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

The 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 13 kb. The recombinant RNase Inhibitor, supplied with the kit, effectively protects RNA from degradation at temperatures up to 55°C. 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)	60μL	120μL	240μL
5X Reaction Buffer 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT	250μL	500μL	1000μL
dNTP Mix 10mM	125μL	250μL	500μL
Oligo dT25 Primer 100μM	60μL	120μL	240μL
Random Hexamer Primer 100μM	60μL	120μL	240μL
RNase Inhibitor 40U/μl	30μL	60μL	120μL
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 or specific RNA	0.1 ng - 5μg 10 pg - 0.5μg 0.01 pg - 0.5μg
Primer	Oligo (dT)25 primer or Random Hexamer primer or gene-specific primer	1μL 1μL 15-20 pmol
Water, nuclease-free		to 12μL
Total volume		12μL

2. Optional. If the RNA template is GC-rich or contains secondary structures, 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
RNase Inhibitor (40U/μL)	0.5 μL
10 mM dNTP Mix	2 μL
M-MuLV RT (200 U/μL)	1 μL
Total volume	20 μL

4. Mix gently and centrifuge briefly.
5. For oligo(dT)25 or gene-specific primed cDNA synthesis, incubate for 60 min at 42°C. For random hexamer primed synthesis, incubate for 5 min at 25°C followed by 60 min at 42°C.
   Note. For GC-rich RNA templates the reaction temperature can be increased up to 45°C.
6. Terminate the reaction by heating at 70°C for 5 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, 2μL of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50μL total volume.

Store condition

-20°C

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