Product Information

ATRScript First Strand cDNA Synthesis Kit

Catalogue Number	Size
ATR-R6021	20 rxns (20 µl/rxn)
ATR-R6022	50 rxns (20 µl/rxn)

Product Description

ATRScript First Strand cDNA Synthesis Kit contains all reagents necessary to synthesize first-strand cDNA from total or polyA$^+$ RNA using **ATRScript Reverse Transcriptase**, a Moloney Murine Leukemia Virus (M-MLV)-derived reverse transcriptase. **ATRScript Reverse Transcriptase** is a novel reverse transcriptase obtained using in vitro molecular evolution technology based on M-MLV (RNase H-) Reverse Transcriptase. Compared with M-MLV Reverse Transcriptase, **ATRScript** further improves thermal stability and is suitable for the reverse transcription of RNA templates with complex secondary structures. The enzyme with reduced RNase activity maintains activity at 55°C which is suitable for reverse transcription of RNA templates with complex secondary structures and for synthesis of cDNA up to 13 kb. 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 that are found in a wide variety of samples that cause other currently available RTs to perform inefficiently.

The enzyme is preblended with an RNase inhibitor, which effectively protects the RNA from degradation. The kit also contained two optimized primers for reverse transcription and nuclease-free water. **An anchored-oligo(dT)18 primer** forces the primer to anneal to the beginning of the polyA tail. The optimized **random hexamer primers** provided random and consistent priming sites covering the entire RNA template, including both mRNAs and non-polyadenylated RNAs. Random primers or gene-specific primers (GSP) can be selected as reverse transcription primers according to the requirements.

Applications

- First strand cDNA synthesis for RT-PCR and RT-qPCR
- Construction of full length cDNA libraries

Highlights

- High yields of full-length cDNA up to 13 kb
- Complete cDNA synthesis in 10 to 60 minutes
- Efficient cDNA synthesis in a wide temperature range (37–55°C)
- Significantly improved resistance to a variety of inhibitors that can interfere with cDNA synthesis

Kit Contents

Components	ATR-R6021 20 rxns	ATR-R6021 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.

Shipping

The kit is shipped with ice gel transport at ≤0°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
	polyA$^+$ RNA	10 pg - 0.5 µg
	specific RNA	0.01 pg - 0.5 µg
Primer	Oligo $(dT)_{18}$ primer	1 µL
	Random Hexamer primer	1 µL
	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 5 min at 25°C followed by 60 min at 42°C. However, many targets can be detected after a much shorter incubation time. For example, 10 minutes incubation is enough for a 2 kb cDNA synthesis.

Note. For GC-rich RNA templates the reaction temperature can be increased up to 55°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. Normally, 2 µL of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 µL total volume. Also, for some PCR enzymes the rate of amplification may be affected by the amount of template. Thus, refer to the PCR enzyme instruction manual to assess the appropriate amount of template to use. If non-specific amplification or no product is obtained after amplification, results can be improved by treating the cDNA with RNase H.

Important Notes for Successful cDNA Synthesis

1. Template RNA

Intact RNA of high purity is essential for sensitive RT-PCR detection. RNA should have a minimum A260/A280 ratio of 1.7 or higher. Assess RNA integrity prior to cDNA synthesis. The most common method is denaturing agarose gel electrophoresis followed by ethidium bromide staining. If both 18S and 28S rRNA appear as sharp bands after electrophoresis of total eukaryotic RNA, the RNA is considered to be intact. The 28S rRNA band should be approximately twice as intense as the 18S rRNA. Any smearing of rRNA bands is an indication of degraded mRNA. If this occurs, a new sample of total RNA should be prepared. Total cellular RNA isolated by standard methods is suitable for use with the kit. Purified RNA must be free of salts, metal ions, ethanol and phenol to avoid inhibiting the cDNA synthesis reaction. Trace contaminants can be removed by ethanol precipitation of the RNA followed by two washes of the pellet with cold 75% ethanol.

For RT-PCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I, RNase-free (#36273) to remove trace amounts of DNA. Always perform a control reaction which includes all components for RT-PCR except for the reverse transcriptase enzyme. PCR product in the negative control (RT-) indicates

the reaction is contaminated with DNA. Perform DNase I digestion prior reverse transcription.

2. Primers selection for Reverse Transcription

For PCR:

For eukaryotic RNA templates, oligo d(T) priming is preferred for most applications because it ensures that all cDNA copies terminate at the 3´ end of the mRNA and produces the longest contiguous cDNA. An anchored Oligo$(dT)_{18}$ Primer forces the primer to anneal to the start of the polyA tail, thereby preventing priming at internal sites in the polyA tail. However, two other priming choices are possible if desired.

When a gene-specific primer is used in a cDNA synthesis reaction, the cDNA product can be used only for amplification of that transcript. This priming method gives good results when the amount of RNA is limiting (below 10 ng) and only one particular cDNA is desired. GSP has the highest specificity. If GSP fails in the 1st strand cDNA synthesis, Oligo$(dT)_{18}$ Primer or Random hexamers can be used for reverse transcription.

Random hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be used as the template of Random hexamers. Random hexamers can be used as primers, when oligo$(dT)_{18}$ or GSP can not effectively guide cDNA synthesis for the target region has complex secondary structure and high GC content, or the template is prokaryotic origin.

For qPCR:

Mixing Oligo$(dT)_{18}$ with Random hexamers enables the same efficiency of cDNA synthesis in each region of the mRNA, which helps to improve the authenticity and reproducibility of quantitative results.

3. Prevent RNase Contamination

It is important to to prevent contamination with RNase derived from equipment and/or solutions. Extra precautions should be taken during sample preparation, including use of clean disposable gloves, dedication of a table exclusively for RNA preparation, and avoiding unnecessary speaking during assembly, to prevent the RNase contamination from sweat or saliva.

Disposable plastic equipment should be used. Glass tools should be treated with the following protocol prior to use. Hot-air sterilization (180℃, 60 min) and Treatment with 0.1% diethylpyrocarbonate (DEPC) at 37℃, for 12 hours, followed by autoclaving at 120℃ for 30 min to remove DEPC. It is recommended that all the equipment be used exclusively for RNA preparation.

Reagents for RNA preparation, including purified water, should be prepared with heat sterilized glass tools (180℃, 60 min) or, if possible, those treated with 0.1% DEPC solution and autoclaved. Reagents and purified water should be exclusively used for RNA preparation.

Precautions and Disclaimer

This product is for R&D use only, not for use in drug, diagnostic procedures, household, or other uses. When working with the product, always wear a suitable lab coat and disposable gloves, protective eyewear. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@atrmed.com). To the extent allowed by law, ATR-MED Inc. will not be liable for special, incidental, indirect, punitive, multiple, or consequential damages in connection with or arising from this document, including your use of it. By use of this product, you accept all the terms and conditions of ATR-MED products. All trademarks are the property ATR-MED unless otherwise specified.

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