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Total RNA isolation from cells using RNAqueous-Micro Total RNA Isolation Kit

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We use this protocol and it's working

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Abstract

For the isolation of total RNA from a small number of cells

Sample disruption

1 Resuspend cell pellet (up to ~500,000 cells) by vortexing vigorously in at least 100 µL Lysis Solution.

RNA isolation

2 For a standard prep of 100 µL of lysate, add 50 µL of 100% ethanol, and vortex briefly but thoroughly.

3 Load the lysate/ethanol mixture (up to 150 µL) onto a Micro Filter Cartridge Assembly and close the cap.

4 Centrifuge for ~10 sec at maximum speed or until all of the mixture has passed through the filter. Longer centrifugation times may be needed to filter the lysate from tissue samples. For lysate/ethanol mixtures >150 µL, load and filter the first 150 µL, then repeat with additional aliquots until the entire sample has passed through the filter. The Collection Tube has a capacity of ~700 µL when assembled with a Micro Filter Cartridge; if more than 150 µL of lysate/ethanol mixture is filtered, empty the Collection Tube before proceeding. The RNA is now bound to the filter in the Micro Filter Cartridge.

5 Open the Micro Filter Cartridge, add 180 µL of Wash Solution 1 (working solution mixed with ethanol) to the filter and close the cap. Centrifuge for ~10 sec to pass the solution through the filter.

6 Open the Micro Filter Cartridge, add 180 µL of Wash Solution 2/3 (working solution mixed with ethanol) to the filter and close the cap. Centrifuge for ~10 sec to pass the solution through the filter. Repeat with a second 180 µL aliquot of Wash Solution 2/3.

7 Open the Micro Filter Cartridge assembly, remove the filter cartridge from the Collection Tube, and pour out the flow-through. Replace the Micro Filter Cartridge into the same Collection Tube, close the cap, and centrifuge at maximum speed for 1 min to remove residual fluid and dry the filter.

8 Label a Micro Elution Tube (1.5 mL tubes provided with the kit) and transfer the Micro Filter Cartridge into it.

9 Apply 5–10 µL of Elution Solution, preheated to 75°C, to the center of the filter. Close the cap and store the assembly for 1 min at room temperature. Centrifuge the assembly for ~30 sec to elute the RNA from the filter.

10 Repeat with a second 5–10 µL aliquot of preheated Elution Solution, collecting the eluate in the same Micro Elution Tube.

Protocol references

https://documents.thermofisher.com/TFS-Assets/LSG/manuals/cms_057352.pdf