

ProScript One Step RT-PCR Kit

Cat# no.: A9001

Store at -20 C

For Research use Only

1.1 Ordering Information

Kit components	A9001-10	A9001-25	A9001-50	A9001-100
2X One Step RT-PCR buffer	100µL	250µL	500µL	1mL
Enzyme mix	10µL	25µL	50µL	100µL
Nuclease-free water	500µL	1mL	1.5mL	1mL x 2

1.2 Introduction

One Step RT-PCR combines the processes of cDNA synthesis and subsequent amplification of the cDNA to billions of copies. It occurs in a single tube and in a single step from RNA to ds DNA. This reaction set-up can help simplify the work-flow, reduces variation and minimizes possible contamination due to frequent sample handling. It is a time-saving process and eliminates the steps of pipetting of cDNA product.

1.3 Description

ProEnz ProScript One Step RT-PCR Kit provides 2X Buffer premixed with dNTPs and Enzyme mix help in easy reaction assembly and aid in cDNA synthesis and subsequent amplification of the cDNA to billions of copies. The enzyme mix contains Reverse transcriptase for reverse transcription of RNA to cDNA, RNase inhibitor to inhibit RNases and minimize risk of contamination and Taq polymerase for amplification of cDNA to ds DNA.

This kit is suitable for detecting small amounts of RNA and it helps achieve convenient experimental set-up with premixed components.

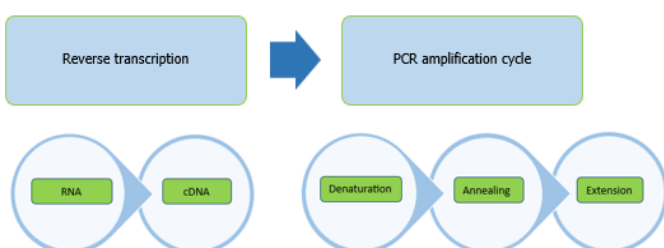
1.4 Salient features

- Quick reaction set-up
- Limited pipetting steps and errors
- Works well with 100pg to 1 µg of RNA

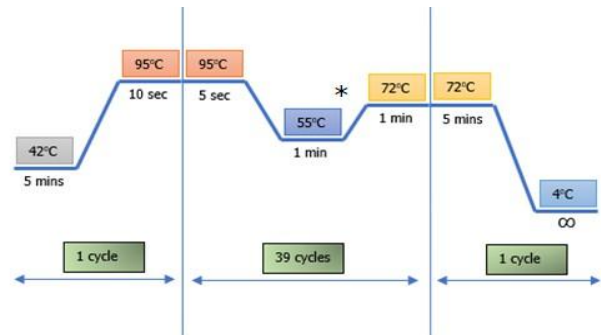
1.5 Principle of the technique

In One Step RT-PCR, RNA is converted to cDNA by Reverse transcriptase using gene-specific primers and with the same primers cDNA is amplified by Taq DNA polymerase, which adds a nucleotide to the pre-existing 3'-OH group of the primers and synthesizes new strand complementary to the template strand, extending the desired target region.

1.6 One Step RT-PCR steps



1.7 One Step RT-PCR conditions



* Annealing temperature can vary with primers.

1.8 Applications

1. For reverse transcription and amplification by PCR.
2. Fast and accurate gene analysis.
3. High-sequence specificity.

1.9 Storage & handling

Store all the components @-20°C

1.10 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
Template RNA	100pg-1µg	Variable
2X One-step RT-PCR buffer	1X	10 µL
Forward Primer	0.1-0.5 µM	0.5-1 µL
Reverse Primer	0.1-0.5 µM	0.5-1 µL
Enzyme mix	-	1 µL
Nuclease free water	-	Make up to 20 µL

4. It is recommended to add the template RNA as the last component to the tube.
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- 1.7
7. Start the run and note the end time.
8. In the meantime, prepare an agarose gel of desired concentration. After solidification, submerge the gel in 1X TAE/TBE buffer.
9. After the PCR run, take out the tubes and add DNA loading dye to the tubes and load the samples on the gel and run till the dye front can be seen 3/4th the gel.
10. Observe the gel under UV light for desired bands.

1.11 Enzyme mix contains

Mixture of Reverse transcriptase, RNase inhibitor and Taq DNA polymerase.

1.12 Must follow

Store enzyme and buffer at -20°C.

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

1.13 Materials required but not supplied

- | | |
|--------------------------------|----------------------|
| 1. Template | 5. Agarose |
| 2. 0.2ml PCR tubes | 6. 1X TAE/TBE buffer |
| 3. Forward and reverse primers | 7. DNA loading dye |
| 4. Pipette tips | 8. DNA ladder |

1.14 Limitations

- The specificity of the generated PCR product may be altered by non-specific binding of the primers to other similar sequences on the DNA.
- 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.
- In order to design primers to generate a PCR product, some prior sequence information is usually necessary.
- DNA polymerases are prone to error, which potentially causes mutations in PCR products.

1.15 2X One-step RT-PCR buffer composition

24mM Tris-SO₄ (pH- 8.6 at 25°C), 3.6mM (NH₄)₂SO₄, 6.0mM MgSO₄ and dNTPs for easy reaction assembly.

1.16 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).

1.17 Optimal results can be obtained by:

- Clean the working bench with 70% ethanol or isopropyl alcohol before placing anything on the bench.
- Wear a fresh pair of protective nitrile powder-free gloves before setting up the reaction.
- Let all the components thaw on ice completely prior to vortex them.
- Use freshly isolated RNA with absorbance readings as given in Table 1.18 for the reaction.
- Avoid repeated freeze-thaw cycles of RNA. To avoid repeated freeze-thaw cycles, aliquot RNA with volume required for single use.
- Use properly calibrated pipettes.

1.18 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
Poor quality of RNA	Preferred absorbance ratios for RNA: A260/280 - ~2 A260/230 - ~2 Ratios less than mentioned results in low cDNA.

Note: Use pre-mixed **ProEnz 2X One Step RT-PCR master mix Cat# MA9001** for easy assembly and reduce pipetting errors. Just add primers, template and Nuclease free water to the recommended volume of the master-mix.

Problem: Primer-dimer formation

Possible cause	Solution
Poor primer specificity	Check the target specificity of the primers using online primer tools. The annealing temperature is 5°C less than the T _m of the primer pair.
Primer concentration	Primer concentration can range anywhere between 0.1µM -1µM.

Problem: False positives

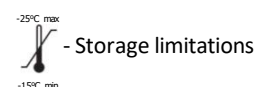
Possible cause	Solution
Contamination of previous results	Make sure to dedicate a different space for pre-PCR set-up and post-PCR run.
Non-specific binding	Set-up the reaction on ice.

Explanation of symbols

REF - Catalogue number



LOT - Lot number/Batch number



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