GO BEYOND RNA
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rtStar™ First-Strand cDNA Synthesis Kit

Cat#: AS-FS-001

Instruction Manual version 1.03

Product summary

Product description

The rtStar™ First-Strand cDNA Synthesis Kit is designed to create cDNA libraries from total RNAs for qPCR. This kit allows reverse transcription at high temperatures and without RNase H activity, ensuring efficient reverse transcription of targets with strong secondary structures and providing increased specificity, higher yields of cDNA. The RNA Spike-in 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 pg to 5 µg of total RNA according to the expression of interested RNA.

Kit components	Amounts	Storage	Shipping
5 × RT Reaction Buffer	50 µl	-20°C	Dry Ice
Reverse Transcriptase	12 µl	-20°C	Dry Ice
RNase Inhibitor	15 µl	-20°C	Dry Ice
2.5 mM dNTP Mix	15 µl	-20°C	Dry Ice
Random Primers	15 µl	-20°C	Dry Ice
Oligo(dT)$_{18}$	15 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

1. Dilute Template Total RNA
   Adjust the volume of template RNA sample to 10 µl using Nuclease-free Water.

2. Prepare reagents
   Gently thaw all kit components except for Reverse Transcriptase, and immediately place on ice. Mix by vortexing. Spin down all reagents.
   Note *The first time to use this kit, please reconstitute the RNA Spike-in by adding 50 µl Nuclease-free Water to the tube. Mix by vortexing and spin down. Leave on ice for 20 ~ 30 min to fully dissolve the RNA Spike-in. Vortex again, then spin down.*

3. Combine Annealing Mix according to Table
   If performing first-strand cDNA synthesis on multiple RNA samples, it is recommended to prepare an Annealing Mix of the Primer, dNTP Mix and RNA Spike-in (in the proportion indicated in Table). 10% excess volume for pipetting losses is recommended.

Oligo(dT)$_{18}$, or Random Primers, or Gene-Specific Primer(GSP)	1.0 µl
2.5 mM dNTP Mix	1.0 µl
RNA Spike-in	2.0 µl
Template Total RNA	10.0 µl
Total volume	14.0 µl

4. Incubate in a thermal cycler at 65°C for 5 min, and then immediately place on ice for at least 1 min. Collect the contents of the tube by brief centrifugation.

5. Combine cDNA Synthesis Mix

cDNA Synthesis Mix is recommended to prepare for multiple RNA samples. It includes the components in the following table. And 10% excess volume for pipetting losses should be well advised to take into account.

5 × RT Reaction Buffer	4.0 μl
RNase Inhibitor	1.0 μl
Reverse Transcriptase	1.0 μl
Total volume	6.0 μl

6. Add cDNA Synthesis Mix to the tube from Step 4. Vortex the sample briefly to mix, and collect by brief centrifugation. Incubate as follows:

   Oligo(dT)$_{18}$ or GSP primed: 30 min at 45°C

   Random Primers primed: 10 min at 25°C, followed by 30 min at 45°C

7. Terminate the reactions at 85°C for 5 min. Chill on ice.

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

Troubleshooting

Problem	Suggestions
Target mRNA contains strong transcriptional pauses	• Use Random Primers instead of Oligo(dT)$_{18}$ in the first-strand reaction. • Increase the temperature of first-strand reaction (up to 55°C). • Use PCR primers closer to the 3´ terminus of the target cDNA.
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. To avoid amplification of contaminating genomic DNA, Primers are recommended to span an exon-exon junction.
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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