Supplementary protocol

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NucleoSpin® RNA – isolation of RNA from fibrous tissue (Rev. 01)

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure. The latest version of the user manual is available at ***www.mn-net.com/usermanuals*** *or can be requested from our technical service (tech-bio@mn-net.com). Material safety data sheets (MSDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

Additional equipment needed:

- Water bath or heating block
- Proteinase K (REF 740506)
- Triton-X-100

Before starting the preparation:

- Heat a water bath or heating block to 55 °C.
- Prepare Proteinase K solution (20 mg/mL).

1 Homogenize sample

Disrupt up to **30 mg tissue** (for homogenization methods see section 2.3 of the NucleoSpin® RNA user manual). Eukaryotic cells (up to 5 x 10^6) can be collected by centrifugation.

2 Lyse cells

Add **350 µL Buffer RA1** supplemented with **1 % Triton-X-100** and **3.5 µL β-mercaptoethanol** to the cell pellet or ground tissue and vortex vigorously.

3 Digest with Proteinase K

Add **590 µL RNase-free water** to the homogenate. Then add **10 µL Proteinase K solution** and mix thoroughly by pipetting.

Incubate at room temperature for **10 min** and then at **55 °C** for **10 min**.

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

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

A small pellet of tissue debris will be formed, sometimes associated with a thin layer or film on top of the supernatant. Pipette the supernatant (approximately 950 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.

4 Adjust RNA binding conditions

Add **0.5 volumes ethanol (96–100 %; around 475 µL)** to the homogenized lysate and **mix** by vortexing. After addition of ethanol a stringy precipitate may become visible which will not affect the RNA isolation. Make sure to load all of the precipitate onto the column.

5 Bind RNA

Pipette **700 µL** of the mixture, including any precipitate that may have formed, into a NucleoSpin® RNA Column (light blue ring) placed in a Collection Tube (2 mL).

Centrifuge for **30 s** at **8,000 x *g***. Discard the flow-through. Reuse Collection Tube in step 6.

6 Repeat step 5 using the remaining sample. Discard the flow-through. Place the NucleoSpin® RNA Column in a new Collection Tube.

Proceed with step 6 of the standard protocol ('Desalt silica membrane').