I. List of Components

Store all components at –20°C.

- SMART MMLV Reverse Transcriptase
- 5X First-Strand Buffer
- 100 mM DTT

II. Additional Materials Required

- RNase-free H_2O
- dNTP Mix (10 mM each); we recommend Advantage® UltraPure PCR Deoxynucleotide Mix (Cat. No. 639125)
- Oligo $(dT)_{12–18}$, random hexamer or gene-specific primers may be used.
- [Optional] 60 mM EDTA

III. For Routine First-Strand cDNA Synthesis Reactions

This 20-µl reaction is suitable for synthesizing first-strand cDNA from 5 ng–5 µg of total RNA or 10 ng–1 µg of poly A^+ RNA.

1. Add 2.5 µl of 20 µM primer stock (final concentration 2.5 µM) to your RNA sample. Add RNase-free H_2O to a final volume of 11.5 µl.
2. Heat the mixture at 70°C for 3 min, and immediately cool on ice.
3. Centrifuge briefly, then add the following:

   4 µl 5X First-Strand Buffer
   2 µl dNTP Mix
   2 µl 100 mM DTT
4. Mix the contents of the tube by gently pipetting up and down. Add 0.5 µl SMART MMLV RT and mix again.
5. Incubate at 42°C for 60 min.

   NOTE: Samples can be incubated for 50–90 min if necessary.
6. Terminate the reaction by heating at 70°C for 15 min, or by adding 4 µl of 60 mM EDTA.

IV. References

Ausubel, F. M., *et al.* (1995) In *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.), Supplement 29, Section 5.5.

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