Product Information

ATRScript First Strand cDNA Synthesis Kit

Kit Contents

Components	ATR-R602-2 (50 reactions)
ATRScript Reverse Transcriptase[a]	50 µL
5X ATRScript Reaction Buffer	250 µL
dNTP Mixture (10 mM each)	50 µL
Oligo(dT)$_{18}$ Primer (50 µM)	50 µL
Random Hexamer Primer (50 µM)	50 µL
DTT (100 mM)	50 µL
Water, nuclease-free	1 mL

[a] Premixed with RNase inhibitor to ensure RNA integrity.

Product Description

The ATRScript First Strand cDNA Synthesis Kit provides a comprehensive set of reagents optimized for the synthesis of first-strand complementary DNA (cDNA) from total RNA or polyadenylated (polyA+) RNA. The kit utilizes ATRScript Reverse Transcriptase, a recombinant enzyme derived from Moloney Murine Leukemia Virus (M-MLV). This novel variant, engineered through *in vitro* directed evolution from an RNase H-minus (RNase H-) form of M-MLV Reverse Transcriptase, exhibits superior thermal stability compared to its wild-type counterpart. This enhanced thermostability enables efficient reverse transcription of RNA templates with complex secondary structures, maintaining enzymatic activity at temperatures up to 55°C and facilitating the synthesis of cDNA fragments up to 5 kb in length.

ATRScript Reverse Transcriptase incorporates targeted point mutations that significantly enhance template binding affinity, processivity, catalytic efficiency, and reaction kinetics during cDNA synthesis. Additionally, the enzyme demonstrates exceptional tolerance to common reverse transcription inhibitors prevalent in diverse biological samples, thereby overcoming performance limitations observed in conventional reverse transcriptases. The inclusion of an RNase inhibitor in the enzyme preparation ensures robust protection against RNA degradation, preserving template integrity during reverse transcription.

The kit includes two optimized primers to accommodate varied experimental needs: an anchored Oligo(dT)$_{18}$ primer, which ensures precise annealing at the 5' initiation site of the polyA tail for efficient cDNA synthesis from polyadenylated mRNAs, and random hexamer primers, which provide broad and uniform binding across the RNA template, including both messenger RNAs (mRNAs) and non-polyadenylated RNAs. The choice of primer—Oligo(dT)$_{18}$, random hexamers, or gene-specific primers (GSP)—depends on the specific requirements of the downstream application, such as reverse transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR (RT-qPCR), or cDNA library construction.

Applications

- Synthesis of first-strand cDNA for RT-PCR and RT-qPCR
- Construction of full-length cDNA libraries

Highlights

- High-yield synthesis of full-length cDNA fragments up to 13 kb
- Rapid cDNA synthesis completed within 10 to 60 minutes
- Robust performance across a broad temperature range (37–55°C)
- Enhanced resistance to common inhibitors of reverse transcription
- Integrated RNase inhibitor for superior RNA template protection
- Versatile primer options for diverse RNA templates and experimental designs

Source

Recombinantly expressed and purified from an *Escherichia coli* strain harboring the cloned gene.

Unit definition

One unit is defined as the amount of enzyme that incorporates 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37°C, utilizing poly(A)•oligo(dT)$_{25}$ as the template-primer.

Storage Buffer Composition

20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% (v/v) NP-40, 50% (v/v) glycerol

5X ATRScript Reaction Buffer Composition

250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$

Storage

Store all kit components at -20°C. Promptly refreeze the DTT (100 mM) solution following use.

Protocols

I. First Strand cDNA Synthesis

Thaw kit components on ice, gently mix, and briefly centrifuge prior to use. Proceed as follows:

1. In a sterile, nuclease-free tube on ice, assemble the following reagents in the indicated order:
2. In a sterile, nuclease-free tube on ice, sequentially add the reagents as listed.

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

3. (Optional) For RNA templates with high GC content or extensive secondary structure, mix gently, centrifuge briefly, and incubate at 65°C for 5 minutes. Immediately chill on ice, centrifuge, and return to ice.
4. Add the following components in the specified sequence:

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

5. Gently mix the reaction and centrifuge briefly.
6. Incubate as follows:

- For Oligo(dT)$_{18}$ or gene-specific primer-initiated synthesis: 42°C for 60 minutes.
- For random hexamer-initiated synthesis: 25°C for 10 minutes, followed by 39°C for 60 minutes.

Note: For GC-rich RNA templates, elevate the reaction temperature to 55°C.

7. Inactivate the enzyme by heating at 80°C for 5 minutes.

The resultant cDNA can be utilized directly in downstream PCR applications or stored at -20°C for short-term use (up to one week). For extended storage, maintain at -70°C to minimize freeze-thaw cycles.

II. PCR Amplification of First Strand cDNA

The first-strand cDNA product may be directly employed in PCR or qPCR reactions. The volume of cDNA input should not surpass 10% of the total PCR reaction volume. Typically, 2 µL of cDNA is sufficient for a 50 µL PCR reaction. Optimize template quantity based on the specific PCR enzyme guidelines, as excessive input may influence amplification efficiency. In cases of non-specific amplification or absence of product, pretreatment of the cDNA with RNase H may enhance outcomes.

Important Considerations for Successful cDNA Synthesis

1. Template RNA

The integrity and purity of RNA are paramount for achieving sensitive and reproducible RT-PCR results. Prior to cDNA synthesis, evaluate RNA quality via denaturing agarose gel electrophoresis with ethidium bromide staining. Intact RNA is evidenced by distinct 18S and 28S rRNA bands, with the 28S band exhibiting approximately twice the intensity of the 18S band. Smearing indicates degradation, warranting sample replacement. Total RNA isolated via standard protocols is compatible, provided it is free from contaminants such as salts, metal ions, ethanol, and phenols. Residual contaminants can be eliminated through ethanol precipitation and subsequent washes with 75% ethanol (chilled). For RT-PCR applications, ensure RNA is devoid of genomic DNA; pretreat with RNase-free DNase I if necessary. Include a no-reverse-transcriptase control to verify the absence of DNA contamination.

2. Primer selection for Reverse Transcription

For PCR:

Oligo(dT) priming is optimal for eukaryotic mRNA, as it initiates synthesis from the poly(A) tail, yielding full-length cDNA copies. Anchored Oligo(dT)$_{18}$ primers mitigate internal priming within the poly(A) tract. Gene-specific primers (GSPs) enable targeted transcript amplification, ideal for low-abundance RNA (<10 ng) or when specificity is required. Although GSPs confer high selectivity, alternative priming with Oligo(dT)$_{18}$ or random hexamers may be employed if initial attempts fail. Random hexamers, exhibiting broad specificity, are versatile for various RNA species, including mRNA, rRNA, and tRNA.

For qPCR:

A combination of Oligo(dT)$_{18}$ and random hexamers optimizes cDNA yield and consistency. Recommended incubation: 40°C for 60 minutes.

3. Prevent RNase Contamination

Rigorous precautions are essential to avert RNase contamination during RNA handling. Utilize disposable gloves, designate RNA-

exclusive workspaces, and avoid sources of airborne RNases (e.g., from skin or breath). Prefer RNase-free plasticware; glassware should be dry-heat sterilized and treated with 0.1% diethyl pyrocarbonate (DEPC), followed by autoclaving. Dedicate equipment solely for RNA workflows. Prepare reagents with sterilized or DEPC-treated glassware, using nuclease-free water reserved exclusively for RNA applications.

Precautions and Disclaimer

This product is designated for research use only and is not intended for therapeutic, diagnostic, household, or other non-research applications. Employ standard laboratory protective equipment, including lab coats, disposable gloves, and safety goggles. When incorporating radioactive nucleotides, comply with institutional radiation safety protocols. For comprehensive safety data, consult the material safety data sheets (MSDSs) available at www.atrmed.com or via email request to info@atrmed.com. To the maximum extent permitted by applicable law, ATR-MED Inc. disclaims all liability for special, incidental, indirect, punitive, or consequential damages arising from the use of this product or associated documentation. Product utilization constitutes acceptance of ATR-MED's terms and conditions. All trademarks referenced herein are owned by ATR-MED unless otherwise indicated.

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