

RNA Extraction from 3D Gels

Materials:

Gels for RNA extraction

RNA miniprep kit (link for more information: [GenElute Mammalian Total RNA Miniprep Kit, Sigma](#))

Lysis solution

2-mercaptoethanol (2-ME)

Wash solution 1

Wash solution 2

Elution solution

GenElute Filtration Column (blue insert with a 2mL receiving tube)

GenElute Binding Column (red insert with a 2mL receiving tube)

2mL collection tubes (colorless)

70% Ethanol (RNase-free)

Small amount of liquid N₂

Dewar flask for liquid N₂

1.5mL microcentrifuge tubes (# of tubes = # of gels)

Lab spatula



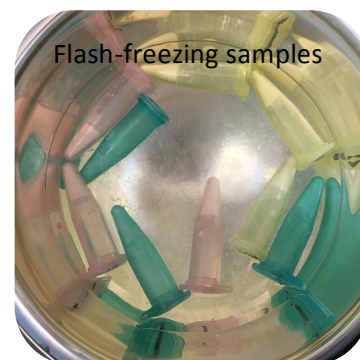
[A]. Preparation:

1. Prepare 70% ethanol solution:
 - a. Make a large volume (e.g. 50mL) at a time, and use this stock solution for RNA extraction.
 - b. Bring an empty 50mL conical tube.
 - c. Put 35mL of 100% Ethanol and 15mL of RNase-free water into the 50mL tube.
 1. **100% Ethanol**
Product name: **Ethyl Alcohol 200 proof**
Company: PHARMCO-AAPER
Location: Under lab benches – normally between Alyssa's and Elizabeth's benches)
 2. **RNase-free water** (1L bottle)
Product name: **Water, Sterile (for RNA work)**
DEPC-treated and Nuclease-free
Company: Fisher Scientific
Location: On the top cabinet next to a stock of pipet tip boxes, near the sink.
 - d. Vortex the tube to mix the solution. This becomes a final 50mL volume of 70% ethanol stock solution, and this can be stored at a room temperature.
2. Prepare Lysis/2-ME solution (follow the Sigma Kit Protocol).
 - a. Add 2-mercaptoethanol (2-ME) to a volume of Lysis Solution sufficient for that day's use, usually 250 or 500µL of Lysis/2-ME Mixture per RNA preparation sample (see the table below). [2-ME is required to fully inactivate RNases.]
 - b. Add 10µL of 2-ME for each 1mL of Lysis solution.
 - c. For example, if there are N=8 samples with less than 5×10^6 cells, prepare 2mL of Lysis/2-ME mixture (250µL/sample * 8 samples = 2000µL): Add 20µL of 2-ME into 2mL Lysis solution.

Sample Size	Lysis solution/2-ME Mixture Volume
Up to 5×10^6 cells	250µL
5×10^6 to 1×10^7 cells	500µL

[B]. Gel Destruction:

1. Using a lab spatula, detach gels from a well plate.
2. Carefully transfer gels into 1.5ml tubes using the lab spatula.
 - a. Each gel goes into a different tube (one gel per one tube).
 - b. Wipe the lab spatula with KimWipes every time you change the sample.
3. Add 250 μ L of Lysis/2-ME solution into each tube and close the cap.
4. Take a small amount of liquid N₂ into a Dewar flask.
5. Flash-freeze the samples by putting the tubes into liquid N₂.
6. After 5-10 seconds, take out the tubes.
7. Keep the tubes at a room temperature for 3-5 seconds.
8. When the samples start thawing, try to break/poke/stir the samples using 1mL pipet tips until the samples become slush-like liquid.
 - a. Use different tips between each sample.
9. Repeat all the steps from 5 to 8 (from flash-freezing to thawing) three times.



[C]. RNA Extraction: Follow the Sigma Kit Protocol from Step 3a on Page 7.

1. Transfer the entire sample solution with pieces of destructed gel into GenElute Filtration Column (blue insert with a 2mL receiving tube).
2. Centrifuge at maximum speed (12000-16000 x g) for 2 minutes.
3. Discard the filtration column.
4. Add an equal volume of 70% ethanol solution (250 or 500 μ L) to the filtered lysate.
5. Vortex or pipette thoroughly to mix.
6. Pipette up to 700 μ L of the lysate/ethanol mixture into a GenElute Binding Column (red insert with a 2mL receiving tube).
 - a. If the volume of lysate/ethanol mixture is greater than 700 μ L, the RNA must be bound to the column in 2 steps.
7. Centrifuge at maximum speed (12000-16000 x g) for 15 seconds.
8. Discard the flow-through liquid, but retain the collection tube.
9. Return the binding column to the collection tube. (If any, apply remaining lysate/ethanol mixture to the column, and repeat the steps 7 to 9).
10. **First Column Wash:** Pipette 500 μ L of Wash Solution 1 into the column, and centrifuge at maximum speed (12000-16000 x g) for 15 seconds.
11. Transfer the binding column into a fresh 2mL collection tube.
12. Discard the flow-through liquid and the original collection tube.
13. **Second Column Wash:** Pipette 500 μ L of ethanol containing Wash Solution 2 into the column, and centrifuge at maximum speed (12000-16000 x g) for 15 seconds.
 - a. Ensure that Wash Solution 2 Concentrate has been diluted with ethanol as described in the Sigma Kit Instructions.
14. Discard the flow-through liquid, but retain the collection tube.
15. **Third Column Wash:** Pipette a second 500 μ L volume of Wash Solution 2 into the column, and centrifuge at maximum speed (12000-16000 x g) for 2 minutes to dry the binding column.
 - a. The binding column must be free of ethanol before eluting the RNA.
 - b. If any residual Wash Solution 2 is seen on the surface of the binding column, centrifuge the column for an additional 1-minute at maximum speed.
 - c. Empty & reuse the collection tube if you need this additional centrifugation step.
16. Transfer the binding column to a fresh 2mL collection tube.

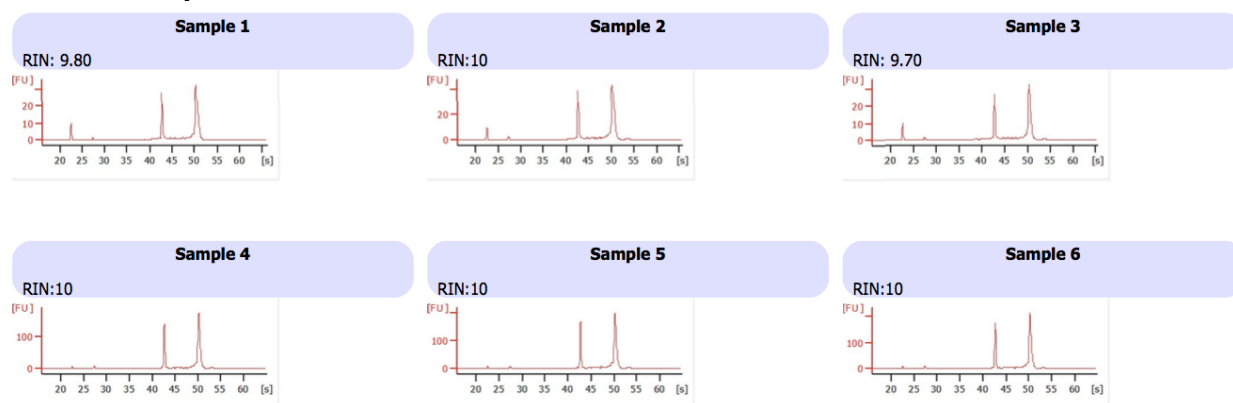
17. **Elute RNA:** Pipette 50 μ L of Elution Solution into the binding column, and centrifuge at maximum speed (12000-16000 x g) for 1 minute.
- If greater than 50 μ g of RNA is expected, repeat the elution with a second 50 μ L volume of Elution Solution, collecting both eluates in the same tube.
 - Expected yield (from pelleted cells): 10-30 μ g of total RNA per million cultured cells. [*Expected yield from gel-seeded cells is lower than this.]
18. Purified RNA is now in the flow-through eluate (~45 or 90 μ L total), and is ready for immediate use or storage at -80°C. Keep the RNA on ice whenever it is thawed for use.

[Notes]: Tested conditions & analyzed results from each condition

- Nanodrop results:

Conditions	Cell seeding density	Average RNA concentration (ng/ μ L)	260/280 ratio	Conditions	Cell seeding density	Average RNA concentration (ng/ μ L)	260/280 ratio
TCPS (12-well plate)	10,000 cells/well	6.24	2.45	Gel (PEG-Mal gel)	10,000 cells/gel	2.06	1.86
	40,000 cells/well	22.49	2.05		40,000 cells/gel	7.48	2.12
	100,000 cells/well	48.48	2.10		100,000 cells/gel	13.22	2.19
	200,000 cells/well	105.82	2.05		200,000 cells/gel	16.84	2.12
	500,000 cells/well	213.78	2.10		500,000 cells/gel	39.67	2.19

- Bioanalyzer results:



- Sample 1-3: Total RNA from 500,000 cells/gel conditions.
 - Sample 4-6: Total RNA from 500,000 cells/well (TCPS) conditions.
- Type of gels tested for RNA extraction using this protocol:
 - PEG gel (PEG-Maleimide)
 - PEG-RGD gel
 - Bone marrow mimic gel (Lauren Jensen's Bone marrow gel)