# ProScript Two Step RT-PCR Kit

**Cat# no.: A9002** **Store @-20 C**

For Research use Only

## 1.1 Kit Information

| Kit components | A9002-10 | A9002-25 | A9002-50 | A9002-100 |
|---|---|---|---|---|
| ProScript M-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 (10mM) | 10µL | 25µL | 50µL | 100µL |
| 10X RT reaction buffer | 20µL | 50µL | 100µL | 200µL |
| Pro Taq DNA polymerase (5U/µL) | 10µL | 25µL | 50µL | 100µL |
| 10X PCR reaction buffer | 20µL | 50µL | 100µL | 200µL |
| Nuclease-free water | 1mL | 1.5ml | 2.5ml | 5ml |

## 1.2 Description

ProEnz Two-step RT-PCR kit consists of a buffer, dNTPs, Oligo (dT)s, Random hexamers, ProScript M-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 -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. The kit additionally provides Pro Taq Polymerase and 10X PCR reaction buffer for amplification of cDNA for user convenience.

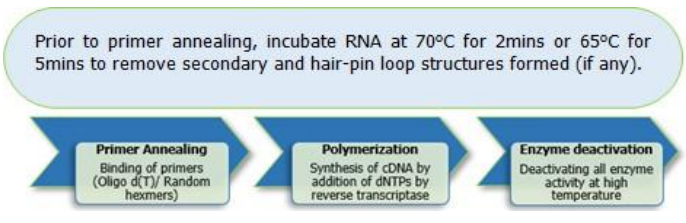
## 1.3 Introduction

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.

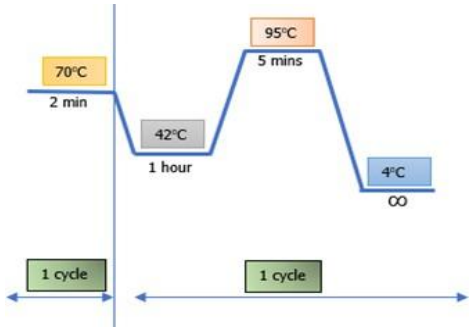
## 1.4 Principle of the technique

In Two-step RT-PCR, 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.

## 1.5 Two-step RT-PCR steps




## 1.6 Two Step RT-PCR conditions




## 1.7 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 components for 20µl reaction volume as given in Table-1.

**Table-1:**

| Components | Concentration | Vol. for 20 µL |
|---|---|---|
| **RNA template** | 0 . 2 µg -1µg | Variable |
| **Oligo (dT)/ Random hexamer(20µM)** | 100-200pm | 1 µL |
| **dNTPs (10mM)** | 0.4mM-0.6mM | 1 µL |
| **Nuclease free water** | - | Variable |
| | Total volume | 10µL |

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

6. 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.
7. After incubation, snap chill the tubes on ice.
8. 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 Reverse transcriptase* (200U/µL)** | 200U | 1 µL |
| **Pro RNase inhibitor (40U/µL)** | 40U | 1 µL |
| **Nuclease free water** | - | Variable |
| | Total volume | 10µL |

9. 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.
10. Place these vials in a thermal cycler and perform PCR with conditions as given in the section- 1.6.
11. Start the run and note the end time.
12. The cDNA synthesized can be stored at -20°C and further used for PCR amplification and gene-studies.



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### 1.8 Applications

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

### 1.9 Salient features

- Used for multiple gene analysis.
- Stabel cDNA, for greater and prolonged study.
- Works well with 0.2µg to 1µg of RNA.
- Flexible priming options.

### 1.10 Storage & handling

Store all the components @-20 C

### 1.11 Must follow

Store enzyme and buffer at -20ºC.

If turbidity/precipitation is observed in buffer and enzyme, discard the vials immediately.

### 1.12 10X RT reaction buffer composition

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

### 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 37ºC using poly (A) oligo d(T) as template

### 1.14 Unit definition of Taq DNA polymerase

One unit is the amount of enzyme that will incorporate 10 nmol of dNTP into acid- insoluble products in 30 minutes at 72ºC with GAPDH mice DNA as template

### 1.15 Materials required but not supplied

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

### 1.16 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 -70ºC to -80ºC 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.

### 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.<br><br>See Table-1 in section-1.7 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 Cat# PE2406** 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:<br><br>A260/280 - ~2<br><br>A260/230 - ~2<br><br>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 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 properly calibrated pipettes.

### Explanation of symbols

REF - Catalogue number

- Expiry

LOT - Lot number/Batch number

-25ºC max / -15ºC min - Storage limitations

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



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