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ISOLATION OF RNA FROM SUCROSE GRADIENTS USING TRI REAGENT® LS

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Sucrose gradients with varying concentrations of salts are used to separate nucleic acids. TRI Reagent® LS can be used to recover RNA from sucrose gradients containing up to 50 % sucrose (wt/wt).

PROTOCOL

Combine 250 µl of a sucrose gradient fraction with 750 µl of TRI Reagent LS (Cat. No. TS 120)and mix thoroughly. Perform the phase separation and precipitate RNA according to the TRI Reagent LS protocol. Take particular care when removing the aqueous phase from the phenol phase. If the sucrose gradient fraction contains a high concentration of sucrose and/or salt, there may be a cloudy region near the interphase that enhances the opportunity to draw phenol into the pipet tip. If necessary, do not recover the cloudy portion of the aqueous phase directly adjacent to the phenol phase.

If the expected yield of RNA is 20 µg or less, add 2 - 4 µl of Polyacryl Carrier (Cat. No. PC 152) to the aqueous phase prior to precipitation. Addition of the carrier improves recovery of small quantities of RNA.

NOTES

Recovery of RNA may vary depending upon the sucrose and salt concentrations of the gradient fractions. When both sucrose content and salt concentration are relatively low (about 5% and 100 mM, respectively), TRI Reagent LS extraction recovers greater than 90% of the total RNA. As sucrose and/or salt contents increase, RNA recovery will tend to decrease. Samples with high sucrose and/or salt contents (about 50% and 1.0 M, respectively) may be diluted to improve RNA recovery. If the dilution step reduces expected RNA yield to 20 µg per sample, use Polyacryl Carrier as described above to improve recovery.

When separated by electrophoresis on a denaturing 1% agarose - formaldehyde gel, RNA isolated from sucrose gradients is of high quality with intact 18S and 28S ribosomal bands.

Cite this protocol by referring to Molecular Research Center, Inc. Technical Bulletin 9.