Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences
RCPN

cDNA synthesis (RT-PCR)

PSR

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Workflow

RNA extraction → cDNA synthesis (RT-PCR) → Real-Time PCR → Data analysis

Detailed procedure

2. cDNA synthesis (RT-PCR)

Materials

product	Cat. No.
Random hexamer primer	-
RNAse inhibitor	00219287
dNTP	10297-018
Reverse transcriptase	18064-022
0.2 mL microfuge tube	-
Ultrapure Dnase/Rnase-Free Distilled water	DW8520

Step 1:
Primer/RNA Mix:

- Template RNA → 1 µg
- Random hexamer primers → 0.5 µM or 20 pmol
- DEPC-Treated water up to → 12.5 µL

Optional: if RNA template is GC rich or is known to contain secondary structure, mix gently; incubate at (65°C for 5 min).

Step 2:
Chill on ice for 1 minute. Centrifuge briefly in a micro centrifuge.

Step 3:
Buffer/Enzyme mix, add on ice:

- 10X reaction buffer → 2 µL
- RNAse inhibitor → 0.5 µL
- dNTP mix, 10 mM each → 2 µl (1mM final concentration)
- Reverse transcriptase (mM LV) → 1 µL
- DEPC-treated water up to → 20 µL

Mix by pippeting up and down.

Step 4:
Add Buffer/Enzyme mix to the first tube, mix gently and transfer in a 0.2 mL microfuge tube.

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Step 5:
Place the tube in the PCR machine programmed as follows:

10 min 25°C
60 min 42°C → for GC reached → 45°C
10 min 70°C

Step 6: Analyze the PCR products by agarose gel electrophoresis

Step 7: Store cDNA at -20ºC.

Good luck!
☺

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