Support protocol

Using NucleoSpin® RNA XS for fibrous tissue (Rev. 03)

Before starting the procedure, heat a water bath or heating block to 55°C. Prepare Proteinase K solution (20 mg/mL; not provided in kit). The additional component Triton-X-100 is required.

If desired Carrier RNA (REF 740514) can be used (working solution: 20 ng).

Procedure

1. Homogenization of sample.
Disrupt up to 5 mg tissue (for homogenization methods see section 2.3). Eukaryotic cells (up to $5x10^5$) can be collected by centrifugation.

2. Cell lysis.
Add **100 µL** of **Buffer RA1** supplemented with **1% Triton-X-100** and **2 µL** of **TCEP** to the cell pellet or ground tissue and vortex vigorously.

3. Proteinase K digestion.
Add **170 µL** of **RNase-free water** to the homogenate. Then add **3.5 µL** of **Proteinase K** solution and mix thoroughly by pipetting.

Incubate at **room temperature** for **10 min**. Then, incubate at **55°C** for **10 min**. Vortex from time to time.

Note: First incubation step at room temperature allows better performance of Proteinase K.

Centrifuge for **3 min** at **10,000 x *g*.**

Note: A small pellet of tissue debris can be formed, sometimes associated with a thin layer or film on top of the supernatant.

4. Load sample.
Pipette the **supernatant** (approximately 270 µL) into a new micro centrifuge tube (not provided).

Note: Avoid transferring any pellet. If unavoidable, however, a small amount of pelleted debris may be carried over without affecting the procedure. Hold the pipette tip under the thin layer or film on top of the supernatant, if present. This layer will usually adhere to the outside of the pipette tip and should not be transferred.

Optional: Add **5 µL** of **Carrier RNA**. Mix by **vortexing** (2 x 5 s). Spin down briefly (approx. 1 s, 1000 x g) to clear the lid.

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5. Adjust RNA binding conditions.
Add **0.5 volumes** of **ethanol** (**96%**; around 135 µL) to the homogenized lysate and **mix** by vortexing.

Note: After addition of ethanol a stringy precipitate may become visible, this will not affect the RNA isolation. Make sure to load all of the precipitate onto the column.

6. Bind RNA.
Pipette the sample, including any precipitate that may have formed, into a **NucleoSpin® RNA XS Column** (light blue ring) placed in a Collection Tube (2 ml). Centrifuge for **30 s** at **8000 x *g***. Discard the flow-through.

Proceed with step 7 of the standard protocol.

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