NucleoSpin® NucleoSpin RNA Plant and Fungi (Rev. 01, June 2018)

NucleoSpin® RNA Plant and Fungi for fatty or lipid rich fruit material

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). Safety data sheets (SDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

NucleoSpin® RNA Plant and Fungi for fatty or lipid rich fruit material

Before starting with the preparation, set incubator or water bath to 56 °C.

Optimal RNA yields from fruit samples can be obtained by using Paraffin Dissolver (REF 740968.25).

1 Homogenize sample

Add **500 µL Buffer PFL** into **NucleoSpin® Bead Tube Type G**.

500 µL PFL
20 µL PFR

Add **20 µL Buffer PFR** to the tube.

Add **500 µL Paraffin Dissolver** to the tube (optional, for ease of handling).

Optional:
500 µL Paraffin Dissolver

Transfer sample (50 mg oil palm fruit flesh or 50 mg avocado fruit flesh) to the **NucleoSpin® Bead Tube Type G**.

Transfer sample

Place the Bead Tube into a swing-mill and **agitate for 30 sec at 30 Hz** until the sample is disintegrated.

Agitate
30 s

Incubate NucleoSpin® Bead Tube Type G for **5 min** at **56°C**.

56 °C, 5 min

Transfer the **lysate** into a fresh 2 mL tube (not provided).

Centrifuge **1 min** at **20,000 x *g*** in oder to sediment cell debris and to achieve phase separation. *Alternatively, centrifuge 3 min at 14,000 x g.*

20,000 x *g*,
1 min

2 Filter Lysate

Insert a **NucleoSpin® RNA Plant and Fungi Filter Column** (green ring) into a Collection Tube (2 mL, provided).

Load aqueous phase

Load the RNA containing **lower aqueous phase** (approximately 300–400 µL) from step 1 onto the column. Do not load the upper lipid (Paraffin Dissolver) phase as well as sedimented cell debris onto the column.

14,000 x *g*, 1 min

Centrifuge for **1 min** at **14,000 x *g***.

3 Adjust RNA binding conditions

500 µL PFB

Add **500 µL Buffer PFB** to the flow through and mix by pipetting.

Incubate for **5 min** at **room temperature**.

RT, 5 min

4 Bind RNA

For further steps, please follow the standard protocol (please see page 16, chapter 5.1, step 4).