

Product Information

ATRScrip Reverse Transcriptase

Contents

Components	ATR-R601 (10,000 Units)
ATRScrip Reverse Transcriptase (200 U/μL)	50 μL
5X ATRscrip Reaction Buffer	1 mL
DTT (100 mM)	50 μL

Product Description

ATRScrip Reverse Transcriptase is a recombinant enzyme derived from Moloney Murine Leukemia Virus (M-MLV). This novel variant has been engineered through in vitro directed evolution from an RNase H-minus (RNase H-) form of M-MLV Reverse Transcriptase, exhibiting superior thermal stability relative to the wild-type enzyme. This enhanced thermostability renders it particularly suitable for the reverse transcription of RNA templates possessing complex secondary structures. The enzyme maintains activity at elevated temperatures up to 55°C, enabling efficient reverse transcription of structurally challenging RNA templates and the synthesis of complementary DNA (cDNA) fragments up to 5 kb in length. Furthermore, ATRscrip incorporates targeted point mutations that augment template affinity, processivity, catalytic efficiency, and reaction kinetics during cDNA synthesis. Notably, it demonstrates increased tolerance to prevalent inhibitors encountered in diverse biological samples, thereby surpassing the performance limitations of conventional reverse transcriptases.

Applications

- Synthesis of first-strand cDNA for reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR (RT-qPCR)
- Construction of comprehensive full-length cDNA libraries

Highlights

- High-yield production of full-length cDNA fragments extending up to 5 kb
- Expedited cDNA synthesis achievable in 10 to 60 minutes
- Robust cDNA synthesis across a wide temperature spectrum (37–55°C)
- Augmented resilience against inhibitors that may compromise cDNA synthesis efficacy

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 ATRscrip Reaction Buffer Composition

250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl₂

Storage

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

Shipping

The kit is shipped on gel ice packs.

Protocols

I. First Strand cDNA Synthesis

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

- In a sterile, nuclease-free tube on ice, assemble the following reagents in the indicated order:
- 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

- (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
ATRScrip 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

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