Guidelines
AMV Reverse Transcriptase

Reaction Buffer Concentrate for AMV Reverse Transcriptase is a multipurpose buffer for use in first strand cDNA synthesis. Using the protocols below, a high percentage of full length cDNA of approximately 7.5 kb may be obtained.

Composition of Reaction Buffer Concentrate: This is used as a 10X or 5X stock depending on the choice of protocol, A or B.

250 mM Tris-HCl (pH 8.3 at 25 °C)
50 mM $MgCl_2$
500 mM KCl
20 mM DTT

Thaw at 37 °C. Triturate with pipet tip to dissolve, if necessary.

Protocol A: The reaction buffer concentrate is used as a 10X buffer for synthesis of first strand cDNA that can then be used in primer extension reactions. The final reaction conditions of the buffer, and suggested concentrations of the other components are as follows:

25 mM Tris-HCl (pH 8.3)
50 mM KCl
2.0 mM DTT
5.0 mM $MgCl_2$
1.0 mM each dGTP, dTTP, dCTP and dATP
1 U /µl RNasin™ (1)
40 µg/ml $p(dT)_{12-18}$ or $p(dT)_{25}$ or
50-100 ng random hexamer (2) or gene specific primer per µg of RNA
Up to 40 µg/ml poly(A)$^+$ mRNA or total RNA
200 U/ml or 400 U/ml AMV Reverse Transcriptase (5 U per µg of poly(A)$^+$ or 10 U per µg total RNA)

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

1. Heat RNA and primer at 70 °C for 10 minutes.
2. Chill mixtures on ice.
3. Microfuge reactions for a few seconds.
4. Add water to obtain reaction volume minus volumes of other components.
5. Add deoxynucleotides (1 mM final conc.).
6. Add buffer concentrate (1/10 reaction volume).
7. Add 0.5 - 1.0 U/µl RNasin™.
8. Add reverse transcriptase.
9. Mix gently and incubate for 40 min at 42 °C.

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Protocol B: This method utilizes the buffer concentrate as a 5X solution and is similar to the Gubler and Hoffman procedure [(a)] for preparing cDNA for cloning purposes.

Suggested Final Reaction Concentrations:

50 mM Tris-HCl (pH 8.3)
100 mM KCl
4 mM DTT
10 mM $MgCl_2$
1.0 mM each dGTP, dTTP, dCTP and dATP
1 U /µl RNasin™ [(1)]
40 µg/ml oligo $(dT)_{12-18}$ primer (varies with different protocols)
40 µg/ml poly(A)$^+$ mRNA (varies with different protocols)
4 mM sodium pyrophosphate
200–400 U/ml AMV Reverse Transcriptase (or 5–10 U per µg of poly(A)$^+$ mRNA)
Reaction volume: 20 µl

Method: A reaction volume of 20 µl should be used for 1 µg of poly(A)$^+$ mRNA.

1. Heat RNA and primer (oligo dT_{12-18}) at 70 °C for 5 minutes.
2. Chill mixture by placing on ice.
3. Microfuge reactions for a few seconds.
4. Add water to obtain reaction volume minus volumes of other components.
5. Add 5X buffer and deoxynucleotides.
6. Add RNase Inhibitor or RNasin™.
7. Add sodium pyrophosphate.
8. Add reverse transcriptase.
9. Mix gently and incubate for 60 min at 41 °C.

[(1)] Although AMV Reverse Transcriptase is RNase-free, the use of RNase inhibitor is recommended to protect the reaction from accidental RNase contamination.
[(2)] The mass ratio of random hexamer to RNA template directly affects the size of cDNA transcripts. Use 50–100 ng of hexamer per 1 µg of RNA template to obtain transcripts up to 7 kb. Larger amounts of hexamer will decrease the transcript size.

Reference:

(a) Guide to Molecular Cloning Techniques. Methods in Enzymology, Volume 152, pp 316-325. Edited by Shelby Berger and Alan R. Kimmel. Academic Press, Inc.

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