Oct 14, 2019

RNA precipitation

DOI
dx.doi.org/10.17504/protocols.io.73chqiw

Liz Hughes[1]

[1]University of Edinburgh

Wallace lab for Fungal RNA
Tech. support email: **Edward.Wallace@ed.ac.uk**

Liz Hughes
University of Edinburgh

DOI: https://dx.doi.org/10.17504/protocols.io.73chqiw

Protocol Citation: Liz Hughes 2019. RNA precipitation . **protocols.io https://dx.doi.org/10.17504/protocols.io.73chqiw**

Protocol status: Working
We use this protocol in our group and it is working.

Created: October 09, 2019

Last Modified: October 14, 2019

Protocol Integer ID: 28484

Keywords: RNA precipitation, RNA clean up, RNA concentration, precipitating nucleic acid, rna precipitation, nucleic acid, rna, purified rna, sodium acetate, nucleic acid backbone, versatile salt, charge on the nucleic acid backbone, salt, dna, extraction

Abstract

This protocol can be used to precipitate RNA to clean it up after extraction or to concentrate it.

Sodium acetate is highly efficient at precipitating nucleic acids and is the most versatile salt because it does not inhibit many of the reactions that are often performed with purified RNAs. The salt neutralizes the charge on the nucleic acid backbone. This causes the DNA to become less hydrophilic and precipitate out of solution.

Guidelines

An RNase free environment is essential when working with RNA samples.
RNases are very hardy and removing them can be very difficult.
RNAses are a ubiquitous component of skin.
Always wear gloves when handling RNA and any associated materials/equipment.
This protocol is for small volumes of RNA using a 1.5ml microcentrifuge tube.

Materials

MATERIALS

- RNAse-free Water
- Propan-2-ol, AR **Catalog #**AR 1162
- Sodium Acetate 3M, pH 5.2 **Thermo Scientific Catalog #**R1181
- Ethanol **Merck Millipore (EMD Millipore) Catalog #**100983

Ensure the sodium acetate and propan-2-ol are at room temperature.
Be aware that Propan-2-ol will precipitate salts at low temperatures.
Make up a 70% solution of ethanol and cool on ice before starting.
Ethanol washes will remove any precipitated salt and exchange the propan-2-ol for ethanol prior to drying.

Troubleshooting

1 Make up the volume of your RNA solution to 500µl with RNase free water.

2 Add 50 µl of room temperature 3M Sodium Acetate (pH 5.2-5.5)

3 Add 500 µl of room temperature Propan-2-ol.
Mix by inverting a few times.
Leave at room temperature for 15-20 mins.

4 Pellet the precipitated RNA by centrifugation at 12,000 g for 15 mins.
Take note of the side of the tube where the pellet will be.

5 Note the pellet will appear clear and glassy after the supernatant has been removed.
Carefully remove the supernatant and wash the pellet with 500µl 70% ice cold ethanol.
Be very carefull not to dislodge the pellet.
It will appear white and more visable after the ethanol wash.
Spin at 12,000g for 2 mins.
Repeat this step one more time. (i.e. 2 ethanol washes)
Carefully remove all of the ethanol.

6 Allow the pellet to dry with the tube upside down on tissue at room temperature for 5-30 mins.

7 Resuspend the pellet in RNase free water in an appropriate volume (usually 30-50µl)
Assess the RNA quantity and integrity using the NanoDrop and Fragment Analyzer.