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Protocol

Precipitation of RNA with Ethanol

Michael R. Green and Joseph Sambrook

Purified RNA may need to be concentrated by precipitation for downstream applications. Precipitation of RNA with ethanol (or isopropanol) is the standard method to recover RNA from aqueous solutions.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

DEPC-treated water
Ethanol (70%), ice cold
Ethanol (100%) or isopropanol, ice cold
RNA solution
Salt (5 M ammonium acetate, 8 M LiCl, or 3 M sodium acetate)

METHOD

Solutions used for precipitation of RNA must be free of RNase.

1. Estimate the volume of the RNA solution.
2. Adjust the concentration of monovalent cations by addition of one of the salt solutions shown in Table 1. If the RNA solution contains a high concentration of salts, dilute with TE (pH 7.0). Mix the solution well.

   To increase the salt concentration, for example, add 0.1 volume of 3 M sodium acetate.

   If the volume of the final solution is 400 µL or less, carry out the precipitation in a single microcentrifuge tube. Larger volumes can be divided among several microcentrifuge tubes, or the RNA can be precipitated and centrifuged in tubes that will fit into a medium-speed centrifuge or ultracentrifuge.
3. Add 2.5–3.0 volumes of ice-cold ethanol (or 1 volume of isopropanol) and mix the solution well. Store the ethanolic solution for 1 h to overnight at −20°C to allow the RNA to precipitate.

   RNA precipitation is faster and more complete at higher RNA concentrations. A general rule of thumb is that RNA concentrations of 10 µg/mL can usually be precipitated in several hours to overnight with no difficulty, but at lower concentrations, glycogen should be added to facilitate precipitation and maximize recovery (see Troubleshooting).

   RNA can be stored for up to 1 yr at −80°C.

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TABLE 1. Salt solutions for precipitating RNA

Salt	Stock solution (M)	Final concentration (M)
Ammonium acetate	5	0.5
Lithium chloride	8	0.8
Sodium acetate	3 (pH 5.2)	0.3

4. Recover the RNA by centrifugation at 12,000*g*–14,000*g* for 10 min at 4°C.
5. Decant the supernatant, and carefully remove remaining traces of the supernatant with an automatic micropipettor or with a disposable pipette tip attached to a vacuum line (see Fig. 1). Take care not to disturb the pellet of nucleic acid (which may be invisible). Use the pipette tip to remove any drops of fluid that adhere to the walls of the tube.
6. Wash the pellet with 0.5 mL of ice-cold 70% ethanol and centrifuge at maximum speed for 10 min at 4°C in a microcentrifuge.
7. Repeat Step 5.
8. Store the open tube on the bench at room temperature until the last traces of fluid have evaporated.

   Do not dry the RNA pellet completely; otherwise, it can be difficult to dissolve the RNA.
9. Dissolve the RNA pellet (which is often invisible) in the desired volume of RNase-free buffer (usually TE; pH between 6 and 7). Rinse the walls of the tube well with the buffer.

   To aid solubilization, the RNA pellet can be incubated in the resuspension solution for 5 min at 65°C with intermittent gentle vortexing.

TROUBLESHOOTING

Problem (Step 3): There is low recovery of RNA from low concentrations (ng/mL).

Solution: Glycogen is an inert coprecipitant that is used for quantitative recovery of RNA at low concentrations. When used at a final concentration of 50–150 µg/mL, glycogen will coprecipitate with RNA in the presence of 0.5 M ammonium acetate and ethanol/isopropanol. Note that glycogen is usually contaminated with nucleic acid, which can affect UV absorbance readings and compete in subsequent enzymatic reactions (e.g., RT-PCR). Therefore, if the precipitated

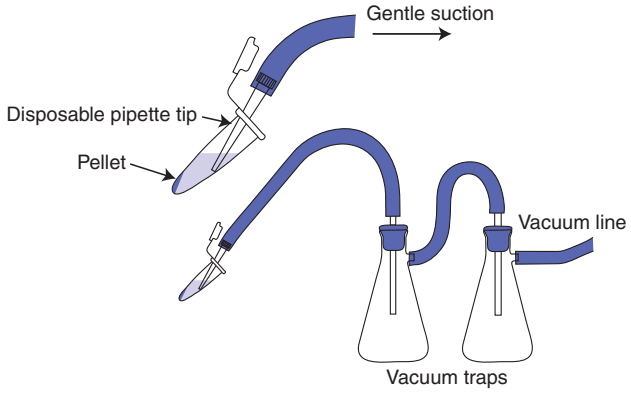

FIGURE 1. Aspiration of supernatants. Hold the open microcentrifuge tube at an angle, with the pellet on the *upper* side. Use a disposable pipette tip attached to a vacuum line to withdraw fluid from the tube. Insert the tip just beneath the meniscus on the lower side of the tube. Move the tip toward the base of the tube as the fluid is withdrawn. Use a gentle suction to avoid drawing the pellet into the pipette tip. Keep the end of the tip away from the pellet. Finally, vacuum the walls of the tube to remove any adherent drops of fluid.

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RNA is to be used subsequently for reverse transcriptase-polymerase chain reaction (RT-PCR), it is strongly advised to use a nucleic acid-free preparation of glycogen (e.g., Ambion catalog number AM9510). Products are also available that consist of a colored dye covalently linked to glycogen (e.g., GlycoBlue from Ambion), which can be used to increase both the size and visibility of the RNA pellet.

DISCUSSION

RNA is efficiently precipitated from solutions containing 0.8 M LiCl, 0.5 M ammonium acetate, or 0.3 M sodium acetate. The choice among these salts is determined by the way in which the RNA will be used later. Because the potassium salt of dodecyl sulfate is extremely insoluble, avoid potassium acetate if the precipitated RNA is to be dissolved in buffers that contain sodium dodecyl sulfate (SDS) (which is sometimes used for storing RNA because it inhibits ribonucleases). For the same reason, avoid potassium acetate if the RNA is already dissolved in a buffer containing SDS. Avoid LiCl when the RNA is to be used for cell-free translation or reverse transcription, as LiCl ions inhibit initiation of protein synthesis in most cell-free systems and suppress the activity of RNA-dependent DNA polymerase.

In addition to the type of salt, the type of alcohol used for the precipitation is also a factor. RNA can be precipitated with either 2.5–3.0 volumes of ethanol or 1 volume of isopropanol; the latter can be advantageous when trying to keep the volume of fluid to a minimum. Although isopropanol is somewhat less efficient at precipitating RNA than ethanol, isopropanol in the presence of ammonium cations is better than ethanol at keeping free nucleotides in solution and thus separating them from the precipitated RNA. However, isopropanol is less volatile than ethanol and is therefore more difficult to remove. For a discussion on the principles of ethanol precipitation, see Protocol: **Precipitation of DNA with Ethanol** (Green and Sambrook 2016).

REFERENCES

Green MR, Sambrook J. 2016. Precipitation of DNA with ethanol. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot093377.

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