

# Support protocol

## Using NucleoSpin® RNA L for purification of total RNA from plants (Rev. 03)

The Buffer RAP and RP1 can be purchased separately:

|                |                     |
|----------------|---------------------|
| REF 740936.50  | Buffer RAP (50 mL)  |
| REF 740936.500 | Buffer RAP (500 mL) |
| REF 740385.50  | Buffer RP1 (50 mL)  |
| REF 740385.125 | Buffer RP1 (125 mL) |

### Procedure

#### 1. Lysis buffer set up.

For each preparation, add **1.8 mL Lysis Buffer (RA1, RAP, or RL1)** and **18 µL β-mercaptoethanol** to a 15 mL centrifugation 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 1g 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 L**. Centrifuge at **4,500 x g** for **3 min**.

#### 5. Adjust RNA binding conditions.

Add **1.8 mL of 70% ethanol** to the 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.*

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### 6. Bind RNA.

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

Repeat this step until the complete mixture has been loaded.

Proceed with step 6 of the standard protocol.

