First Strand cDNA Synthesis Kit MR9098

Technical literature is available at: www.mesgenbio.com. E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

MESGEN™ INNOVATION BIOTECHNOLOGY

Product overview

MesGen™ First Strand cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit uses Reverse Transcriptase (RT), which has lower RNase H activity compared to AMV reverse transcriptase. The enzyme maintains activity at 42-50°C and is suitable for synthesis of cDNA up to 7 kb. First strand cDNA synthesized with this system can be directly used as a template in PCR or real-time PCR. It is also ideal for second strand cDNA synthesis or linear RNA amplification. Radioactively and non-radioactively labeled nucleotides can be incorporated into first strand cDNA for use as a probe in hybridization experiments, including microarrays.

Kit Components

	50 Assays	100 Assays	200 Assays
M-MLV Reverse Transcriptase (200 U/μL)	50 μL	100 μL	200 μL
5X Reaction Buffer	200 μL	400 μL	800 μL
0.1M DTT	100 μL	200 μL	400 μL
dNTP Mix	50 μL	100 μL	100 μL
Random primer	50 μL	100 μL	200 μL
RNase free ddH_2O	1×1.25 mL	2×1.25 mL	4×1.25 mL

Assay Protocol

I. First Strand cDNA Synthesis

After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

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

Template RNA	total RNA or poly(A) mRNA	1 ng – 5 μg 10 pg - 0.5 μg
Random primer		1 μL
10 mM dNTP Mix		1 μL
Water, nuclease-free		to 12 μL

2. Mix gently, centrifuge briefly and incubate at 65°C for 5 min. Chill on ice, spin down and place the vial back on ice.

3. Add the following components in the indicated order :

5X Reaction Buffer	4 μL
0.1M DTT	2 μL
Water, nuclease-free	1 μL

4. Mix gently and centrifuge briefly. Incubate for 2 min at 37°C.
5. Add 1 μL M-MLV Reverse Transcriptase and Mix gently and centrifuge briefly. Incubate for 10 min at 25°C.
6. Incubate for 50 min at 37°C.
7. Terminate the reaction by heating at 70°C for 15 min.
   The reverse transcription reaction product can be directly used in PCR applications or stored at -20°C for less than one 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.

Store condition

-20°C

For Research Use Only. Not For Use In Diagnostic Procedures.