Reverse transcriptase reaction

Please note that the concentrations listed below are the final concentrations in the reaction

Reagents available from PerkinElmer

- 0.1 mM dATPgammaS35 (Cat. No. NEG034H)

Reagents needed and available from other suppliers

- 1 mM dCTP, 1 mM dTTP and 1 mM dGTP
- 20 mM DTT
- 50 mM Tris-HCl, pH 8.3
- 100 µg/mL actinomycin D
- 60 mM NaCl
- 6 mM $MgCl_2$
- 5-10 µg/mL RNA to be 5 µg/mL oligo dT reverse transcribed 280 units/mL reverse transcriptase

1. The reaction is initiated by adding of reverse transcriptase. The incubation temperature is 37°C and the length of the incubation is at least 90 min.
2. The reaction may be terminated in the usual manner or by the addition of two volumes of 0.5% SDS, 5 mM EDTA.

Tips

Note that NEG034H (^{35}S-dATP-gammaS) is supplied in 10 mM Tricine and 1mM DTT. If this solution needs to be concentrated prior to use with reverse transcriptase caution must be exercised to ensure that the final DTT does not inhibit the enzyme activity. If necessary remove the DTT stabilizer as follows:

- Perform entire procedure in the cold (0-4°C)
- Prepare a glass column (0.6 cm x 18 cm) containing a glass wool plug packed with 5.0 mL of settled Sephadex G-10 (a 5 mL disposal glass pipet is suitable)
- Equilibrate column with water, pH 7-8
- Apply the ^{35}S nucleotide as shipped to the column in a *maximum volume of 0.5 mL*. Immediately begin collecting 1.0 mL fractions
- Elute column with water, pH 7-8. The ^{35}S nucleotide will elute in fraction of 3 and 4 (approx. 80-85% of the nucleotide elutes in fraction 3). The DTT, Tricine and ethanol begin to elute at 4.5-5 mL.
- The ^{35}S in water may be used directly or further concentrated. Concentration may be rapidly accomplished by adding an equal volume of ethanol followed by rotary evaporation or under a stream of nitrogen (this last step should be carried out at RT: 20-25°C).
- Use the non-stabilized ^{35}S nucleotide in 1-2 days (*without stabilizer the* ^{35}S w*ill decompose at the rate of a few percent per day*)

Q. **Do I need to use siliconized glass tubes and pipets?**
A. Yes, all procedures should be carried out using polypropylene, polyethylene or siliconized glass tubes and pipets. DNA adheres strongly to untreated glass as well as some plastic which will results in undesirable losses in nick translated products.

Q. **How do I prepare my nucleic acid material?**
A. For this particular protocol, we recommend that your nucleic acid starting material is prepared in Tris-EDTA buffer (10 mM Tris,1 mM EDTA) at pH 8.1-8.3, or to be precipitated and then reconstituted in this buffer solution before use. The concentrations of DNA, enzymes and radioactive triphosphate can be adjusted to achieve maximum incorporation of label.

Citation

1. Friedman, E.Y. & Rosbash, M. The syntheis of high yields of full-length reverse transcripts of globin mRNA. Nucleic Acids Res 4, 3455-3471 (1977). Link