NucleoSpin® RNA – Support Protocol for difficult-to-lyse tissue (Rev.02)

Protocol Details

Application	RNA purification from blood
Kit	NucleoSpin® RNA Mini
REF	740955/.10/.50/.250
Protocol name	NucleoSpin® RNA – Support Protocol for difficult-to-lyse tissue Rev02

This supplementary protocol is developed for the isolation of RNA from difficult to-lyse tissue. 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 ***https://www.mn-net.com/media/pdf/b0/51/ee/Instruction-NucleoSpin-RNA.pdf*** 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***.

Reagents

- 96–100 % ethanol (to prepare Wash Buffer RA3)
- 70 % ethanol (to adjust RNA binding conditions)
- Reducing agent (ß-mercaptoethanol, or DTT (dithiothreithol), or TCEP (BisTris (Bis-(2-hydroxyethyl)-imino-tris(hydroxymethyl)-methane)) as supplement forLysis Buffer RA1

Consumables

- 1.5 mL microcentrifuge tubes (NucleoSpin® RNA)
- Sterile RNase-free tips

Equipment

- Manual pipettors
- NucleoSpin® RNA: centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

Protocol Steps

Steps	Procedure
Prepare sample	Add 400 µL of Buffer RA1 to the sample. Homogenize with NucleoSpin® Filter, homogenizer, or syringe/needle method.
	Incubate 5 min on ice, then vortex well.
	Centrifuge at 14,000 x g for 5 min to remove debris.
	Carefully transfer supernatant to a new 1.5 mL tube. Avoid pipetting pelleted material.
	Add 300 µL of 96 - 100% ethanol to sample, mix very well by vortexing.
	Centrifuge at 14,000 x g for 10 min. Discard as much supernatant as possible.
	Air-dry 5 min.

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany
www.mn-net.com

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com

	Add 25 µL of RNase-free water to the pellet and resuspend completely.
	Add 375 µL Buffer RA1, mix by pipetting and light vortexing.
	Continue with step 4 of the standard protocol.

Ordering information

Product	Content	REF
NucleoSpin® RNA kit	Mini spin kit for isolation of RNA from cultured cells and tissue; The kit includes buffers, NucleoSpin® RNA Columns, Proteinase K, and collection tubes	740200.10 740200.50 740200.250

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11
52355 Düren · Germany
www.mn-net.com

DE Tel.: +49 24 21 969-0 info@mn-net.com
CH Tel.: +41 62 388 55 00 sales-ch@mn-net.com
FR Tel.: +33 388 68 22 68 sales-fr@mn-net.com
US Tel.: +1 888 321 62 24 sales-us@mn-net.com