RNA Extraction for Cells in Hydrogel Protocol

Description

This protocol provides instructions to extract RNA with good quality (A260/280 ratio close to 2.0 and yield more than 200 ng RNA per sample) RNA for qPCR experiment from cells cultured in the hydrogels.

Materials Needed

- Hydrogel cultured with cells
- TRIzol (Thermo-Fisher Scientific Cat # 15596026)
- Isopropanol
- RNase-free water
- Qiagen RNeasy Kit (Qiagen Cat #74104)
- Sterile, RNase-free pipette tips
- Microcentrifuge tubes
- Microcentrifuge
- Centrifuge equipped with cooling system
- 75% ethanol
- Tissue homogenizer
- RNase Zap wipes/spray
- Cell culture plates
- Centrifuge tubes
- Serological pipettes
- Micropipette and tips
- Vortex mixer
- Microplate reader/Nanodrop reader

Procedure

Sample preparation

1. Remove cell culture medium and rinse the hydrogels with PBS.

2. Either extract the RNA immediately or freeze scaffolds sample at -80 °C for storage.

RNA Extraction

1. Transfer samples into the tissue homogenizer and break down the samples completely (~5 min).

2. Turn on the microcentrifuge and cool to 4°C.

3. Transfer the disrupted hydrogels into a well-plate and lyse the cells with 500 µL of TRIzol per gel.

4. Pipette the lysate using the micro-pipette tip and transfer the lysate into a microtube. Avoid transferring the hydrogel debris into the microtube as much as possible.

5. Add 200 µL of chloroform to the lysate and vortex the mixture for 15 seconds. Centrifuge the mixture at 12000 × g under 4°C for 10 minutes.

6. After centrifugation, there should be three separate phases present in the microtube. From top to bottom, it contains: (1) an aqueous phase (containing RNA), (2) an interphase (containing DNA), and (3) an organic phase (containing proteins and lipids).

7. Carefully collect the top-most layer without touching the middle white layer and transfer to a new microtube.

8. To the new microtube, add an equal volume of isopropanol and vortex the mixture for 15 seconds. Centrifuge the mixture at 12000 x g under 4°C for 5 minutes.

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9. After centrifugation, there might be a white pellet sticking at bottom of the tube. Aspirate the supernatant and add 350 μL of RLT buffer supplied in the RNeasy kit.

10. Pipette the solution several times to break up the white pellet. Add 100 μL of RNease free water and 250 μL of 100% ethanol to the tube.

11. Transfer the ~700 μL mixture to a RNeasy spin column placed inside a 2 mL collection tube. Close the lid gently and centrifuge for 30 seconds at 13000 rpm in a microcentrifuge. Discard the flow through. The RNA is on filter now. Reuse the collection tube for the next step.

12. Add 700 μL of Buffer RW1 to the RNeasy spin column, close the lid and wait for 5 minutes. Centrifuge for 30 seconds at 13000 rpm to wash the spin column membrane. Discard the flow through.

    ***Notes:**
    a. Remove spin column carefully to avoid contact with the flow through.
    b. Empty the collection tube completely.
    c. Skip this step if performing column DNase digestion.

13. Add 500 μL of Buffer RPE (reconstituted with ethanol) to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at 13000 rpm to wash the spin column membrane. Discard the flow through. Reuse the collection tube.

14. Add 500 μL of Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 minutes at 13000 rpm to wash the spin column membrane. Discard flow through.

    ***Notes:**
    a. Carefully remove RNeasy spin column to prevent contacting the flow through.
    b. Make sure all liquid is removed. If not, spin again.

    Centrifuge the spin column without any buffer for 1 minute to fully remove the RPE buffer since the salts in RPE buffer will affect the result.

15. Place the RNeasy spin column in a new 1.5 mL collection tube (supplied with the kit).

16. Add 25 μL of RNase-free water **<u>directly to the spin column membrane</u>**. Close the lid gently and incubate for 5 minutes on ice. Then centrifuge for 2 minutes at 13000 rpm to elute the RNA.
    ***Notes:**
    a. Check for liquid on the membrane – re-spin if needed.

17. Add the 25 μL of RNA elutent back to the spin column. Incubate for 5 minutes on ice and then centrifuge for 2 minutes at 13000 rpm to elute the RNA again.

18. If the expected RNA yield is >30 μg, repeat the previous step using another 30 – 50 μL of RNase-free water or using eluate from the previous step (if high RNA concentration is required). Reuse the collection tube from the previous step.

19. Save the eluent and <u>this has the RNA</u>. Samples may be stored at -80 °C (stopping point).

20. Take 2 μL of RNA elute from each sample and load onto the microplate/nanodrop reader. Read the sample to obtain RNA purity and concentration.

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***Notes:**

For typical nanodrop reading, we obtain:

1) RNA Concentration (ng/µL):

This value represents the amount of RNA present in the sample. Nanodrop measures absorbance at 260 nm (A260), which is used to calculate the RNA concentration based on the Beer-Lambert law.

2) A260/A280 Ratio:

This ratio indicates the purity of the RNA with respect to protein contamination. A ratio of ~2.0 is generally considered pure for RNA. Ratios significantly lower than 2.0 suggest the presence of proteins or other contaminants that absorb at 280 nm.

3) A260/A230 Ratio:

This ratio provides an additional measure of RNA purity, specifically indicating contamination by organic compounds, such as phenol, guanidine, or other chemicals that might be present after extraction. An ideal A260/A230 ratio is typically between 2.0 and 2.2. Lower values may indicate the presence of contaminants that absorb at 230 nm.

21. Normalize the sample RNA concentration to lowest RNA yield for all the samples by diluting the RNA with RNase-free water.

22. The RNA can be stored at -80 °C before cDNA synthesis.

***Notes:**

a. Pure RNA is not stable and cDNA synthesis should be performed as soon as possible. The cDNA samples are more stable and can be stored at -20 °C for months.

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