

First-strand cDNA Synthesis Kit

PROTOCOL

Reverse transcription reaction

Mix the followings in a sterile thin-wall microtube:

Total RNA	0.5~1 µg
(dN) ₉ or oligo(dT) ₁₈	100 pmol

- Denature the mixture at **70°C for 5 min**, and cool the tube **on ice rapidly**, then add the following components:

5×M-MLV Buffer	4 µl
dNTPs (10 mM each)	1 µl
RNasin	10 U
100 mM DTT	2 µl
M-MLV Reverse Transcriptase	100 U
DEPC- treated water	up to 20 µl

- Mix the solution and centrifuge briefly, then incubate 1 hr at the appropriate temperature: 42 °C for using oligo(dT)₁₈ primers, and 37 °C for using (dN)₉ primers.
- Stop the reaction by incubating at 94 °C for 5 min and cool the tube on ice.
- The cDNA synthesized using this system can be used directly in PCR amplification or other downstream applications.

PCR amplification (optional)

- Mix followings in a sterile thin-wall microtube:

Synthesized cDNA	2-5 µl
10×PCR Buffer	2 µl
Forward primer	10 pmol
Reverse primer	10 pmol
dNTPs (10 mM each)	0.5 µl
Taq DNA Polymerase	1.5 U
ddH ₂ O	up to 20 µl

- Mix the solution and centrifuge briefly, then begin the PCR amplification:

Denature the template at 94 °C for 2,5 min. Then enter the following 30~40 cycles (this amplification parameter is just for reference): 94 °C - 30s, 60 °C - 45s, 72 °C - 1~3min. Extend at 72 °C for 7 min lastly.

Remarks

1. Ensure the integrity and purity of your RNA. The quality of RNA is the key for first-strand cDNA synthesis. The integrity and purity of RNA can be inspected by the ratio of OD_{260}/OD_{280} and agarose gel analysis. The common ratio of purified RNA is 1.8~2.0, if not, the RNA should be purified further. The ratio of eukaryotic RNA 28S/18S is about 2:1, if not, the RNA has been degraded.
2. Avoid RNase contamination. All vessels, reagents and solutions must be sterile, and all procedures must be carried out with gloves.
3. Select the right primers for first-strand cDNA synthesis. Primer oligo (dT)₁₂₋₁₈ can ensure the synthesized cDNAs have intact 3'-end, and get the first-strand cDNA close to full length. Primer (dN)₆ or (dN)₉ can anneal to many sites of the mRNA, and produce short length first-strand cDNA segments, which is often used to acquire 5'-end sequence and to obtain cDNA from RNA with secondary structure or stop site that stops the reverse transcription.

Troubleshooting Guide:

1. Why the yield of cDNA is low?

Possible causes: (1) The quality of template RNA was too low. (2) The concentration of RNA was estimated too high. (3) Reverse transcriptase inhibitor existed or reverse transcriptase was insufficient. (4) Reaction volume was too large. The common volume should not be more than 50 μ l.

2. Why the long cDNA can't be synthesized?

- (1) RNA has been degraded: all vessels and reagents should be sterile and treated with DEPC to avoid RNase. At the same time, RNase inhibitor should be added into the reverse transcription reaction.
- (2) Improper reaction condition: condition can be optimized, including quantity of reverse transcriptase, salt concentration, reaction temperature (37~56 °C) and concentration of DTT (0.5~10mM).
- (3) Secondary structure of RNA: increase reaction temperature or use random primer.

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FFS-25	First-strand cDNA Synthesis Kit - 25 reactions