

Protocol

I. First strand cDNA synthesis

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Template RNA	(A) mRNA or specific RNA	1-5 µg
Prime	oligo (dT) ₁₅ primer or Random hexamer primer	1 µl
DEPC-treated water		to 13.4 µl
Total volume		13.4 µl

2. Mix gently, centrifuge briefly and incubate at 70°C for 5 min. Chill on ice, spin down and place the vial back on ice.

3. Prepare the following cDNA Synthesis Mix, add the following components in the indicated order:

5x first-strand buffer	4 µl
dNTPs(10 mM each)	1 µl
RNasin	0.6 µl
M-MLV	1 µl

4. Mix gently and centrifuge

5. For oligo(dT)₁₅, incubate for 60 min at 42°C. For random hexamer primed synthesis, incubate for 60 min at 37°C.

6. Terminate the reaction by heating at 70°C for 5 min.

The reverse transcription reaction product can be used immediately in second strand cDNA synthesis reactions or stored at -20°C for less than a week. For longer storage, -70°C is recommended.

II. PCR Amplification of First Strand cDNA

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2 µl of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 µl total volume.



Hy-RT-PCR Kit

#ER1011 20preps

For research use only

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Components of the kit

Component	R1011(20 rxns)
M-MLV (200U/μl)	20 μl
RNasin (40U/μl)	12 μl
Oligo(dT) ₁₅ (50μM)	20 μl
Random hexamer primer (50μM)	20 μl
5x First-strand Buffer	80 μl
RNase-free ddH ₂ O	1 ml
dNTPs(10mM each)	50 μl

Storage

All components of the kit should be stored at -20°C. Keep control RNA at -70°C for longer storage.

Description

Hy-RT-PCR kit is optimized to synthesize first-strand cDNA from purified poly(A)⁺ or total RNA. The RT-pcr kit for RT-PCR delivers increased cDNA yields, high sensitivity, and full-length transcripts in a convenient format. You get all of the components you need for successful first-strand cDNA synthesis, saving your time and ensuring your success with every experiment. The kit Reverse Transcriptase is a version of M-MLV RT that has been engineered to reduce RNase H activity and provide increased thermal stability. The enzyme is used to synthesize cDNA at a temperature range of 42-55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases.

Applications

First strand cDNA synthesis for RT-PCR .

Construction of cDNA libraries.

One-step RT-PCR

Primer extension

Important notes

Avoiding ribonuclease contamination

RNA purity and integrity is essential for synthesis of full-length cDNA. RNA can be degraded RNase A, which is a highly stable contaminant found in any laboratory environment. All components of the kit have been rigorously tested to ensure that they are RNase free. To prevent contamination both the laboratory environment and all prepared solutions must be free of RNases. General recommendations to avoid RNase contamination:

- DEPC-treat all tubes and pipette tips to be used in cDNA synthesis or use certified nuclease-free labware.
- Wear gloves when handling RNA and all reagents, as skin is a common source of

RNases. Change gloves frequently.

- Use RNase-free reagents, including high quality water (e.g., DEPC-treated Water).
- Use an RNase inhibitor, such as Hy-RNasin (#R2011) to protect RNA from the activity of RNases.
- Keep all kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

Template RNA

Total cellular RNA isolated by standard methods is suitable for use with the kit. Purified RNA must be free of salts, metal ions, ethanol and phenol to avoid inhibiting the cDNA synthesis reaction. Trace contaminants can be removed by ethanol precipitation of the RNA followed by two washes of the pellet with cold 75% ethanol. For RT-PCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I to remove trace amounts of DNA. DNase I must be obtained by the user. Always perform a control (RT-minus) reaction which includes all components for RT-PCR except for the reverse transcriptase enzyme.

RNase H Digestion

The sensitivity of the PCR step can be increased (especially for long templates) by removing the RNA template from the cDNA:RNA hybrid molecule by digestion with RNase H after first-strand synthesis. Presence of RNase H during first-strand synthesis will degrade the template mRNA, resulting in decreased full-length cDNA synthesis and decreased yields of first-strand cDNA.

Primers

Synthesis of first strand cDNA can be primed with either oligo(dT)₁₅ primer, random primers or gene-specific primers.

Oligo(dT)₁₅ primes cDNA synthesis from the poly(A) tail present at the 3'-end of eukaryotic mRNA. Random Primers initiate cDNA synthesis from the total RNA population (rRNA and mRNA). Therefore, using random primers for first strand synthesis results in a greater complexity of the generated cDNA compared with the oligo(dT)₁₅ primer. As a consequence, the sensitivity and specificity of subsequent PCR reactions may be reduced. However, there are several applications where it is beneficial to use random primers, such as cDNA synthesis using mRNAs without a poly(A) tail, or cDNA synthesis using poly(A)-enriched RNA samples.

Gene-specific primers are used to synthesize specific cDNA from a pool of total RNA or mRNA and must be obtained by the user.

First Strand cDNA synthesis procedure

The first strand cDNA reaction can be performed as an individual reaction or as a series of parallel reactions with different RNA templates. Therefore, the reaction mixture can be prepared by combining reagents individually or a master mix containing all of the components except template RNA can be prepared. Depending on the structure of the RNA template, separate steps for RNA denaturation and primer annealing may improve RT-PCR results.