

M-MuLV Reverse Transcriptase RNase H⁻

Standard protocol for the first strand synthesis of single-stranded cDNA from RNA

Using M-MuLV Reverse Transcriptase RNase H⁻ single stranded cDNA can be synthesized for cloning purposes or for use as a PCR template. Either total RNA or poly(A)⁺ RNA can be used as a template. New cDNA strand is synthesized at a site or sites determined by the type of primer used: at the 3' end of poly(A)⁺ RNA when oligo dT is used, at non-specific sites when random primers are used or at a specific site determined by sequence-specific primer used.

Standard protocol:

The final concentration of reaction buffer and suggested concentrations of other components are as follows:

50 mM Tris-HCl (pH 8.3)
75 mM KCl
3 mM MgCl₂
10 mM DTT
0.5 mM each dGTP, dTTP, dCTP and dATP
RNase inhibitor ⁽¹⁾
40 µg/ml p(dT)₁₂₋₁₈ or p(dT)₂₅ or
50-100 ng random primer or gene-specific primer per µg of RNA
Up to 40 µg/ml poly(A)⁺ RNA or total RNA
10 000 U/ml M-MuLV RNase H⁻ reverse transcriptase

A reaction volume of 20 or 25 µl may be used per µg of RNA.

1. In a sterile RNase-free microcentrifuge tube, add RNA and primer(s) in a total volume of 15 µl RNase-free water.
2. Heat the tube to 70°C for 5 minutes.
3. Chill the tube immediately on ice.
4. Spin briefly.
5. Add water to obtain reaction volume minus volumes of other components.
6. Add deoxynucleotides (0.5 mM final concentration)
7. Add 10x M-MuLV reaction buffer (1/10 reaction volume)
8. add RNase inhibitor⁽¹⁾
9. Add reverse transcriptase.
10. Mix gently and incubate for 60 minutes at 37–42°C.

⁽¹⁾ Although M-MuLV reverse transcriptase RNase H⁻ is free of contaminating RNases, the use of RNase inhibitor is strongly recommended. See manufacturer's recommendation for the unit amount.