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Total RNA Isolation

User Manual

NucleoMag 96 RNA

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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoMag 96 RNA	
	1x 96 preps	**4 x 96 preps**
Cat. No.	**744350.1**	**744350.4**
NucleoMag B-Beads	3 ml	12 ml
Lysis Buffer MR1	50 ml	200 ml
Binding Buffer MR2	80 ml	320 ml
Wash Buffer MR3	80 ml	320 ml
Wash Buffer MR4	250 ml	1000 ml
Elution Buffer MR5**	25 ml	100 ml
Reducing Agent TCEP	1 vial (107 mg/vial)	4 vials (107 mg/vial)
rDNase, lyophilized*	3 vials (size D)	12 vials (size D)
Reaction Buffer for rDNase	35 ml	4 x 35 ml
RNase-free H_2O	5 ml	20 ml
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* For preparation of working solutions and storage conditions see section 3.

** Elution Buffer MR5: RNase-free water

1.2 Material to be supplied by user

Product	Cat. No.	Pack of
Separation plate for magnetic beads separation, e.g., Square-well Block (96-well block with 2.1 ml square-wells)	740481 740481.24	4 24
Lysis tubes for incubation of samples and lysis, e.g., Rack of Tubes Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 ml wells) each, and 12 Cap Strips)	740477.4 740477.24	4 sets 24 sets
Elution plate for collecting purified RNA, e.g., Elution Plate, U-bottom (96-well 0.3 ml microtiterplate with 300 µl u-bottom wells) e.g., Elution Plate, Flat-bottom (96-well 0.3 ml microtiterplate with 300 µl flat-bottom wells)	740486.24 740673	24 20
For use of kit on KingFisher 96 instrument: KingFisher® 96 Accessory Kit B (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag 96 RNA preps using KingFisher® 96 platform)	740951	1 set

2 Product description

2.1 The basic principle

The **NucleoMag 96 RNA** procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved by homogenization in a solution containing chaotropic ions. For the adjustment of conditions under which nucleic acids bind to the paramagnetic beads Buffer MR2 and the NucleoMag B-Beads are added to the lysate. After magnetic separation the paramagnetic beads are incubated with a recombinant DNase to remove co-purified DNA. Following a RNA rebinding step contaminants and salts are removed using the Wash Buffers MR3 and MR4. Residual ethanol from previous wash steps is removed by air drying. Finally, highly pure RNA is eluted with Elution Buffer MR5* and the RNA can directly be used for downstream applications. The **NucleoMag 96 RNA** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag 96 RNA is designed for rapid manual and automated small-scale preparation of highly pure total RNA from tissue or cell samples. The kit is designed for use with NucleoMag SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified RNA can be used directly as template for RT-PCR, or any kind of enzymatic reactions.

Due to the recombinant DNase provided with the kit, eluted RNA is virtually DNA-free.

NucleoMag 96 RNA allows easy automation on common liquid handling instruments or automated magnetic separators, for example Thermo Fisher Scientific KingFisher® instruments. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag SEP on automation platforms.

The kit provides reagents for the purification of up to 30 µg of pure RNA from suitable samples. Depending on the elution volume used concentrations of 10 – 30 ng/µl can be obtained.

NucleoMag 96 RNA can be processed completely at room temperature.

NucleoMag B-Beads are highly reactive superparamagnetic beads. The binding capacity is approx. 1 µg of RNA per 1 µl of NucleoMag-B-Bead Suspension. 1 µl of suspension contains 130 µg beads.

* Elution Buffer MR5: RNase-free water

2.3 Magnetic separation systems

For use of **NucleoMag 96 RNA** the use of the magnetic separator NucleoMag SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators. See suppliers ordering information for suitable separation plates.

Magnetic separator	Separation plate or tube
NucleoMag SEP (MN Cat. No. 744900)	Square-well Block (MN Cat. No. 740670)
Tecan Te-MagS™	1.5 ml tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example NucleoMag SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins, for example Te-MagS™ (for automated use only): Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets, for example Thermo Fisher Scientific KingFisher® instruments: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps the speed settings have to be adjusted carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

Adjusting shaker speed for wash steps:

- Load 900 µl dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again and use this setting for the washing step.

Adjusting shaker speed for the elution step:

- Load 100 µl dyed water to the wells of the collection plate and proceed as described above.

2.5 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Number of tips needed
Magnetic mix	+	++	Low
Shaker	++	++	Low
Pipetting	+++	+*	High

* 8-channel pipetting device

2.6 Elution procedures

Purified total RNA can be eluted directly with the supplied Elution Buffer MR5. Elution can be carried out in a volume of ≥ 50 µl. It is essential to cover the NucleoMag B-Beads completely with Elution Buffer MR5 during the elution step. The volume of dispensed Elution Buffer MR5 depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution the magnetic bead pellet should be resuspended completely in the Elution Buffer MR5. For some separators high elution volumes might be necessary to cover the whole magnetic bead pellet.

3 Storage conditions and preparation of working solutions

Attention:

Buffers MR1 and MR3 contain chaotropic salt! Wear gloves and goggles!

- All components of the **NucleoMag 96 RNA** kit should be stored at room temperature (20 – 25°C) and are stable for up to one year.
- All buffers are delivered ready-to-use.

Before starting **NucleoMag 96 RNA** protocol prepare the following:

- **rDNase working solution:** Add 800 µl of RNase-free H_2O to each rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. If not used completely this working solution can be stored at -20°C for up to 6 month. Do not freeze/thaw the rDNase working solution more than three times.
- **rDNase reaction mixture:** Add 9.2 ml Reaction Buffer for rDNase to 800 µl rDNase working solution and mix. The resulting rDNase reaction mixture will be sufficient for 32 isolations and should be used up. When performing less than 32 reactions prepare a smaller amount of the reaction mixture. For each isolation combine 276 µl of reaction buffer for rDNase with 24 µl of rDNase working solution.
- **Reducing Agent TCEP**: Add 750 µl of RNase-free H_2O to the TCEP vial and incubate for several minutes at room temperature. Mix the vial to dissolve the TCEP completely. Store dissolved TCEP at -20°C.

	NucleoMag 96 RNA	
	1 x 96 preps	**4 x 96 preps**
Cat. No.	**744350.1**	**744350.4**
rDNase (lyophilized)	3 vials (size D)	12 vials (size D)
	Add 800 µl RNase-free H_2O to each vial	Add 800 µl RNase-free H_2O to each vial
TCEP	1 vial (107 mg)	4 vials (107 mg/vial)
	Add 750 µl RNase-free H_2O	Add 750 µl RNase-free H_2O to each vial

4 Safety instructions – risk and safety phrases

The following components of the **NucleoMag 96 RNA** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
MR1	Guanidine thiocyanate	Xn*	Harmful by inhalation, in contact with the skin, and if swallowed	R 20/21/22	S 13
MR2	Isopropanol >90%	F Xn**	Highly flammable - Irritating to eyes - Vapours may cause drowsiness and dizziness	R 11-36-67	S 7-16-24-25-26
MR3	Guanidine thiocyanate + ethanol <40%	Xn*	Flammable - Harmful by inhalation, in contact with the skin, and if swallowed	R 10-20/21/22	S 13-16
MR4	Ethanol <80%	F**	Highly flammable	R11	S 7-16
rDNase, RNase-free	rDNase, lyophilized	Xi**	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24
Reducing Agent TCEP	Tris (2-carboxyethylphosphine Hydrochloride)	Xi**	Causes burns	R 34	S 26-27-36/37/39

Risk phrases

R 10	Flammable
R 11	Highly flammable
R 20/21/22	Harmful if swallowed
R 34	Causes burns
R 36	Irritating to eyes
R 42/43	May cause sensitization by inhalation and skin contact
R 67	Vapours may cause drowsiness and dizziness

* Hazard labeling not necessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

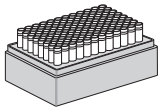
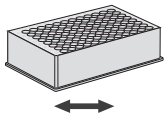
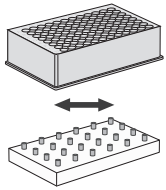
** Hazard labeling not necessary if quantity per bottle below 25 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

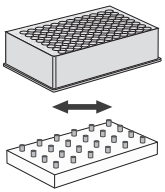
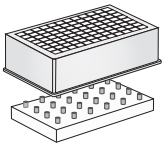
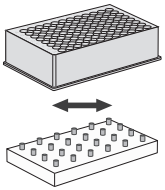
Safety phrases

S 7	Keep container tightly closed
S 13	Keep away from food, drink, and animal feedstuffs
S 16	Keep away from sources of ignition - No smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 24/25	Avoid contact with skin and eyes
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 27	Take off immediately all contaminated clothing
S 36/37/38	Wear suitable protective clothing and gloves

5 Procedure

5.1 General procedure

1	Homogenize/lyse sample	**Up to 20 mg tissue or 2 x 10^6 cells** **350 µl MR1** **6 µl TCEP** **Mix or use mechanical disruption**	
2	Bind RNA to NucleoMag B-Beads	**28 µl B-Beads** **350 µl MR2** **Mix** **Incubate 5 min at RT** *(Optional: Mix by shaking)* **Remove supernatant after 2 min separation** **Dry for 5 min at RT**	
3	rDNase digestion	**300 µl rDNase reaction mixture** **Mix** **Incubate 15 min at RT**	
4	Rebinding	**350 µl MR2** **Mix** **Incubate 5 min at RT** **Remove supernatant after 2 min separation**	

5	MR3 wash	**600 µl MR3** **Resuspend, separate, 2 min** **Aspirate and discard supernatant**	
6	MR4 wash	**900 µl MR4** **Resuspend, separate, 2 min** **Aspirate and discard supernatant** **Repeat washing step once**	
7	Drying	**Incubate for 10 – 15 min at RT**	
8	Elution	**50 – 200 µl MR5** **Shake 5 – 10 min, RT** ***(Optional: Mix by pipetting up and down)*** **Transfer eluted RNA after 2 min separation**	

5.2 Protocol for the isolation of total RNA from cells or tissue

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag SEP) and suitable plate shakers. It is recommended using a Square-well Block for separation (see ordering information). Alternatively isolation of RNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

1 Homogenize/lyse sample

Lyse up to **20 mg of tissue** or **2 x 10^6 cells** in **350 µl Buffer MR1**.

For tissue samples: Use a suitable homogenization tool to homogenize samples in Buffer MR1. Samples can be disrupted using bead based homogenization tools, for example GenoGrinder* or Mixer Mill MM400** (see instrument manufacturer's recommendations for suitable plates or tubes for homogenization) or any other suitable homogenization tools. Centrifuge the crude homogenate to pellet debris or remaining tissue particles. Alternatively NucleoSpin® RNA Filter Tubes or Plates can be used to clear the crude lysate (see ordering information). Transfer the clear supernatant to the Square-well Block (see ordering information) for further processing.

For cells: Add Buffer MR1 to cell pellet. Pipette up and down several times to lyse the cells. *Optionally: Use NucleoSpin® RNA Filter Tubes or Plates (see ordering information) or a syringe to reduce the viscosity of the lysate. Transfer lysate to the Square-well Block for further processing.*

2 Bind RNA to NucleoMag B-Beads

Add **28 µl resuspended NucleoMag B-Beads** and **350 µl Buffer MR2** to the lysed sample. Mix by pipetting up and down 6 times and **incubate for 5 min** at **room temperature**. NucleoMag B-Beads and Buffer MR2 can be premixed.

Be sure to resuspend the NucleoMag B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting. Remove supernatant completely.

Do not disturb the attracted beads while aspirating the supernatant.

* GenoGrinder: *http://www.spexcsp.com/sampleprep/*

** Mixer Mill MM400 *http://www retsch.com/products/milling/ball-mills/mm-400/*

Dry beads for **5 min** at **room temperature**. Keep the Square-well Block on the NucleoMag SEP magnetic separator for the drying step.

3 rDNase digestion

Remove the Square-well Block from the NucleoMag SEP magnetic separator. Add **300 µl rDNase reaction mixture** and resuspend the beads by pipetting up and down. Incubate for **15 min** at **room temperature**. Do not separate the beads! Following incubation proceed with step 4.

4 Rebinding

Add **350 µl Buffer MR2** to each sample. Mix by pipetting up and down 6 times and incubate for **5 min** at **room temperature**.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on NucleoMag Sep magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

5 MR3 wash

Remove the Square-well Block from the NucleoMag SEP magnetic separator. Add **600 µl Buffer MR3** resuspend the beads by pipetting up and down. Incubate for **1 min.**

Separate the magnetic beads by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

6 MR4 wash

Remove the Square-well Block from the NucleoMag SEP magnetic separator. Add **900 µl Buffer MR4** and resuspend the beads by pipetting up and down. Incubate for **1 min.**

Separate the magnetic beads by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Repeat washing step once with **900 µl Buffer MR4**. Leave the Square-well Block on the NucleoMag SEP magnetic separator for the following step.

7 Drying

Air dry the beads for **10 – 15 min** at **room temperature**.

8 Elution

Remove the Square-well Block from the NucleoMag SEP magnetic separator. Add desired volume of **Buffer MR5 (at least 50 µl, 50 – 200 µl)** and resuspend the beads by pipetting up and down.

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

Separate the magnetic beads by placing the Square-well Block on the NucleoMag SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified total RNA to a suitable collection plate (see ordering information).

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded/ no RNA obtained	*RNase contamination* • Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes or plates is recommended. Glassware should be oven-baked for at least 2 h at 250°C before use.
Poor RNA yield	*Elution buffer volume insufficient* • Beads pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step.* • Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation too short or aspiration speed too high.
Low purity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, for example Square-well Block in combination with NucleoMag SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.

Problem	Possible cause and suggestions
Poor performance of RNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Low purity* • See above.
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the Square-well Block when transferring the tissue lysate. If the rim of the wells is contaminated, seal the Square-well Block with Self-adhering PE Foil (see ordering information) before starting the shaker.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoMag 96 RNA	744350.1 744350.4	1 x 96 preps 4 x 96 preps
NucleoSpin® Filters	740606	50
NucleoSpin® RNA Filter Plates	740711	4
NucleoMag SEP	744900	1
Square-well Blocks	740481.4 740481.24	4 24
Self-adhering PE Foil	740676	50 sheets
Rack of Tube Strips (set consists of 1 Rack, 12 Tube Strips with 8 tubes each and Cap Strips)	740477.4 740477.24	4 sets 24 sets
Cap Strips	740638	30 strips
KingFisher® 96 Accessory Kit B Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag 96 RNA preps using KingFisher® 96 platform	744951	1 set

Visit *www.mn-net.com* for more detailed product information.

6.3 Product use restriction / warranty

NucleoMag 96 RNA kit components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY.** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoMag 96 RNA** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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