ProScript cDNA synthesis Kit

Cat# no.: A9003

Store at -20°C

For Research use Only

1.1 Ordering Information

Kit components	A9003-10	A9003-25	A9003-50	A9003-100
ProScript MuLV Reverse transcriptase (200U/µL)	10µL	25µL	50µL	100µL
Pro RNase Inhibitor (40U/µL)	10µL	25µL	50µL	100µL
Random hexamers (20µM)	10µL	25µL	50µL	100µL
Oligo (dT)s (20µM)	10µL	25µL	50µL	100µL
dNTPs mix (10mM)	10µL	25µL	50µL	100µL
10X RT reaction buffer	20µL	50µL	100µL	200µL
Nuclease-free water	500µL	1.2mL	2.5ml	5mL

1.2 Description

ProEnz ProScript cDNA synthesis kit consists of a buffer, dNTPs, Oligo (dT)s, Random hexamers, ProScript MULV Reverse transcriptase and Pro RNase inhibitor which aids in cDNA synthesis. This kit is efficient in conversion of RNA to first strand cDNA, from a wide range of 0.2µg to 1µg. The RNase inhibitor protects the RNA from degradation. The cDNA produced can be utilized for further amplification and also in real-time gene expression analysis.

1.3 Salient features

- Used for multiple gene analysis.
- Stable cDNA, for greater and prolonged study.
- Works well with 0.2µg to 3µg of RNA.
- Flexible priming options.

1.4 Applications

1. For reverse transcription of RNA to cDNA.
2. Stable cDNA pool generation.
3. Archiving of cDNA for further gene-analysis.

1.5 Introduction

Reverse transcription is the process of synthesis of DNA from RNA template. This process is driven by RNA-dependent DNA polymerases, also known as reverse transcriptases. A reverse transcriptase synthesizes a complementary DNA (cDNA) strand from the given RNA template strand aiding to produce cDNA libraries. cDNA libraries contain DNA copies of mRNA from cells and tissues and are used to understand actively expressed genes and their functions at a specific point of time.

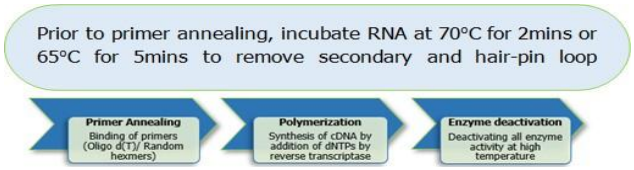
1.6 Principle of the technique

In cDNA synthesis, the reverse transcriptase binds to the RNA template in the presence of an annealed primer. It starts synthesizing complementary DNA (cDNA) strand by incorporating dNTPs. This is known as first strand synthesis which generates a cDNA: RNA hybrid. The RNase H activity of the reverse transcriptase enzyme cleaves the RNA from the hybrid.

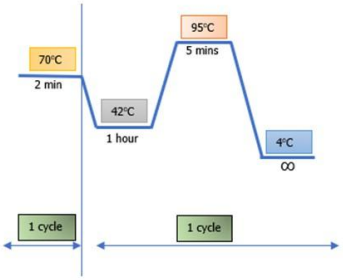
1.7 Must follow

Store enzyme and buffer at -20°C.
If turbidity/precipitation is observed in buffer and enzyme, discard the vials immediately.

1.8 cDNA synthesis steps



1.9 cDNA synthesis reaction conditions



1.10 10X RT reaction buffer composition

500mM Tris-HCl (pH- 8.3 at 25°C), 750mM KCl, 30mM $MgCl_2$, 100mM DTT.

1.11 Storage & handling

Store all the components at -20°C and make sure to thaw on ice prior use.

1.12 Quality control tests

Compositions of the 10X reaction buffers have been optimized to assure quality performance of the enzyme under a variety of conditions. Good performance of DNA amplification by PCR was confirmed by using λDNA as the template (amplified fragment: 8kb).

1.13 Unit definition of Reverse transcriptase

One unit is the amount of enzyme that is required to incorporate 1 nmole of dTTP into acid precipitable material at 37°C using poly (A) oligo d(T) as template primer.

1.14 Materials required but not supplied

1. Template RNA
2. 0.2ml PCR tubes
3. Pipette tips

1.15 Optimal results can be obtained by:

1. Clean the working bench with 70% ethanol or isopropyl alcohol before placing anything on the bench.
2. Wear a fresh pair of protective nitrile powder-free gloves before setting up the reaction.
3. Let all the components thaw on ice completely prior to vortexthem.
4. Use freshly isolated RNA with absorbance readings as given in Table 1.17 for the reaction.
5. Avoid repeated freeze-thaw cycles of RNA. To avoid repeated freeze-thaw cycles, aliquot RNA with volume required for single use.
6. Use calibrated pipettes.

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1.16 Protocol

1. Place all the components on ice for thawing 20-30 mins prior.
2. After ensuring that all the components are thawed completely, gently vortex the components and give a brief spin and place the vials on ice.
3. Place 0.2mL PCR tubes, for the required number of reactions, on ice and add the as given in Table-1.

Table-1:

Components	Concentration	Vol. for 20 µL
RNA template	0.2µg-1µg	Variable
Oligo (dT)/ Random hexamer(20µM)	100-200pm	1 µL
dNTPs (10mM)	0.4mM-0.6mM	1 µL
DEPC water	-	Variable
	Total volume	10µL

Note: Use Oligo (dT) primers for mRNA and Random hexamers for whole RNA reverse transcription.

4. Incubate the above reaction at 70°C for 2 minutes either in PCR or in water bath to remove secondary structures formed (if any) in RNA.
5. After incubation, snap chill the tubes on ice.
6. To these tubes, add the components as given in Table-2

Table-2:

Components	Concentration	Vol. for 20 µL
10X RT Reaction buffer	1X	2 µL
ProScript M-MuLV *Reverse transcriptase* (200U/µL)	200U	1 µL
Pro RNase inhibitor (40U/µL)	40U	1 µL
DEPC water	-	Variable
	Total volume	10µL

5. After ensuring that all components have been added to the tube, spin the tubes in a microcentrifuge and ensure no bubbles are visible in the tubes.
6. Place these vials in a thermal cycler and perform PCR with conditions as given in the section- 1.9
7. Start the run and note the end time.
8. The cDNA synthesized can be stored at -20°C and further used for PCR amplification and gene-studies.

1.17 Troubleshooting

The first step is to confirm that all the reagents are added to the vial and the reagents are not contaminated.

Problem: No or low PCR yield

Possible cause	Solution
Low or high reagent concentration	Adjusting final concentration. See Table-1 in section-1.10 for appropriate concentrations.
Cycling conditions	Tweak the cycling conditions as per template & primer concentration.
Pipetting errors	Make sure to use calibrated pipettes

Note: Use pre-mixed **ProEnz 2X MuLV RT master mix** for easy assembly and reduce pipetting errors. Just add primers, template and water to the given volume of the master-mix.

Problem: Truncated cDNA formation

Possible cause	Solution
Poor quality of RNA	Preferred absorbance ratios for RNA: A260/280 - ~2 A260/230 - ~2 Ratios less than mentioned results in low cDNA.
Repeated freeze-thaw cycles of RNA	Aliquot RNA into with volume required for single use.
Contamination of RNA	Use nuclease-free water or DEPC water (Cat# PE2413) to suspend and dilute RNA to ensure no contamination.

1.18 Limitations

1. The specificity of the generated PCR product may be altered by non-specific binding of the primers to other similar sequences on the DNA.
2. RNA is highly sensitive to contamination. Contamination with genomic DNA can result in false positives. It is also highly unstable and should be stored at -70°C to -80°C to avoid degradation.
3. In order to design primers to generate a PCR product, some prior sequence information is usually necessary.
4. DNA polymerases are prone to error, which potentially causes mutations in PCR products.
5. PCR is highly sensitive to contamination.

Explanation of symbols

REF - Catalogue number

- Expiry

LOT - Lot number/Batch number

-25°C max
-15°C min
- Storage limitations

-Manufacturing: Plot No. 147/D, Phase II, Cherlapally IDA, Telangana, Hyderabad - 500051, India.

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