

## NucleoMag® RNA – Support Protocol

### Protocol Details

Application	RNA from PAXgene® tubes
Kit	NucleoMag® RNA
REF	744350 (.1 or .4)



### Protocol Steps

Steps	Procedure
Harvest sample	Centrifuge PAXgene® Blood RNA Tube for 10 min at 3,000-5,000 x g using a swing-out rotor
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 by gently decanting the supernatant and blot on a paper towel. Thoroughly resuspend the pellet in 300 µL RNase-free water by pipetting. Do not vortex.
Lyse sample	Transfer the sample into a 1.5 mL microcentrifuge tube. Add 150 µL MR1, 150 µL RNase-free-water supplemented with 3 µL DTT or TCEP and 40 µL Proteinase K. <i>Note: Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer mixture.</i> Mix by vortexing and incubate for 20 min at 55 °C using a shaker incubator, heating block, or water bath.
Filtrate sample	Clear the lysate by filtration through NucleoSpin® Filter: Place the NucleoSpin® Filter Column in a Collection Tube (2 mL), apply the mixture, and centrifuge for 1 min at 11,000 x g. Alternatively, clear the lysate by filtration through NucleoSpin® RNA Filter Plate by vacuum or centrifugation. Collect the cleared lysates in an appropriate collection plate.
Adjust DNA binding conditions	Add 28 µL resuspended B-Beads and 350 µL Binding Buffer to the lysate. Pipette up and down at least 10 times, until a homogenous solution is achieved and incubate for 5-10 min. at room temperature.
Proceed with standard protocol	Proceed with the removal of the supernatant of step 3 in the NucleoMag RNA user manual and follow the standard protocol for the subsequent steps.
Note	<i>Reduce lysate input if the beads cannot be resuspended completely during the binding step.</i>

### Additional Consumables

Product	Content	REF
NucleoSpin® RNA Filter Plate	96-well plates for filtration of cell and tissue homogenates, for use under vacuum or centrifugation	740711
NucleoSpin® Filters	50 filter columns	740606

### Trademarks

NucleoMag® (MACHEREY-NAGEL), PAXgene® (PreAnalytiX)

[www.mn-net.com](http://www.mn-net.com)



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