

Product Description

NeoScript One Step RT-PCR Kit is an end-point PCR detection reagent for RNA templates. Using gene-specific primers, RNA-to-cDNA conversion and PCR amplification are performed consecutively in the same reaction system without additional reagent addition, thereby simplifying the workflow. This kit combines a high-temperature reverse transcriptase, a novel hot-start enzyme, and an RNase inhibitor with an optimized buffer system, enabling amplification of fragments longer than 10 kb.

Components

NeoScript One Step RT-PCR Kit is supplied in the following package configurations.

Components	BR3M321-03 (24 T)	BR3M321-06 (96 T)
2× One Step Buffer	300 µL	1,200 µL
One Step Enzyme Mix	72 µL	288 µL

Storage

Store at $-20\pm 5^{\circ}\text{C}$.

Notes

1. Use RNase-free consumables and clean the experimental area regularly.
2. Other reagents required for amplification should be prepared by the user or purchased separately.
3. For Research Use Only.

Protocol

1. Reaction Setup

1.1 Thaw 2× One Step Buffer on ice, mix thoroughly, and briefly centrifuge before use. Gently mix One Step Enzyme Mix without introducing bubbles, briefly centrifuge, and keep on ice before use. Prepare the reaction mixture in an RNase-free centrifuge tube according to Table 1.

Table 1. RT-PCR Reaction Setup

Component	Volume (µL)	Final Concentration
2× One Step Buffer	12.5	
One Step Enzyme Mix	3	
Gene Specific Primer Forward (10 µM)	1	0.4 µM
Gene Specific Primer Reverse (10 µM)	1	0.4 µM
RNase-free ddH ₂ O	7.5-X	
RNA Template	X	
Total	25	

Note: The reaction volume can be adjusted according to experimental needs; scale all components proportionally.

2. Amplification Program

2.1 Mix gently by pipetting, briefly centrifuge to collect the reaction mixture at the bottom of the tube, and run the following program on a thermal cycler.

Table 2. Amplification Program

Step	Temperature	Time	Cycles
Heated Lid	105°C	—	—
Reverse Transcription	50°C	30 min	1
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	20 s	30-35
Annealing	60°C ^a	15 s	
Extension	72°C	20 s-1 min/kb ^b	
Hold	4°C	∞	1

Note a: Adjust the annealing temperature according to the primer T_m values; in general, set it 1-2°C below the primer annealing temperature.

Note b: Adjust the extension time as needed. In general, a longer extension time helps improve amplification yield.

2.2 Analyze the amplification products by agarose gel electrophoresis.