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rtStar™ First-Strand cDNA Synthesis Kit (3' adaptor)

Cat#: AS-FS-002

Instruction Manual version 1.0

Product summary

Product description

The rtStar™ First-Strand cDNA Synthesis Kit (3' adaptor) is designed to create cDNA libraries from small RNAs for qPCR detection. The method is based on ligation of a 5'-adenylated/3'-blocked oligonucleotide adapter (Adenylated 3' Ligation Adapter) to the 3' ends of the small RNAs, which provides the universal binding site for the reverse transcription (RT) primer. The protocol uses a single-tube format for ligation, reverse transcription, and subsequent dilution of the cDNA library with 10 ng ~ 2.0 µg starting total RNA. The Spike-in RNA can be used for monitoring the cDNA synthesis efficiency and as a reference for qPCR data comparison.

Kit components

The volumes provided are sufficient for preparation of up to 12 reactions. The recommended amount of starting material can vary from 10 ng to 2.0 µg total RNA according to the expression of interested RNA.

Kit components	Amounts	Storage	Shipping
3' Ligation Adapter	12 µl	-20°C	Dry Ice
RNA Ligase	12 µl	-20°C	Dry Ice
5×Ligase Reaction Mix	50 µl	-20°C	Dry Ice
RNase Inhibitor	12 µl	-20°C	Dry Ice
Universal RT Primer Mix	12 µl	-20°C	Dry Ice
Reverse Transcriptase	12 µl	-20°C	Dry Ice
Universal Reverse PCR Primer	200 µl	-20°C	Dry Ice
RT Reaction Master Mix	150 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
- Pipettors and tips
- Microcentrifuge for 200 µl tubes
- Thermal cycler

Protocol

3'-Adapter ligation to the small RNAs

1. Dilute the 3' Ligation Adapter supplied in the Kit with Nuclease-free Water at 1/10 for 10 ~ 500 ng total RNA, or at 1/3 for 500 ng ~ 2 µg total RNA. Dissolve RNA Spike-in with 20 µl Nuclease-free Water.
2. Mix the following components in a 200 µl PCR tube for each sample.

Total RNA in Nuclease-free Water	4.5 µl
diluted 3' Ligation Adapter	1 µl
RNA Spike-in	1 µl
Total volume	6.5 µl

3. Incubate the mix in a thermal cycler at 70°C for 2 min; Chill on ice immediately. Add the following components orderly.

5×Ligase Reaction Mix	2 µl
RNase Inhibitor	0.5 µl
RNA Ligase	1 µl
Final volume	10 µl

4. Incubate at 22°C for 60 min, 72°C for 2 min, and chill on ice for 2 min.

First-Strand cDNA synthesis

5. Add 1 µl Universal RT Primer Mix to the tube from Step 4, mix gently.
6. Incubate at 65°C for 2 min. Place on ice for at least 2 min.
7. Prepare Reverse Transcription Master Mix and add 10 µl to each sample above.

RT Reaction Master Mix	8.5 µl
RNase Inhibitor	0.5 µl
Reverse Transcriptase	1 µl
Total volume	10 µl

8. Incubate at 42°C for 60 min; inactivate the reaction at 85°C for 5 min. Chill on ice.

Note *Store the cDNA synthesis reaction at -20°C, or proceed directly to PCR. Reconstitute the RNA Spike-in qPCR Primer Mix by adding 100 µl Nuclease-free Water. Use 1 µl RNA Spike-in qPCR Primer Mix in 10 µl qPCR reaction system.*

Small RNAs cDNA library validation

9. The generated Small RNAs cDNA library can be verified by running a qPCR on the RNA Spike-in control. Mix the following reagents:

RNA Spike-in qPCR Primer Mix	1 µl
Small RNAs cDNA library	2 µl
qPCR Mix (SYBR® Green)	5 µl
Nuclease-free Water	2 µl
Total volume	10 µl

10. Run the qPCR cycles.

Cycles	Temperature	Time
1	95°C	10 min
40	95°C	10 s
	55 ~ 65°C	1 min
Melting curve analysis		

11. A Ct value less than 30 for the RNA Spike-in indicates a successful Small RNAs cDNA synthesis.

Troubleshooting

Problem	Suggestion
PCR signal in samples amplified from first-strand synthesis reactions performed without reverse transcriptase	This typically indicates contamination of the template RNA with genomic DNA. Perform DNase treatment of the RNA sample. If this does not solve the problem, RNA samples or other reagents may be contaminated with PCR products.
PCR signal in no-template PCR reaction	This typically indicates contamination of the cDNA template or PCR reagents with 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 cool (on ice or 4°C) at all times.
Generated signals are weak	• On some real-time PCR cyclers, gain-settings are adjustable. Make sure the gain settings of your real-time PCR cycler have been set to accommodate the signals generated from the specific assay. • RNA samples may contain PCR inhibitors. Further purification or an alternative RNA extraction method may be necessary. Check positive controls. In order to reduce the inhibition, you can try to perform real-time PCR with less cDNA volume.
No fluorescent signal is detected during the PCR	Confirm whether have procedural error during first-strand cDNA synthesis. Check whether have a PCR product by agarose gel electrophoresis.
No fluorescent signal detected during the PCR, but a PCR amplicon can be detected by agarose gel electrophoresis	• Check that the filter in the real-time PCR cycler was set to either SYBR® Green or FAM/FITC. • Check that the optical read is at the correct step of the real-time PCR cycles. • Adjust the baseline in the real-time PCR cycler software.

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