MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.1

Product name: First Strand cDNA Synthesis Kit

Cat #: FSCS-100, FSCS-200

Size: 50 reactions, 250 reactions

Description:

First Strand cDNA Synthesis Kit can be used to synthesize first strand cDNA by reverse transcription (RT) at higher temperatures than the wild type M-MuLV and to reach higher cDNA yields for difficult RNA transcription, which is based on MCLAB's QuantumScript™ HD Reverse Transcriptase, a unique mutation with increased thermostability and reduced RNase H activity.

Components:

Cat #: FSCS-100, 50 reactions

Components	Cap Color	Volume
5X RT Buffer	Red	125 µl
RT Enzyme Mix	Clear	100 µl
DTT	Yellow	50 µl
dNTP	Blue	50 µl
Oligo dT Primer	Green	50 µl
Random Primer	Purple	50 µl

Cat #: FSCS-200, 250 reactions

Components	Cap Color	Volume
5X RT Buffer	Red	625 µl
RT Enzyme Mix	Clear	500 µl
DTT	Yellow	250 µl
dNTP	Blue	250 µl
Oligo dT Primer	Green	250 µl
Random Primer	Purple	250 µl

Upon receipt of the kit, immediately store the components at -20 °C in a freezer without a defrost cycle. It is recommended to reduce freeze-thaw cycles as much as possible.

Additional Materials Required:

The following reagents, instruments and consumables are supplied by the user:
- Template RNA
- Gene specific RT Primer
- Microcentrifuge
- Thermal cycler
- PCR tubes
- Pipettes and tips

Protocol:

1. Set up reaction in a PCR tube on ice as below:

Component	Quantity *	Note
RNA Template	x µL	1ng-5µg Total RNA
or 100pg-500 ng mRNA		
RT Primer	1 µL	Oligo (dT)12-18 (500 µg/ml), Random Primers (125 µg/ml)
or GSP Primer (2 pmol)		
10 mM dNTP	1 µL	
Nuclease-free water	To 6 µL	

*: For multiple reactions, master mix should be made with 5% extra reagents to reduce pipette error.

2. Gently mix thoroughly and then centrifuge briefly.

3. Incubate the tube in a thermal cycler at 65°C for 5 minutes, then place on ice immediately for 3 minutes.

4. Set up reaction mix in another PCR tube on ice as below:

Component	Quantity *
5x RT Buffer	2.5 µL
0.1M DTT	1 µL
RT Enzyme Mix	2 µL
RNsae inhibitor	1 µL
Total Volume	6.5 µL

*: For multiple reactions, master mix should be made with 5% extra reagents to reduce pipette error.

5. Gently mix thoroughly and then centrifuge briefly.

6. Transfer this 5.5 ul reaction mix to previous reaction tube, mix gently by pipetting and centrifuge briefly.

7. Incubate the tube in a thermal cycler at 50°C for 45 to 60 minutes. Run 25°C for 10 minutes first if using Random Primers.

8. Inactive the enzyme at 70°C for 15 minutes and chill on ice.

9. Store the products at -20°C or proceed to the next step.