**First Strand cDNA Synthesis Kit MR9098**

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**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**

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/L) | 60L | 120L | 240L |
| **5X Reaction Buffer** 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM MgCl2, 50 mM DTT | 250L | 500L | 1000L |
| **dNTP Mix** 10mM | 125L | 250L | 500L |
| **Oligo dT25 Prime**r 100M | 60L | 120L | 240L |
| **Random Hexamer Primer** 100M | 60L | 120L | 240L |
| **RNase Inhibitor** 40U/l | 30L | 60L | 120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 RNA  or poly(A) mRNA  or specific RNA | 0.1 ng - 5g  10 pg - 0.5g  0.01 pg - 0.5g |
| Primer | Oligo (dT)25 primer  or Random Hexamer primer  or gene-specific primer | 1L  1L  15-20 pmol |
| Water, nuclease-free | | to 12L |
| Total volume | | 12L |

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L |
| RNase Inhibitor (40U/L) | 0.5L |
| 10 mM dNTP Mix | 2L |
| M-MuLV RT (200 U/L) | 1L |
| Total volume | 20L |

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, 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.***