Standard operating procedure to isolate total RNA with Macherey – Nagel NucleoSpin RNA XS Kit from blastocysts

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RESEARCH INSTITUTE FOR
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Samples

- pelleted blastocysts

Equipment

- 1.5 ml microcentrifuge tubes
- sterile RNase-free tips
- pipettes
- centrifuge for microcentrifuge tubes
- vortex mixer
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Kits & Chemicals

- MN NucleoSpin RNA XS kit (Cat.: 740902.50)
- 96 % - 100 % ethanol
- 70% ethanol

Attention: Buffers RA1, RA2, and MDB contain chaotropic salt and detergents. Wear gloves and goggles!

CAUTION: Buffers RA1, RA2, and MDB contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste

Preparation

- **rDNase**: Add indicated volume (see following table or label on the rDNase vial) of RNase-free H2O to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 °C. The frozen working solution is stable for 6 months. Do not freeze/thaw the aliquots more than three times.
- **Reducing Agent TCEP**: Add indicated volume of RNase-free H2O to the TCEP vial and incubate for several minutes at room temperature. Mix the vial to completely dissolve the TCEP. Store dissolved TCEP at -20 °C.
- **Carrier RNA**: Prepare a stock solution before first time using: Dissolve the Carrier RNA in 750 µL Buffer RA1 to obtain a 400 ng/µL stock solution. Prepare a working solution before RNA extraction: Dilute 1:100 with Buffer RA1 (e.g., 1 µL Carrier RNA stock solution + 99 µL Buffer RA1) to obtain the working solution of 4 ng/µL. Add 5 µL of this working solution (20 ng) to every lysate (protocol step 3 in section 5). Store stock solution at -20 °C; do not store working solution, prepare it freshly immediately before use.
- **Wash Buffer RA3**: Add the indicated volume of 96 – 100 % ethanol to Wash Buffer RA3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RA3 at 15 – 25 °C for up to one year

Procedure

1. Add 100 µL Buffer RA1 and 2 µL TCEP to the cell sample and vortex vigorously (2 × 5 s). If multiple samples are processed, the preparation of a master-premix is recommended (e.g., 1.1 mL Buffer RA1 and 22 µL TCEP for 10 preparations). Use 102 µL of the premix.
2. Add 5 µL Carrier RNA working solution (20 ng) to the lysate. Mix by vortexing (2 × 5 s). Spin down briefly (approx. 1 s 1000 x g) to clear the lid.

3. Add 100 µL ethanol (70 %) to the homogenized lysate and mix by pipetting up and down (5 times).
4. For each preparation, take one NucleoSpin ® RNA XS Column (light blue ring) placed in a Collection Tube. Load the lysate to the column. Centrifuge for 30 s at 11,000 x g. Place the column in a new Collection Tube (2 mL). *Note:* The maximum loading capacity of NucleoSpin® RNA XS Columns is 600 µL. Repeat the procedure if larger volumes are to be processed.
5. Add 100 µL MDB (Membrane Desalting Buffer) and centrifuge at 11,000 x g for 30 s to dry the membrane. It is not necessary to use a fresh Collection Tube after this centrifugation step. If the column outlet has come into contact with the flowthrough for any reason, discard the flowthrough and centrifuge again for 30 s at 11,000 x g.
6. Prepare rDNase reaction mixture in a sterile microcentrifuge tube: for each isolation, add 3 µL reconstituted rDNase to 27 µL Reaction Buffer for rDNase. Mix by flicking the tube. Apply 25 µL rDNase reaction mixture directly onto the center of the silica membrane of the column. Close the lid. Incubate at room temperature for 20 min.
7. Add 100 µL Buffer RA2 to the NucleoSpin® RNA XS Column. Incubate for 2 min at RT. Centrifuge for 30 s at 11,000 x g. Place the column into a new Collection Tube (2 mL).
8. Add 400 µL Buffer RA3 to the NucleoSpin® RNA XS Column. Centrifuge for 30 s at 11,000 x g. Discard flowthrough and place the column back into the Collection Tube. *Note*: Make sure that residual buffer from the previous steps is washed away with Buffer RA3, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer RA3.
9. Add 200 µL Buffer RA3 to the NucleoSpin® RNA XS Column. Centrifuge for 2 min at 11,000 x g to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL). *Note*: If for any reason the liquid level in the Collection Tube has reached the NucleoSpin® RNA XS Column after centrifugation, discard flowthrough and centrifuge again.
10. Elute the RNA in 10 µL H_2O (RNase-free) and centrifuge at 11,000 x g for 30 s. If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5 – 30 µL.