

I. List of Components

Always store any unused product at 20–22°C, either (1) back in the original foil pouch, re-sealed with desiccant; or (2) in a desiccator.

- RNA to cDNA EcoDry Premix 8-tube strips

NOTE: Each tube contains lyophilized master mix with the following components:

- SMART® MMLV Reverse Transcriptase
- Random hexamer and/or oligo(dT)₁₈ primers
- MgCl₂ (6 mM final conc.)
- BSA
- DTT
- dNTP Mix
- Reaction Buffer
- Cryoprotectant
- Stabilizers
- Optically Clear Cap Strips (8 caps/strip)

II. Additional Materials Required

- RNase-free H₂O
- Total or poly A⁺ RNA template
- [Optional] 60 mM EDTA
- [Optional] Benchtop centrifuge

III. First-Strand cDNA Synthesis Reaction

1. The 20- μ l reaction described below is suitable for synthesizing first-strand cDNA from total or poly A⁺ RNA. To measure yield by monitoring the incorporation of [α -³²P]dCTP, see Ausubel *et al.*, 1995.
2. Add 1 ng–1 μ g of poly A⁺ RNA or 5 ng–5 μ g of total RNA in RNase-free H₂O to obtain a final volume of 20 μ l.

NOTE: Prepare sufficient RNA/ H₂O to reconstitute the total number of wells required.

3. Pipet 20 μ l of the RNA/H₂O into each tube of RNA to cDNA EcoDry Premix that you require. Vortex or pipet the solution to mix.

NOTE: It is not necessary to pre-heat the RNA at 70°C prior to starting the first-strand cDNA synthesis reaction. Do **not** pre-heat the EcoDry tubes—this will inactivate the reverse transcriptase.

4. Incubate at 42°C for 60 min.
5. Stop the reaction by heating at 70°C for 10 min or by adding 4 μ l of 60 mM EDTA.

IV. Reference

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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This document has been reviewed and approved by the Clontech Quality Assurance Department.