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NucleoSpin® 8/96 RNA – isolation of RNA from PAXgene® tubes (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:

- Water bath or heating block
- Proteinase K (REF 740506)
- NucleoSpin® Filters (REF 740606)

Additional preparations before starting:

- Heat a water bath or heating block to 55 °C.
- Prepare Proteinase K solution (20 mg/mL).

1 Harvest sample

Centrifuge PAXgene® Blood RNA Tube for **10 min** at **3,000–5,000 x *g*** using a swing-out rotor.

2 Wash pellet

Remove the supernatant by decanting or pipetting. Add **5 mL RNase-free water** to the pellet, and close the tube.

Thoroughly **resuspend the pellet** by vortexing and centrifuge for **10 min** at **3,000–5,000 x *g***. Remove and discard the entire supernatant. Gently decant the supernatant, and blot on a paper towel.

Thoroughly **resuspend** the pellet in **300 µL RNase-free water** by pipetting. Do not vortex.

3 Lyse sample

Pipette the sample into a 1.5 mL microcentrifuge tube. Add **300 µL RA1** supplemented with **3 µL ß-mercaptoethanol**, and **40 µL Proteinase K**.

Note: As alternative to ß-ME, the reducing agent DTT or TCEP may be used. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RA1.

Mix by vortexing and incubate for **20 min** at **55 °C** using a shaker incubator, heating block, or water bath.

4 Filtrate sample

Clear the lysate by filtration through **NucleoSpin® Filter (violet ring)**: Place the NucleoSpin® Filter Column in a Collection Tube (2 mL), apply the mixture, and centrifuge for **1 min** at **11,000 x *g***.

5 Adjust RNA binding conditions

Discard the NucleoSpin® Filter and add **300 µL ethanol (96 %)** to the homogenized lysate and mix by pipetting up and down (5 times).

6 Transfer lysates

Load the lysate to the NucleoSpin® RNA Binding Strips or NucleoSpin® RNA Binding Plate.

Proceed with step 5 of the standard protocol ('Bind RNA to silica membrane').