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NucleoSpin® RNA – disruption and homogenization of RNA*later*®* stabilized tissue using the Mixer Mill MM300 (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****.*

The Mixer Mill MM 300 (e.g., RETSCH GmbH) allows high-throughput, rapid, and effective disruption of 48 biological samples in 2–4 minutes. Homogenization and disruption with the Mixer Mill MM 300 gives results comparable to using rotor–stator homogenization.

The following guidelines can be used for disruption and homogenization of RNA*later*® stabilized tissue using the Mixer Mill MM 300.

Additional equipment needed:

- Mixer Mill MM 300
- Water bath or heating block

Before starting the preparation:

- Heat a water bath or heating block to 55 °C.

1 Stabilize tissue in RNA*later*® RNA Stabilization Reagent as described in the manufacturer's protocol.

2 Pipette **600 µL Buffer RA1** into a 2 mL collection tube.

Optional: Add 6 ***µL β-mercaptoethanol*** *to 600 µL Buffer RA1.*

3 Add **one stainless steel bead** to each tube. For best results, we recommend using a 5 mm (mean diameter) stainless steel bead.

4 Take **up to 30 mg tissue** from the RNA*later*® RNA Stabilization Reagent and transfer it into the lysis tube.

* RNA*later*®*:* Stabilization solution, supplied by Ambion RNA Diagnostics, for additional information contact moinfo@ambion.com

5 Homogenize on the **Mixer Mill MM 300** for **2 min** at **20 Hz**. Homogenization time depends on the tissue used and can be extended until the tissue is completely homogenized.

6 Rotate the Mixer Mill rack to allow even homogenization, and homogenize for another **2 min** at **20 Hz**.

7 Apply the tissue sample (without the bead) to a NucleoSpin® Filter (violet ring) and centrifuge for **1 min** at **11,000 x *g***. Carefully transfer the supernatant of the flow-through to a new microcentrifuge tube (not supplied) by pipetting.

Use only this supernatant (lysate) in subsequent steps. To avoid cross contamination, do not reuse the stainless steel bead.

8 Add **one volume** (usually 600 µL) of **70 % ethanol** to the cleared lysate, and mix immediately and vigorously. Do not centrifuge.

9 Apply **up to 700 µL** of the **sample** to the NucleoSpin® RNA Column (light blue ring) and centrifuge for **30 s** at **8,000 x *g***. Place the column in a new collection tube.

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