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NucleoSpin® RNA Midi – isolation of RNA from plants

(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:

- Buffer RAP or RL1:
  Buffer RAP (50 mL): REF 740936.50
  Buffer RAP (500 mL): REF 740936.500
  Buffer RL1 (50 mL) REF 740385.50
  Buffer RL1 (125 mL): REF 740385.125

1 Prepare lysis buffer

For each preparation, add **1.8 mL Lysis Buffer** (RA1, RAP, or RL1) and **18 µL ß-mercaptoethanol** to a 15 mL collection tube.

Note: For some sample types, it might be necessary to increase the lysis buffer volume and subsequently the ethanol volume accordingly (some samples soak more buffer and thus a higher buffer volume will be needed to keep them always covered in order to prevent RNase activation). In this case, multiple sample loading steps will be necessary.

2 Homogenize sample

Grind **up to 1 g plant tissue** in liquid nitrogen with a mortar and pestle. Make sure to keep the sample powder always frozen.

3 Lyse cells

Transfer the frozen, powdered sample with a pre-cooled spatula to the already mixed lysis buffer and mix immediately and thoroughly. Vortex carefully for **30 s** and centrifuge at **4,500 x *g*** for **10 min**.

4 Filtrate lysate

Load the lysate onto a NucleoSpin® Filter Midi. Centrifuge at **4,500 x *g*** for **3 min**. Transfer the filtrate to a new 15 mL centrifuge tube.

In case of visible formation (depending on sample amount and nature) transfer supernatant without any formed pellet to a new 15 mL centrifuge tube.

5 Adjust RNA binding conditions

Add **1.8 mL of 70 % ethanol** to the cleared lysate and vortex carefully.

Note: After addition of ethanol a stringy precipitate may become visible; however, this will not affect RNA isolation. Be sure to disaggregate any precipitate by mixing and load all of the precipitate onto the column. Do not centrifuge the ethanolic lysate before loading it onto the column.

6 Bind RNA

Load the lysate-ethanol mixture (maximal 3.8 mL) onto a NucleoSpin® RNA Midi Column. Centrifuge at **4,500 x *g*** for **3 min**. Discard the flow-through.

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