cDNA Synthesis

Materials

RNA template (7 μL)
dNTP Mix (10 mM each) (Takara)
Gene-specific Reverse Primer (2 μM)
5× RTase Reaction Buffer (Accurate Biology)
RNase Inhibitor (Accurate Biology)
Evo Super M-MLV RTase II (Accurate Biology)
RNase-free water

Procedure

1. Primer Annealing

Prepare the following 10 μL RNA-primer mixture in a PCR tube.

Reagent	Volume
dNTP Mix (10 mM each)	2 μL
Gene-specific Reverse Primer (2 μM)	1 μL
RNA template	7 μL
Total	10 μL

Perform primer annealing using the following thermal program:

Temperature	Time
65°C	5 min
4°C	Hold

2. Reverse Transcription

Add the following reagents directly to the annealed RNA mixture.

Reagent	**Volume**
Annealed RNA-primer mixture	10 μL
5× RTase Reaction Buffer	4 μL
RNase Inhibitor	0.5 μL
Evo Super M-MLV RTase II	0.5 μL
RNase-free water	5 μL
Total	20 μL

Perform reverse transcription using the following program:

Temperature	**Time**
55°C	45 s
85°C	5 min
4°C	Hold

The final product is 20 μL of first-strand cDNA, which can be used directly for downstream quantitative PCR.