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ISOLATION OF PLASMID-FREE RNA FROM TRANSIENTLY TRANSFECTED CELLS

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Isolation of RNA from transiently transfected cells yields RNA contaminated with plasmid DNA. Typically, this contaminant is removed by DNAse treatment of the isolated RNA and subsequent phenol extraction. The following protocol combining the use of TRI Reagent® (Cat. No. TR 118) and DNAzol® (Cat. No. DN 127) allows for the isolation of plasmid-free RNA in a short time without DNAse treatment and phenol extraction.

PROTOCOL

1. Lyse cells in TRI Reagent, perform the phase separation, and precipitate RNA from the aqueous phase by addition of isopropanol according to the standard TRI Reagent protocol (RNA Isolation, Steps 1 - 3).
2. Following isopropanol precipitation and centrifugation to obtain the RNA pellet, wash the RNA pellet in a solution containing one volume of DNAzol and 0.3 volumes of 100% ethanol. The total volume of this solution should be 250 - 500 µl. Further increasing this volume may reduce the recovery of RNA. Gently agitate or shake the solution for washing and store at room temperature for 5 - 10 minutes. The RNA pellet will not resuspend in this solution, but the pellet may become dispersed.
3. Centrifuge the RNA pellet at 12,000 g for 10 minutes at 4 - 20 C. Wash the RNA pellet twice in ethanol and centrifuge at 7,500 - 10,000 g for 5 minutes at 4 - 20 C. Briefly air dry the pellet and resuspend in the desired buffer. Do not over-dry the RNA pellet.

Cite this protocol by referring to Molecular Research Center, Inc. Technical Bulletin 4.
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