Product Information

ATRScript Reverse Transcriptase

Kit Contents

Components	ATR-R6021 10,000 Units
ATRScript Reverse Transcriptase, (200 U/µL)	20 µL
5X ATRScript Reaction Buffer	100 µL

Product Description

ATRScript Reverse Transcriptase, an enzyme derived from Moloney Murine Leukemia Virus (M-MLV). ATRScript Reverse Transcriptase, a novel variant developed through in vitro molecular evolution based on M-MLV (RNase H-) Reverse Transcriptase, offers enhanced thermal stability compared to M-MLV Reverse Transcriptase. This attribute renders it apt for reverse transcription of RNA templates characterized by intricate secondary structures. The enzyme, with reduced RNase activity, remains functional at 55°C, facilitating reverse transcription of RNA templates featuring complex secondary structures and cDNA synthesis up to 13 kb. Moreover, ATRScript incorporates several point mutations to bolster template binding affinity, processivity, efficiency, and reaction speed during cDNA synthesis. Notably, it exhibits heightened tolerance to common reverse transcription inhibitors commonly present in diverse samples, overcoming inefficiencies observed with other currently available reverse transcriptases.

Applications

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

Highlights

- **High yields of full-length cDNA, extending up to 13 kb**
- **Rapid completion of cDNA synthesis within 10 to 60 minutes**
- **Effective cDNA synthesis across a broad temperature range (37–55°C)**
- **Enhanced resistance to various inhibitors that may impede cDNA synthesis**

Storage

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

Shipping

The kit is shipped with ice gel.

Protocols

I. First Strand cDNA Synthesis

Upon thawing, gently mix and centrifuge the kit components before storing on ice. Follow the steps below:

1. 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

2. Optional. For GC-rich or secondary structured RNA templates, gently mix the contents, centrifuge briefly, and incubate at 65°C for 5 minutes. Chill on ice, spin down, and return the vial to ice.
3. Add the components in the specified order.

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

4. Gently mix and briefly centrifuge the mixture.
5. For oligo$(dT)_{18}$ or gene-specific primed cDNA synthesis, incubate at 42°C for 60 minutes. For random hexamer primed synthesis, incubate at 25°C for 10 minutes followed by 39°C for 60 minutes.

Note. For GC-rich RNA templates, the reaction temperature can be increased to 55°C

6. Terminate the reaction by heating at 80°C for 5 minutes.

The reverse transcription product can be used directly in PCR applications or stored at -20°C for up to one week, with -70°C recommended for longer storage, to avoid repeated freezing and thawing.

II. PCR Amplification of First Strand cDNA

The first strand cDNA synthesis product can be directly used in PCR or qPCR. The volume of the first strand cDNA synthesis reaction mixture should not exceed 1/10 of the total PCR reaction volume. Typically, 2 µL of the first strand cDNA synthesis reaction mixture is used as a template for PCR in a total volume of 50 µL. The amount of template may affect the amplification rate, thus, refer to the PCR enzyme instruction manual for guidance. If non-specific amplification or no product is obtained, treating the cDNA with RNase H may improve results.

Important Considerations for Successful cDNA Synthesis:

1. Template RNA

High-quality RNA with minimal contamination is crucial for sensitive RT-PCR detection. RNA integrity should be assessed prior to cDNA synthesis, typically through denaturing agarose gel electrophoresis followed by ethidium bromide staining. Sharp bands of both 18S and 28S rRNA indicate intact RNA, with the 28S band ideally twice as intense as the 18S band. Smearing of rRNA bands suggests mRNA degradation, necessitating sample replacement. Total cellular RNA isolated using standard methods is suitable for kit use, provided it is free of contaminants such as salts, metal ions, ethanol, and phenol. Trace contaminants can be removed via ethanol precipitation followed by washes with cold 75% ethanol. For RT-PCR, RNA templates must be devoid of DNA contamination. Before cDNA synthesis, treatment with RNase-free DNase I can eliminate trace DNA. A control reaction without reverse transcriptase can confirm absence of DNA contamination.

2. Primers selection for Reverse Transcription

For PCR:

Oligo(dT)priming is preferred for eukaryotic RNA templates, ensuring termination of cDNA copies at mRNA 3′ ends, yielding contiguous cDNA. Anchored Oligo$(dT)_{18}$ Primer prevents priming at internal sites in the polyA tail. Gene-specific primers (GSP) allow amplification of specific transcripts, suitable for limited RNA amounts (<10 ng) and when only one cDNA is desired. GSP offers high specificity, but if unsuccessful, Oligo$(dT)_{18}$ Primer or random hexamers can be used. Random hexamers, with low specificity, are suitable for diverse RNA templates, including mRNA, rRNA, and tRNA.

For qPCR:

Combining Oligo$(dT)_{18}$ with random hexamers enhances cDNA synthesis efficiency and reproducibility. Incubation at 40°C for 60 minutes is recommended for Oligo$(dT)_{18}$ and random hexamer-primed cDNA synthesis.

Prevent RNase Contamination

Strict measures are necessary to prevent RNase contamination during sample preparation, including use of disposable gloves, dedicated RNA preparation areas, and minimizing oral contamination. Plastic equipment is preferred, while glass tools should undergo hot-air sterilization and treatment with 0.1% DEPC followed by autoclaving. Equipment dedicated solely for RNA preparation is recommended. Reagents, including purified water, should be prepared with heat-sterilized glass tools or those treated with 0.1% DEPC solution and autoclaved, exclusively for RNA preparation.

Precautions and Disclaimer

This product is intended for research and development (R&D) purposes only and is not suitable for use in drugs, diagnostic procedures, households, or other applications. When handling the product, always wear appropriate laboratory attire, including a lab coat, disposable gloves, and protective eyewear. When working with radioactive tracers, adhere to standard safety protocols for handling radioactive materials. For detailed safety information, refer to the relevant material safety data sheets (MSDSs), which are available online as PDF

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