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Ethanol precipitation of RNA from small or large volumes

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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

NAME	CATALOG #	VENDOR
GlycoBlue™ Coprecipitant	AM9516	Thermo Scientific
70% Ethanol		
100% Ethanol (KOPTEC)	89125-186	Vwr

MATERIALS TEXT

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.

Precipitating RNA (< 500 ul sample volume)

- 1 Record the sample volume. If unknown, you can estimate it using the side of the tube or measure it by pipetting it.
- 2 Add NaOAc pH 5.2 to sample to a final concentration of 0.3M



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



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

4 Vortex briefly

5 Add 2 volumes 100% ethanol



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.



[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 - glycoblu 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



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



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



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.



[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 $> \sim 5000g$, for 25 minutes

27 Aspirate supernatant carefully with a serological pipet or vacuum aspirator



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

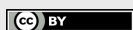
28 Add 750 ul of 100% ethanol at -20 °C

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



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