ATRScript First Strand cDNA Synthesis Kit

Instructions for Use of Products ATR-R6021 and ATR-R6022.

Quick Protocol

ATRScript First Strand cDNA Synthesis Kit contains all reagents necessary to synthesize first-strand cDNA from total or polyA$^+$ RNA. Compared with M-MLV Reverse Transcriptase, **ATRScript** further improves thermal stability (maintains activity at 55°C) and is suitable for the reverse transcription of RNA templates with complex secondary structures. In addition, **ATRScript** adds several point mutations that further enhance the template-binding affinity, processivity, efficiency, and reaction speed of cDNA synthesis and has a higher tolerance to common reverse transcription inhibitors. **The enzyme is preblended with an RNase inhibitor, which effectively protects the RNA from degradation**. The kit also contained ATRScript Reaction Buffer, two optimized primers, dNTP Mixture, DTT and nuclease-free water.

Kit Contents

Components	ATR-R6021 20 rxns	ATR-R6022 50 rxns
ATRScript Reverse Transcriptase [a]	20 µL	50 µL
5X ATRScript Reaction Buffer	100 µL	250 µL
dNTP Mixture (10 mM each)	20 µL	50 µL
Oligo(dT)$_{18}$ Primer, 50 µM	20 µL	50 µL
Random Hexamer Primer, 50 µM	20 µL	50 µL
DTT (100 mM)	20 µL	50 µL
Water, nuclease-free	1.25 mL	1.25 mL

[a] It contains RNase inhibitor.

Storage

All components of the kit should be stored at -20°C.

Protocols

I. First Strand cDNA Synthesis

After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Template RNA	total RNA	0.1 ng - 5 µg
	or polyA$^+$ RNA	10 pg - 0.5 µg
	or specific RNA	0.01 pg - 0.5 µg
Primer	Oligo (dT)$_{18}$ primer	1 µL
	or Random Hexamer primer	1 µL
	or Gene-specific primer	15-20 pmol
Water, nuclease-free		to 13 µL
Total volume		13 µL

2. Optional. If the RNA template is GC-rich or contains secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min. Chill on ice, spin down and place the vial back on ice.
3. Add the following components in the indicated order:

5X ATRScript Reaction Buffer	4 µL
DTT (100 mM)	1 µL
dNTP Mixture (10 mM each)	1 µL
ATRScript Reverse Transcriptase	1 µL
Total volume	20 µL

4. Mix gently and centrifuge briefly.
5. **For oligo(dT)$_{18}$ or gene-specific primed cDNA synthesis, incubate for 60 min at 42°C. For random hexamer primed synthesis, incubate for 60 min at 39°C. For oligo(dT)$_{18}$ and random hexamer primed cDNA synthesis, incubate for 60 min at 40°C.**
6. Terminate the reaction by heating at 80°C for 5 min.

The reverse transcription reaction product can be directly used in PCR applications or stored at -20°C for less than one week. For longer storage, -70°C is recommended. cDNA should avoid repeated freezing and thawing.

II. PCR Amplification of First Strand cDNA

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume, typically 2 µL.

Additional protocol information is available in Product Information #ATR-R6021 and #ATR-R6022, available online at: www.atrned.com