cDNA Synthesis Kit

For research use only

Cat No: YT4500

Size: 50 Rxn

Store at –20°C

Contents:	volume
M-MLV (10,000 U) 200u/ µl	**50 µl**
5X first-strand Buffer	**200 µl**
Oligo(dT)18 primer	*
Random hexamer primer	**
RNasein (40u/µl)	**25 µl**
dNTP 10mM	**50 µl**

Important Note:

Primers are lyophilized.

Please add DEPC water used for Con.50 µM

* Oligo(dT)18 primer : 60.96 µl

** Random Hexamer : 184.48 µl

cDNA synthesis kit offer whole components you need for cDNA synthesize include RNase inhibitor, Primer, dNTP mixture, Buffer and M-MuLV RT enzyme . M-MuLV is a reverse transcriptase enzyme with optimum activity at 37C and no RNase H activity. for greater application flexibility we provide both random hexamer and Oligo dt primers. This kit provides superior reagents that ensure efficient first-strand cDNA synthesis from mRNA or total RNA templates.

Features :

- Have ease of use and consistency
- High sensitivity reverse transcription from low abundance template
- broad linear dynamic range of total input RNA (0.1ng – 5µg) with a highly efficient MMLV reverse transcriptase
- Complete cDNA synthesis and qPCR on the same day

Specification :

- store at -20°C
- Primers are lyophilized
- Contains both Oligo(dT)18 primer and Random Hexamer
- It can be used with either sequence-specific primers, poly(dT)18primers, or random Hexamer

Applications :

- First-strand cDNA synthesis for RT-PCR and qPCR
- Gene expression profiling
- Reagents can be used for miRNA synthesis

Protocol (50 Test)

First-Strand cDNA Synthesis Using M-MLV RT

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

Template RNA	Total RNA Poly (A) mRNA Or specific RNA	0.1ng - 5µg 1 to 500 ng 1-5 µg
Prime	oligo (dT)18 primer(50µM) or Random hexamer primer(50µM)	1.0 µl 1.0 µl
DEPC-treated water		To 13.5 µl
Total Volume		13.5 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 (40U/µl)	0.5 µl
M-MLV	1µl

4. Mix gently and centrifuge
5. For oligo(dT)18, 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.

Protocol (100 Test final volume 10µl)

The kit contains high-quality reagents, allowing it to be used for 100 samples. To utilize it, follow these steps:

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

Template RNA	Total RNA Poly (A) mRNA Or specific RNA	0.1ng - 3µg 1 to 300 ng 1-3 µg
primer	oligo (dT)18 primer(50µM) or Random hexamer primer(50µM)	1µl or 1µl
DEPC-treated water		To 6.75
Total Volume		6.75

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	2µl
dNTPs(10 mM each)	0.5µl
RNasin (40U/µl)	0.25µl
M-MLV	0.5µl

4- Mix gently and centrifuge

5- For oligo(dT)18, 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.

Notice that the final volume of your cDNA will be 10µl.

Protocol (100 Test final volume 20µl)

First-Strand cDNA Synthesis Using M-MLV RT

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

Template RNA	Total RNA Poly (A) mRNA Or specific RNA	0.1ng - 5µg 1 to 500 ng 1-5 µg
Prime	oligo (dT)18 primer(50µM) or Random hexamer primer(50µM)	1.0 µl 1.0 µl
DEPC-treated water		To 13.5 µl
Total Volume		13.5 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	2 µl
dNTPs(10 mM each)	0.5 µl
RNasin (40U/µl)	0.25 µl
M-MLV	0.5 µl
Nuclease free water	Up to 6.5

4- Mix gently and centrifuge

5- For oligo(dT)18, 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.