

# RNA isolation

## User manual

NucleoMag<sup>®</sup> RNA

November 2015 / Rev. 05

**MACHERY-NAGEL**

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



DOMINIQUE DUTSCHER SAS

## Table of contents

1	Components	4
1.1	Kit contents	4
1.2	Equipment and consumables to be supplied by user	5
2	Product description	6
2.1	The basic principle	6
2.2	Kit specifications	6
2.3	Magnetic separation systems	7
2.4	Adjusting the shaker settings	8
2.5	Handling of beads	8
2.6	Elution procedures	9
3	Storage conditions and preparation of working solutions	10
4	Safety instructions	11
5	Protocol for the isolation of RNA	14
6	Appendix	20
6.1	Troubleshooting	20
6.2	Ordering information	22
6.3	Product use restriction/warranty	22

# 1 Components

## 1.1 Kit contents

NucleoMag® RNA		
REF	1x 96 preps 744350.1	4 x 96 preps 744350.4
NucleoMag® B-Beads	2 x 1.5 mL	12 mL
Lysis Buffer MR1	60 mL	250 mL
Binding Buffer MR2	80 mL	400 mL
Wash Buffer MR3	80 mL	320 mL
Wash Buffer MR4	250 mL	1000 mL
Elution Buffer MR5*	30 mL	125 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	30 mL	2 x 60 mL
RNase-free H <sub>2</sub> O	13 mL	30 mL
User manual	1	1

\* Elution Buffer MR5: RNase-free water

\*\* For preparation of working solutions and storage conditions see section 3.

## 1.2 Equipment and consumables to be supplied by user

Product	REF	Pack of
<ul style="list-style-type: none"> <li>• <b>Magnetic separation system</b> e.g., NucleoMag® SEP (see section 2.3)</li> </ul>	744900	1
<ul style="list-style-type: none"> <li>• <b>Separation plate for magnetic beads separation,</b> e.g., Square-well Block (96-well block with 2.1 mL square-wells)</li> </ul>	740481 740481.24	4 24
<ul style="list-style-type: none"> <li>• <b>Lysis tubes for incubation of samples and lysis,</b> e.g., Rack of Tube Strips (1 set consists of 1 Rack, 12 Strips with 8 tubes (1.2 mL wells) each, and 12 Cap Strips)</li> </ul>	740477 740477.24	4 sets 24 sets
<ul style="list-style-type: none"> <li>• <b>Elution plate for collecting purified nucleic acids,</b> 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)</li> </ul>	740486.24	24
<ul style="list-style-type: none"> <li>• <b>For use of kit on KingFisher® Flex instrument:</b> e.g., KingFisher® Accessory Kit B (Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® RNA preps using KingFisher® Flex platform)</li> </ul>	744951	1 set

## 2 Product description

### 2.1 The basic principle

The **NucleoMag<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> RNA** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

### 2.2 Kit specifications

**NucleoMag<sup>®</sup> RNA** is designed for rapid manual and automated small-scale preparation of highly pure RNA from tissue or cell samples. The kit is designed for use with NucleoMag<sup>®</sup> SEP magnetic separator plate (see ordering information, section 6.2) 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<sup>®</sup> RNA** allows easy automation on common liquid handling instruments or automated magnetic separators, for example Thermo Fisher Scientific KingFisher<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> RNA** can be processed completely at room temperature.

NucleoMag<sup>®</sup> B-Beads are highly reactive superparamagnetic beads. The binding capacity is approx. 0.4 µg/µL of RNA per 1 µL of NucleoMag<sup>®</sup> B-Bead Suspension. 1 µL of suspension contains 130 µg beads.

## 2.3 Magnetic separation systems

For use of **NucleoMag® RNA**, the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information, section 6.2). 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 REF 744900)	Square-well Block (MN REF 740481/.24)
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: 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: 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 using 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

+: acceptable, ++: good, +++: excellent, \* 8-channel pipetting device

## 2.6 Elution procedures

Purified RNA can be eluted directly with the supplied Elution Buffer MR5. Elution can be carried out in a volume of  $\geq 50 \mu\text{L}$ . It is essential to cover the NucleoMag<sup>®</sup> 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!

**CAUTION:** Buffers MR1 and MR3 contain guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All components of the **NucleoMag® RNA** kit should be stored at room temperature (18–25 °C) and are stable for at least one year.
- All buffers are delivered ready to use.

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







- **rDNase working solution:** Add 800 µL of RNase-free H<sub>2</sub>O 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 at least 6 months. 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<sub>2</sub>O 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® RNA		
REF	1 x 96 preps 744350.1	4 x 96 preps 744350.4
rDNase (lyophilized)	3 vials (size D) Add 800 µL RNase-free H <sub>2</sub> O to each vial	12 vials (size D) Add 800 µL RNase-free H <sub>2</sub> O to each vial
TCEP	1 vial (107 mg) Add 750 µL RNase-free H <sub>2</sub> O	4 vials (107 mg/vial) Add 750 µL RNase-free H <sub>2</sub> O to each vial

## 4 Safety instructions

The following components of the NucleoMag® RNA kits contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section.*

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g. *Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.*

Component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
<i>Inhalt</i>	<i>Gefahrstoff</i>	<i>GHS-Symbol</i>	<i>H-Sätze</i>	<i>P-Sätze</i>
MR1	Guanidinium thiocyanate 45–60 % <i>Guanidiniumthiocyanat</i> 45–60 % CAS 593-84-0	 WARNING ACHTUNG	302, 412	264W, 273, 301+312, 330
MR2	2-propanol 95–100 % <i>2-Propanol 95–100 %</i> CAS 67-63-0	 DANGER GEFAHR	225, 319, 336	210, 233, 260D, 280sh
MR3	Guanidinium thiocyanate 15–30 % + ethanol 35–55 % <i>Guanidiniumthiocyanat</i> 15–30 % + <i>Ethanol</i> 35–55 % CAS 593-84-0, 64-17-5	 WARNING ACHTUNG	226, 413	210, 273
MR4	Ethanol 75–90 % <i>Ethanol 75–90 %</i> CAS 64-17-5p	 DANGER GEFAHR	225	210, 233,
rDNase, RNase-free	rDNase 90–100 % <i>(Enzym) rDNase 90–100 %</i> CAS 9003-98-9	 WARNING ACHTUNG	334	261sh, 342+311
Reducing Agent TCEP	Tris-(2-carboxyethyl) phosphine hydrochloride, TCEP(•HCl) 70–100 % <i>tris-(2-carboxyethyl)phosphine</i> <i>hydrochloride, TCEP(•HCl)</i> 70–90 % CAS 64-17-5	 WARNING ACHTUNG	315, 319	280sh

**Hazard phrases**

- H 225 Highly flammable liquid and vapour.  
*Flüssigkeit und Dampf leicht entzündbar.*
- H 226 Flammable liquid and vapour.  
*Flüssigkeit und Dampf entzündbar.*
- H 302 Harmful if swallowed.  
*Gesundheitsschädlich bei Verschlucken.*
- H 315 Causes skin irritation.  
*Verursacht Hautreizungen.*
- H 319 Causes serious eye irritation.  
*Verursacht schwere Augenreizung.*
- H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.  
*Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
- H 336 May cause drowsiness or dizziness.  
*Kann Schläfrigkeit und Benommenheit verursachen.*
- H 412 Harmful to aquatic life with long lasting effects.  
*Schädlich für Wasserorganismen, mit langfristiger Wirkung.*
- H 413 May cause long lasting harmful effects to aquatic life.  
*Kann für Wasserorganismen langfristig schädlich sein.*

**Precaution phrases**

- P 210 Keep away from heat/sparks/open flames/hot surfaces. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
- P 233 Keep container tightly closed.  
*Behälter dicht verschlossen halten.*
- P 260D Do not breathe vapors.  
*Dampf nicht einatmen.*
- P 261sh Avoid breathing dust/vapors.  
*Einatmen von Staub/Dampf vermeiden.*
- P 264W Wash with water thoroughly after handling.  
*Nach Gebrauch mit Wasser gründlich waschen.*
- P 273 Avoid release to the environment.  
*Freisetzung in die Umwelt vermeiden.*
- P 280sh Wear protective gloves/eye protection.  
*Schutzhandschuhe/Augenschutz tragen.*
- P 301+312 IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell.  
*BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt anrufen.*
- P 330 Rinse mouth  
*Mund ausspülen.*
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.  
*Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM / Arzt anrufen.*

For further information please see Material Safety Data Sheets ([www.mn-net.com](http://www.mn-net.com)).  
*Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ([www.mn-net.com](http://www.mn-net.com)).*



The symbol shown on labels refers to further safety information in this section.  
*Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.*

DOMINIQUE DUTSCHER SAS

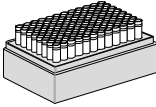
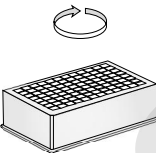
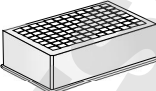

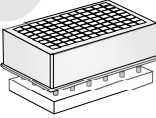

## 5 Protocol for the isolation of RNA

### Protocol at a glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 18.

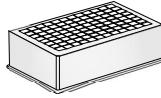
### Before starting the preparation:

- Check that rDNase was prepared according to section 3.

1	<b>Homogenize / lyse</b> samples	<b>Up to 20 mg tissue</b> <b>or 2 x 10<sup>6</sup> cells</b>  <b>350 µL MR1</b>  <b>6 µL TCEP</b>  <b>Mix or use</b> <b>mechanical disruption</b>	
2	<b>Clear lysates</b> by centrifugation, transfer 350 µL of cleared lysate to a Square-well Block for further processing	<b>5,600 x g,</b> <b>5 min</b>  <b>350 µL cleared lysate</b>	
3	<b>Bind RNA to</b> NucleoMag® B-Beads	<b>28 µL NucleoMag® B-Beads</b>  <b>350 µL MR2</b>	
		<b>Mix by shaking</b> <b>for 5 min at RT</b> <i>(Optional: Mix by pipetting</i> <i>up and down)</i>	
		<b>Remove supernatant</b> <b>after 2 min separation</b>  <b>Dry for 5 min at RT</b>	
4	<b>Digest DNA</b>	Remove Square-well Block from NucleoMag® SEP  <b>300 µL rDNase reaction</b> <b>mixture</b>  <b>Mix</b>  <b>Incubate 15 min at RT</b>	

5 **Rebind**

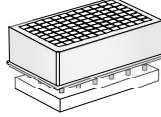
350 µL MR2



**Mix by shaking  
for 5 min at RT**  
*(Optional: Mix by pipetting  
up and down)*



**Remove supernatant  
after 2 min separation**



6 **Wash with MR3**

Remove Square-well Block  
from NucleoMag® SEP

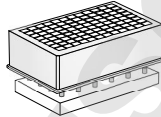
600 µL MR3



**Resuspend: Shake 5 min at  
RT**  
*(Optional: Mix by pipetting  
up and down)*



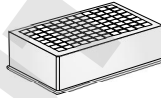
**Remove supernatant  
after 2 min separation**



7 **Wash with MR4 (1<sup>st</sup>)**

Remove Square-well Block  
from NucleoMag® SEP

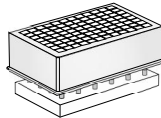
900 µL MR4



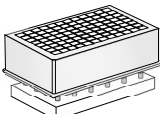
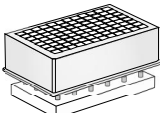


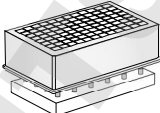


**Resuspend: Shake 5 min at  
RT**  
*(Optional: Mix by pipetting  
up and down)*



**Remove supernatant  
after 2 min separation**



<p>8 <b>Wash</b> with MR4 (2<sup>nd</sup>)</p>	<p>Remove Square-well Block from NucleoMag® SEP</p> <p><b>900 µL MR4</b></p>	
<p><b>Resuspend: Shake 5 min at RT</b> (Optional: Mix by pipetting up and down)</p>		
<p><b>Remove supernatant after 2 min separation</b></p>		
<p>9 <b>Dry</b> samples</p>	<p><u>Leave</u> Square-well Block on NucleoMag® SEP</p> <p><b>Air dry 10–15 min at RT</b></p>	
<p>10 <b>Elute</b> RNA</p>	<p>Remove Square-well Block from NucleoMag® SEP</p> <p><b>50-200 µL MR5</b></p> <p><b>Shake 5–10 min at RT</b> (Optional: Mix by pipetting up and down)</p>	  
<p><b>Separate 2 min and transfer RNA into elution plate / tubes</b></p>		

## Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). 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.

### Before starting the preparation:

- Check that rDNase was prepared according to section 3.

### 1 Homogenize/lyse samples

Lyse up to **20 mg of tissue** or **2 x 10<sup>6</sup> cells** in **350 µL Buffer MR1 + 6 µL TCEP**.

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.

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, section 6.2) or a syringe to reduce the viscosity of the lysate. Transfer lysate to the Square-well Block for further processing.

### 2 Clear lysates

Centrifuge the samples for **5 min** at a full speed (**5,600–6,000 x g**). Remove cap strips.

Transfer **350 µL of the cleared lysate** to a Square-well Block. Do not moisten the rims of the well.

*Note:* See recommendations for suitable plates or tubes and compatible magnetic separators section 1.2.

Alternatively, NucleoSpin® RNA Filter Tubes or Plates can be used to clear the crude lysate (see ordering information, section 6.2). Transfer the clear supernatant to the Square-well Block (see ordering information, section 6.2) for further processing.

\* GenoGrinder: <http://www.spexcsp.com/sampleprep/>

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



---

### 3 Bind RNA to NucleoMag® B-Beads

Add **28 µL resuspended NucleoMag® B-Beads** and **350 µL Buffer MR2** to the lysed sample. **Mix** by shaking for 5 min at room temperature or by pipetting up and down 6 times and a subsequent incubation 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.

*Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.*

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

---

### 4 Digest DNA

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!

---

### 5 Rebind

Add **350 µL Buffer MR2** to each sample. **Mix** by shaking for 5 min at room temperature or by pipetting up and down 6 times and a subsequent incubation 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.

---

### 6 Wash with MR3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MR3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). 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.

---

**7 1<sup>st</sup> Wash with MR4**

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **900 µL Buffer MR4** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times). 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.

---

**8 2<sup>nd</sup> Wash with MR4**

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

---

**9 Dry samples**

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

---

**10 Elute RNA**

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 shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads by repeated pipetting up and down (15 times).

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 RNA to a suitable collection plate (see ordering information, section 6.2).

---

## 6 Appendix

### 6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded / no RNA obtained	<p><i>RNase contamination</i></p> <ul style="list-style-type: none"> <li>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.</li> </ul>
	<p><i>Elution buffer volume insufficient</i></p> <ul style="list-style-type: none"> <li>Beads pellet must be covered completely with elution buffer.</li> </ul> <p><i>Insufficient performance of elution buffer during elution step.</i></p> <ul style="list-style-type: none"> <li>Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps.</li> </ul>
	<p><i>Beads dried out</i></p> <ul style="list-style-type: none"> <li>Do not let the beads dry as this might result in lower elution efficiencies.</li> </ul>
Poor RNA yield	<p><i>Aspiration of attracted bead pellet</i></p> <ul style="list-style-type: none"> <li>Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate.</li> </ul> <p><i>Aspiration and loss of beads</i></p> <ul style="list-style-type: none"> <li>Time for magnetic separation is too short or aspiration speed is too high.</li> </ul>
	<p><i>Insufficient washing procedure</i></p> <ul style="list-style-type: none"> <li>Use only the appropriate combinations of separator and plate, for example Square-well Block in combination with NucleoMag® SEP.</li> <li>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.</li> </ul>

<b>Problem</b>	<b>Possible cause and suggestions</b>
Poor performance of RNA in downstream applications	<p><i>Carry-over of ethanol from wash buffers</i></p> <ul style="list-style-type: none"><li>• Be sure to remove <b>all</b> of the ethanolic wash solution, as residual ethanol interferes with downstream applications.</li></ul> <p><i>Low purity</i></p> <ul style="list-style-type: none"><li>• See above.</li></ul>
Carry-over of beads	<p><i>Time for magnetic separation too short</i></p> <ul style="list-style-type: none"><li>• Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.</li></ul> <p><i>Aspiration speed too high (elution step)</i></p> <ul style="list-style-type: none"><li>• High aspiration speed during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.</li></ul>
Cross contamination	<p><i>Contamination of the rims</i></p> <ul style="list-style-type: none"><li>• 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, section 6.2) before starting the shaker.</li></ul>

## 6.2 Ordering information

Product	REF	Pack of
NucleoMag® RNA	744350.1	1 x 96 preps
	744350.4	4 x 96 preps
NucleoSpin® Filters	740606	50
NucleoSpin® RNA Filter Plates	740711	4
NucleoMag® SEP	744900	1
Square-well Blocks	740481.4	4
	740481.24	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	4 sets
	740477.24	24 sets
Cap Strips	740638	30 strips
<b>For use of kit on KingFisher® Flex instrument:</b>		
e.g., KingFisher® Accessory Kit B Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag® RNA preps	744951	1 set

Visit [www.mn-net.com](http://www.mn-net.com) for more detailed product information.

## 6.3 Product use restriction/warranty

**NucleoMag® RNA** kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:

MACHEREY-NAGEL GmbH & Co. KG

Tel.: +49 24 21 969-270

tech-bio@mn-net.com

---

**Trademarks:**

KingFisher is a registered trademark of Thermo Fisher Scientific

NucleoMag is a registered trademark of MACHEREY-NAGEL GmbH & Co KG

Te-MagS is a trademark of Tecan Group Ltd.

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.



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

**MACHEREY-NAGEL**



MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany

DE/International:

Tel.: +49 24 21 969-0

Fax: +49 24 21 969-199

E-mail: [info@mn-net.com](mailto:info@mn-net.com)

CH:

Tel.: +41 62 388 55 00

Fax: +41 62 388 55 05

E-mail: [sales-ch@mn-net.com](mailto:sales-ch@mn-net.com)

FR:

Tel.: +33 388 68 22 68

Fax: +33 388 51 76 88

E-mail: [sales-fr@mn-net.com](mailto:sales-fr@mn-net.com)

US:

Tel.: +1 484 821 0984

Fax: +1 484 821 1272

E-mail: [sales-us@mn-net.com](mailto:sales-us@mn-net.com)

A037220/0200.1