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Ethanol precipitation of RNA from small or large volumes

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Stephen Floor[1]

[1]University of California, San Francisco

Stephen Floor Lab

Stephen Floor
University of California, San Francisco

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We use this protocol and it's working

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Abstract

How to precipitate RNA with ethanol and resuspend it! Two separate protocols - one for small and one for large volumes. The procedure for large volume precipitation is similar, but begins with an initial precipitation and transfers to a small tube for the final steps to avoid loss in the large tube. Strongly recommended to move to a smaller tube.

Recommended reading: Walker & Lorsch **RNA purification--precipitation methods**.

Materials

MATERIALS

GlycoBlue™ Coprecipitant **Thermo Scientific** **Catalog #**AM9516

70% Ethanol

100% Ethanol (KOPTEC) **VWR International (Avantor)** **Catalog #**89125-186

0.3 M NaOAc pH 5.2
3 M NaOAc pH 5.2

Note: the protocol requires 70% ethanol pre-chilled to -20 C and requires 100% ethanol pre-chilled to -20 C for large volumes.

Troubleshooting

Precipitating RNA (< 500 ul sample volume)

1 Record the sample volume. If unknown, you can estimate it using the side of the tube or meausure it by pipetting it.

2 Add NaOAc pH 5.2 to sample to a final concentration of 0.3M

Note

This can be accomplished by diluting the sample by at least five volumes with 0.3M NaOAc or adding 3M NaOAc to a final concentration of 0.3M.

[CRITICAL] Do not exceed 500 ul NaOAc-diluted volume per 1.5 ml tube. For large volumes, see alternate protocol below.

3 Add 1 ul glycoblue per 250 ul sample volume

Note

volume here is the volume after adding NaOAc, not the original volume.

4 Vortex briefly

5 Add 2 volumes 100% ethanol

Note

volume here is the volume after NaOAc, not the original volume. For example, add 1 ml ethanol to 500 ul NaOAc solution.

6 Vortex briefly

7 Incubate at -80 degrees for at least 1 hour or in a dry ice ethanol bath for 30 minutes.

Note

[PAUSE] RNA is more stable for long periods when stored in ethanol at low temperature than as a pellet. Shorter incubation times are probably ok - glycoblue protocol recommends 30 minutes at -20 C.

8 Pellet RNA at 16000 x g , in tabletop microfuge 16k g for 25 minutes at 4 °C

Note

Tip: orient the tubes so the hinge is pointed outwards and the pellet will be easier to identify along the hinge-side of the tube.

9 Aspirate supernatant carefully without dislodging pellet, first using a P-1000 for most volume and then a P-20 for the end. Using a vacuum aspirator is not recommended as it is easy to aspirate the pellet.

10 Gently add 500 ul of 70% ethanol at -20 °C

Note

the warmer the 70% ethanol is, the more RNA will re-solubilize. Take the 70% out from the -20 right before adding it and then return it to the -20.

11 Pellet RNA at 16000 x g , in tabletop microfuge 16k g for 5 minutes at 4 °C

12 Aspirate supernatant carefully without dislodging pellet, first using a P-200 for most volume and then a P-20 for the end.

13 Quick spin to bring down remaining ethanol

14 Remove as much ethanol as possible without aspirating pellet using a P-20

15 Dry for 5 minutes at RT and not longer - fully dried pellets are harder to resuspend

16 Add the desired volume of water or buffer

17 Scrape the tube along a tube rack to dislodge pellet

18 Quick spin

19 Measure concentration using nanodrop or qubit

Precipitation of RNA (large sample volume)

20 Add NaOAc to sample to a final concentration of 0.3M

Note

This can be accomplished by diluting the sample by at least five volumes with 0.3M NaOAc or adding 3M NaOAc to a final concentration of 0.3M.

21 Add 1 ul glycoblue per 250 ul sample volume (after adding NaOAc)

22 Vortex briefly

23 Add 2 volumes 100% ethanol (1 ml 100% ethanol to 500 ul NaOAc-diluted sample)

24 Vortex briefly

25 Incubate at -80 degrees 1 hour to overnight.

Note

[PAUSE] RNA is more stable for long periods when stored in ethanol at low temperature than as a pellet.

Pelleting and resuspension of RNA

26 Centrifuge in a conical tube at > ~5000g, for 25 minutes

27 Aspirate supernatant carefully with a serological pipet or vacuum aspirator

Note

don't worry about getting all the ethanol off - trying to get all the ethanol off will likely remove some pellet as this is a loose pellet

28 Add 750 ul of 100% ethanol at -20 °C

29 Resuspend chunks of pellet in the 100% ethanol by pipetting up and down

30 Transfer ethanol and chunks to a microfuge tube

31 Spin for 5 minutes at 16k g at 4 °C

32 go to step #12 to wash and resuspend pellet