

cDNA synthesis (RT-PCR)

Workflow

RNA Extraction → **cDNA synthesis (RT-PCR)** → **Real-Time PCR**

cDNA synthesis, also known as reverse transcription, generates complementary DNA (cDNA) from an RNA template.

Specimen

RNA template

Materials

Random hexamer primer

Reverse transcriptase

RNase inhibitor

dNTP

microfuge tube

Ultrapure DNase/RNase

Free Distilled water

Equipment and supplies

Refrigerated Microcentrifuge

Centrifuge tubes

Pipette tips

Nanodrop

Vortex Mixer

Thermocycler

water bath

Safety (Warning and Biohazard consideration):

Microcentrifuge tubes and Pipette tips must be autoclaved before use. A major source of RNase contamination is from the hands of the researcher. Gloves should be worn all stages during the preparation of materials and solutions used for the isolation and analysis of RNA, and during all manipulations involving RNA.

Procedure:

Step1. Prepare the following mixture in a microtube.

Reagent	volume
Oligo dT Primer (50 μ M) or Random 6 mers (50 μ M)	1 μ l or 1 μ l (0.4 - 2 μ l)
dNTP Mixture (10 mM each)	1 μ l
Template RNA	Total RNA: < 5 μ g
RNase Free dH ₂ O	PolyA+ RNA: < 1 μ g
	x μ l
total	10 μ l

Step2. Incubate for 5 min at 65°C, then cool immediately on ice

Step3. Prepare the reaction mixture in a total volume of 20 μ l.

Reagent	volume
Template RNA Primer Mixture (from step 2)	10 μ l
RNase Inhibitor (40 U/ μ l)	0.5 μ l (20 U)
5X PrimeScript Buffer	4 μ l
PrimeScript RTase (200 U/ μ l)	1.0 μ l (200 U)
RNase Free dH ₂ O	x μ l
Total	20 μ l

Step4. Mix by pipetting up and down.

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

Step5. Incubate the reaction mixture using the following conditions.

30°C	10 min (required when using Random 6 mers)
42°C (50°C)	30 - 60 min
95°C	5 min

Step 6. Analyze the PCR products by agarose gel electrophoresis

Step 7. Store cDNA at -20°C

References:

<https://www.neb.com/protocols/1/01/01/first-strand-cdna-synthesis-e6300>

<https://www.takara-bio.com>

شناسنامه سند: Core Lab.105

نام سند پروتکل استاندارد سنتز cDNA

تاریخ صدور ۱۳۹۹/۷/۷

نام کامل فایل پروتکل سنتز cDNA

شرح سند روش سنتز cDNA را شرح می‌دهد.

تهیه کننده شهربانو نادری
