**First Strand cDNA Synthesis Kit MR9098**

****

**Technical literature is available at:** [**www.mesgenbio.com**](http://www.mesgenbio.com)**. E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com**

**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/L) | 50 L | 100 L | 200 L |
| 5X Reaction Buffer | 200 L | 400 L | 800 L |
| 0.1M DTT | 100 L | 200 L | 400 L |
| dNTP Mix | 50 L | 100 L | 100 L |
| Random primer | 50 L | 100 L | 200 L |
| RNase free ddH2O | 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 RNAor poly(A) mRNA | 1 ng – 5 g10 pg - 0.5 g |
| Random primer | 1 L |
| 10 mM dNTP Mix | 1 L |
| Water, nuclease-free | to 12 L |

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 L |
| 0.1M DTT | 2 L |
| Water, nuclease-free | 1 L |

4. Mix gently and centrifuge briefly. Incubate for 2 min at 37°C.

5.Add 1 L 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 L of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 L total volume.

**Store condition**

-20°C

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