Mix	2 µL
RNase Inhibitor	1 µL
Reverse Transcriptase	1 µL
Total volume	40 µL

12. Incubate at 45°C for 60 min and chill on ice. The cDNA may be used immediately for qPCR. If not used immediately, heat inactivate the enzyme at 70°C for 15 min and store at -20°C.

OPTIONAL

The RNA Spike-in qPCR Primer Mix contains the PCR primers for the RNA Spike-in. Add 100 µL Nuclease-free Water to reconstitute the primers. Use 1 µL primer in a 10 µL qPCR reaction.

Troubleshooting

Problem	Cause and suggestion
Low 5' Adaptor ligation efficiency	• Degradation of single stranded 5' RNA adaptor. Keep it cold at all times and store in single-use aliquots. • Free 3' Adaptor from Step 4 ligated with 5' Adaptor. Ensure 3' Adaptor and Reverse Transcription Primer are used in the indicated amounts.
PCR signal from the first-strand synthesis reaction even without reverse transcriptase	• Contamination of genomic DNA in the template RNA. Perform DNase treatment of the RNA sample. If this does not solve the problem, RNA samples or other reagents may have been contaminated with PCR products.
PCR signal in no-template PCR reaction	• Contamination of the cDNA template or PCR reagents by amplified PCR product. • Exposing the reactions to elevated temperatures (*i.e.* room temperature) during any part of the protocol increases the risk of background signals. It is important that the reagents and assembled reactions are kept cold (on ice or 4°C) at all times.
Weak PCR signals	• Verify PCR positive control has good signals • Adjust real-time PCR cycler gain settings if available • RNA samples may contain PCR inhibitors. Re-purify or use an alternative RNA extraction method. Less cDNA volume may be used to lower overall PCR inhibitor.
No fluorescent signal detected during qPCR	• Check the presence of PCR product by gel electrophoresis. • Check any procedural errors during first-strand cDNA synthesis.
No fluorescent signal detected during PCR, but PCR product is present by gel electrophoresis	• Check qPCR cycler settings are correct for SYBR® Green or FAM/FITC. • Check qPCR optical reading is at the correct step of qPCR cycles. • Adjust the baseline in the qPCR cycler software.