Disruption and Homogenization of RNA*later** Stabilized Tissue using the Mixer Mill MM 300 in combination with NucleoSpin® RNA II (Rev. 02)

The Mixer Mill MM 300 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.

Procedure

1. Stabilize tissue in RNA*later** RNA Stabilization Reagent as described in the manufacturers Protocol.

2. Pipet 600 µL of Buffer RA1 into a 2 mL collection tube.

Optional: Add 6 µL β-mercaptoethanol to 600 µLl of 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. Add up to **30 mg tissue** (stabilized in RNA*later** RNA Stabilization Reagent) per tube.

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 unit** (violet) and centrifuge for **1 min** at **11000 x *g***. Carefully transfer the supernatant of the flowthrough 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.

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Support protocol

9. Apply up to **700 µL** of the sample to the **NucleoSpin® RNA II column** (light blue) and centrifuge for **30 s** at **8000 x *g***. Place the column in a new collecting tube.

Continue with step(Membrane Desalting Buffer) as described in the NucleoSpin® RNA II protocol.

*RNA*later*□ Stabilization Solution, supplied by Ambion RNA Diagnostics, for additional information contact moinfo@ambion.com

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