

MACHEREY-NAGEL

User manual



RNA isolation

- NucleoSpin® RNA Plus

January 2021 / Rev. 05

RNA isolation

Protocol at a glance (Rev.05)

NucleoSpin® RNA Plus












| | | | | |
|-----------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| 1 | Homogenize sample and lyse sample |  | | 350 µL LBP |
| 2 | Remove gDNA and filtrate lysate |  |  | 11,000 x g, 30 s |
| 3 | Ajust RNA binding conditions |  | | 100 µL BS Mix |
| 4 | Bind RNA |  |  | Load sample 11,000 x g, 15 s |
| 5 | Wash and dry silica membrane |  | 1 st wash | 200 µL WB1 |
| | | | 2 nd wash | 600 µL WB2 |
| | | | 3 rd wash | 250 µL WB2 |
| | | | 1 st and 2 nd |  |
| 3 rd |  | 11,000 x g, 2 min | | |
| 6 | Elute RNA |  |  | 30 µL RNase-free H ₂ O |
| | | | | 11,000 x g, 1 min |
| | | | | 30 µL RNase-free H ₂ O |
| | | | | 11,000 x g, 1 min |

Table of contents

| | | |
|-----|-------------------------------------------------------------|----|
| 1 | Components | 4 |
| 1.1 | Kit contents | 4 |
| 1.2 | Reagents, consumables, and equipment to be supplied by user | 5 |
| 1.3 | RNase-free work environment | 5 |
| 1.4 | About this user manual | 6 |
| 2 | Product description | 7 |
| 2.1 | The basic principle | 7 |
| 2.2 | Kit specifications | 7 |
| 2.3 | Handling, preparation, and storage of starting materials | 9 |
| 2.4 | Elution procedures | 11 |
| 3 | Storage conditions and preparation of working solutions | 12 |
| 4 | Safety instructions | 13 |
| 5 | NucleoSpin® RNA Plus protocol | 14 |
| 6 | Appendix | 17 |
| 6.1 | rDNase digestion in solution | 17 |
| 6.2 | Troubleshooting | 19 |
| 6.3 | Ordering information | 22 |
| 6.4 | Product use restriction / warranty | 23 |

1 Components

1.1 Kit contents

| REF | NucleoSpin® RNA Plus | | |
|-------------------------------------------------------------------------------|-----------------------|-----------------------|-------------------------|
| | 10 preps 740984.10 | 50 preps 740984.50 | 250 preps 740984.250 |
| Lysis Buffer LBP | 5 mL | 25 mL | 125 mL |
| Binding Solution BS | 1.5 mL | 6 mL | 30 mL |
| Wash Buffer WB1 | 3 mL | 12 mL | 60 mL |
| Wash Buffer WB2 (Concentrate)* | 6 mL | 12 mL | 50 mL |
| RNase-free H ₂ O | 13 mL | 13 mL | 60 mL |
| NucleoSpin® gDNA Removal Column (yellow rings) | 10 | 50 | 250 |
| NucleoSpin® RNA Plus Columns (light blue rings – plus Collection Tubes) | 10 | 50 | 250 |
| Collection Tubes (2 mL) | 20 | 100 | 500 |
| Collection Tubes (1.5 mL) | 10 | 50 | 250 |
| User manual | 1 | 1 | 1 |

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

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to prepare Wash Buffer WB2, non-denatured ethanol recommended)

Consumables

- 1.5 mL or 2.0 mL microcentrifuge tubes (to prepare sample lysate)
- Sterile RNase-free tips

Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization (see section 2.3)
- Personal protection equipment (e.g., lab coat, gloves, goggles)
- RNase-free working environment

Note: Reducing agents (e.g. β -mercaptoethanol) often used for RNA isolation is typically not required for NucleoSpin[®] RNA Plus preparations.

1.3 RNase-free work environment

Kit components have been tested to ensure they are RNase-free. However, a RNase-free working environment is also a critical factor for performing successful RNA isolation and handling. Therefore, general recommendations to avoid RNase contamination should be followed:

- Maintain a separate area, dedicated pipettors and materials when working with RNA.
- Wear gloves when handling RNA and reagents to avoid contact with skin, which is a source of RNases. Change gloves frequently.
- Use sterile RNase-free plastic tubes. Collection Tubes (2 mL, for column flowthrough and 1.5 mL for elution) are provided in the kit. Tubes for lysate preparation have to be supplied by user.
- Use RNase-free water contained in kit for elution.
- Keep all kit components sealed when not in use and all tubes tightly closed when possible.

1.4 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® RNA Plus** kit is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at **www.mn-net.com**.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The **NucleoSpin® RNA Plus** kit is designed to purify RNA from a variety of cell and tissue types. This kit introduces the NucleoSpin® gDNA Removal Column, a spin column, which quickly and effectively removes genomic DNA contamination without the need of DNase digestion.

One of the most important aspects during the isolation of RNA is to prevent degradation of the RNA. Cells and tissues are first lysed by incubation in a chaotropic ion lysis buffer solution, which immediately inactivates RNases. The lysate is added to the NucleoSpin® gDNA Removal Column (yellow rings) to clarify the lysate and to remove contaminating gDNA. After the addition of the Binding Solution to the flowthrough, the RNA is bound to the **NucleoSpin® RNA Plus** Column (light blue rings). Subsequent wash steps remove salts, metabolites, and macromolecular cellular components. High quality RNA is eluted with RNase-free H₂O.

The RNA preparation using **NucleoSpin® RNA Plus** kits can be performed at room temperature.

The eluate should be treated with care because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints and dust. Keep RNA frozen at -20 °C for short term or -70 °C for long term storage to ensure RNA stability.

2.2 Kit specifications

- **NucleoSpin® RNA Plus** kits are recommended for the isolation of RNA from cultured cells and tissue. The **NucleoSpin® RNA Plus** kits allow purification of high quality RNA. The **NucleoSpin® RNA Plus** kits allow purification of RNA with an A_{260}/A_{280} ratio generally exceeding 1.9 (measured in TE buffer, pH 7.5).
- The isolated RNA is ready to use in diverse downstream applications.
- RNA isolated with the **NucleoSpin® RNA Plus** kits is of high integrity. RIN (RNA Integrity Number) of RNA isolated from fresh high quality sample material (e.g. eukaryotic cells or fresh mouse liver) generally exceeds 9. However, RNA integrity strongly depends on the sample quality.
- RNA molecules isolated with **NucleoSpin® RNA Plus** are longer than approximately 200 nucleotides. This kit provides an enrichment for mRNA since most RNAs < 200 nucleotides (e.g. 5.8 S rRNA, 5 S rRNA, tRNA, miRNA – which together comprise approximately 15–20% of total RNA) are selectively excluded.
- RNA isolated with the **NucleoSpin® RNA Plus** kits may generally contain minute amounts of genomic DNA due to carryover from the NucleoSpin® gDNA Removal Column. The probability of DNA detection with PCR increases with:
 1. the number of DNA copies per preparation: single copy target < plasmid / mitochondrial target < plasmid transfected into cells.
 2. decreasing PCR amplicon size.

Table 1: Kit specifications at a glance

| Parameter | NucleoSpin® RNA Plus |
|------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Technology | Two column silica membrane system: 1. Column for DNA removal 2. Column for RNA isolation |
| Format | Mini spin column |
| Sample material | < 1 x 10 ⁷ cultured cells, < 10 ⁹ bacterial cells, < 10 ⁸ yeast cells, < 30 mg tissue |
| Fragment size | > 200 nt |
| Typical yield | HeLa cells 5 x 10 ⁶ : 40–60 µg Mouse liver 20 mg: 80–100 µg Mouse kidney 20 mg: 40–70 µg Mouse spleen 5 mg: 30–60 µg |
| A ₂₆₀ /A ₂₈₀ | 1.9–2.1 |
| A ₂₆₀ /A ₂₃₀ | 1.8–2.5 |
| Typical RIN (RNA integrity number) | > 9 |
| Elution volume | 30–120 µL |
| Preparation time | 20 min/6 preps |
| Binding capacity | 200 µg |

2.3 Handling, preparation, and storage of starting materials

It is important, to use an appropriate amount of sample material in order to obtain optimal RNA yield and purity. The maximum amount of sample material that can be used with the NucleoSpin® RNA Plus kit depends on type of sample and its RNA and DNA content.

Maximal amount of sample material to be used per preparation (approximate values):

- Eukaryotic cells (e.g. HeLa cells): 10⁷ cells
- Animal tissue: 30 mg (wet weight)
- Plant tissue: 100 mg (wet weight)
- Microorganisms (e.g. yeast): 30 mg

Sample harvest and RNase-inhibition

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents.

Sample harvest methods:

- Use freshly harvested sample for immediate lysis and RNA purification.
- Samples can be stored in lysis buffer after disruption at -70 °C for up to one year, at 4 °C for up to 24 hours or up to several hours at room temperature. Frozen samples in lysis buffer should be thawed slowly before starting with the isolation of RNA.
- Flash freeze sample in liquid N₂ immediately upon harvest and store at 70 °C. Frozen samples are stable up to 6 months. Mortar and pestle can be used to pulverize the sample in a frozen state. Make sure that the sample does not thaw prior to contact with lysis buffer.
- Samples can be submerged and stored in RNA later ®. Before using such samples, remove excess RNA later ® solution from the tissue before use.

Disrupting and homogenizing sample material

Cells - Lysis of adherent cells in cell culture dish

Completely aspirate cell-culture medium and immediately add Lysis Buffer to the cell-culture dish. Avoid incomplete removal of the cell-culture medium in order to allow full lysis activity of the lysis buffer. Mixing cultured cells with lysis buffer is usually sufficient for complete lysis.

Cells - Lysis of adherent cells collected after trypsinization

Aspirate cell-culture medium, and add an equal amount of PBS in order to wash the cells. Aspirate PBS. Add 0.1–0.3% trypsin in PBS and incubate for an appropriate time to detach the cells from the dish surface. After cell detachment, add cell culture medium, transfer cells to an appropriate tube (not supplied), and pellet by centrifugation for 5 min at 300 x g. Remove supernatant and continue with the addition of lysis buffer to the cell pellet. Mixing cultured cells with lysis buffer is usually sufficient for complete lysis.

Animal tissues are often solid and must therefore be broken up mechanically as well as lysed. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption, and if viscosity is present the sample needs to be homogenized further to reduce the viscosity.

The most common technique used for disruption of animal tissues is grinding with a pestle and mortar. Grind the sample to a fine powder in the presence of liquid N₂. Take care that the sample does not thaw during or after grinding or weighing and add the frozen powder to an appropriate aliquot of lysis buffer and mix immediately. The broken-up and lysed tissue is then applied to the NucleoSpin[®] gDNA Removal Column (included in the kit) in order to bind DNA and remove insoluble matter.

Thawing of undisrupted animal tissue should be exclusively done in the presence of lysis buffer during simultaneous mechanical disruption, for example, with a rotor-stator homogenizer. This ensures that the RNA is not degraded by RNases before the preparation has started. The spinning rotor disrupts and simultaneously homogenizes the sample by mechanical shearing of DNA within seconds up to minutes prior to RNA isolation (homogenization time depends on sample). Take care to keep the rotor tip submerged in order to avoid excess foaming. Select a suitable sized homogenizer (5–7 mm diameter rotors can be used for homogenization in microcentrifuge tubes).

Bacteria and yeasts have to be incubated in lysozyme or lyticase/zymolase solutions, respectively. By this treatment, the robust cell walls of these organisms are digested or at least weakened, which is essential for effective cell lysis by lysis buffer. For microorganisms with extremely resistant cell walls – like some Gram positive bacterial strains – it may be necessary to optimize the conditions of the treatment with lytic enzymes or the cultivation conditions. After lysis in lysis buffer, homogenization is achieved by the use of a NucleoSpin[®] gDNA Removal Column.

Alternatively, bacteria and yeast cells can be broken up by bead beating. Therefore, resuspend cell pellet in Lysis Buffer RA1, transfer the solution into a MN Bead Tube (see ordering information) and disrupt samples by bead beating (e.g., by using MN Bead Tube Holder on a Vortex Genie[®] 2). Based on data generated by R&D, we suggest the use of MN Bead Tubes Type B for both bacteria and yeast. We highly recommend to optimize the bead beating procedure for your application and starting material. It may be necessary to test different MN bead tubes and disruption times to achieve the best results.

Enzymatic treatment: Resuspend the bacterial cell pellet (Gram negative strains) in 100 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8) containing 1 mg/mL lysozyme by vigorous vortexing. Incubate at 37 °C for 10 min. For preparation of RNA from Gram positive bacteria, resuspend cells in 100 µL TE containing 2 mg/mL lysozyme. It may be necessary to optimize incubation time and lysozyme concentration, depending on the bacterial strain.

Note: Due to the much higher concentration of genome equivalents in a nucleic acid preparation of bacteria compared with eukaryotic material, it may be necessary to use a lower quantity of cells for the preparation.

Plant material can be disrupted e.g., with mortar and pestle in a frozen state (liquid N₂) prior to addition of lysis buffer, or using Dispomix[®] (e.g., Xiril/VWR), or gentleMACSTM Dissociator (Miltenyi Biotec) immediately after addition of lysis buffer. If frozen plant material is used as sample, make sure that it will not thaw before disruption. Keep time from sample contact with lysis buffer to disruption as short as possible. For some plant materials, up to 100 mg can be used; for other plant materials, maximum sample amount is in the range of 20–50 mg.

2.4 Elution procedures

It is possible to adapt elution method and volume of water used for the subsequent application of interest. There are several elution procedures possible.

- **High yield:** Perform two elution steps with 30 μL . About 90–100 % of bound nucleic acid will be eluted.
- **High yield and high concentration:** Elute with the standard elution volume and apply the eluate once more onto the column for reelution.
- **Convenient elution:** Elute once with 60 μL water.

Eluted RNA should immediately be put and always kept on ice for optimal stability because almost omnipresent RNases (general lab ware, fingerprints, dust) will degrade RNA. For short term storage freeze at $-20\text{ }^{\circ}\text{C}$, for long term storage freeze at $-70\text{ }^{\circ}\text{C}$.

3 Storage conditions and preparation of working solutions

Attention:

Buffers LBP and WB2 contain chaotropic salt. Wear gloves and goggles!

CAUTION: Buffers LBP and WB2 contain chaotropic salt 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 kit components should be stored at room temperature (18–25 °C) and are stable for at least one year. Storage at lower temperatures may cause precipitation of salts.

Before starting any **NucleoSpin® RNA Plus** protocol, prepare the following:

- Wash Buffer WB2:** Add the indicated volume of 96–100 % ethanol (see table below) to Buffer WB2 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer WB2 can be stored at room temperature (18–25 °C) for at least one year.

| NucleoSpin® RNA Plus | | | |
|----------------------------------|---------------------------|----------------------------|-----------------------------|
| | 10 preps | 50 preps | 250 preps |
| REF | 740984.10 | 740984.50 | 740984.250 |
| Wash Buffer WB2 (Concentrate) | 6 mL Add 24 mL ethanol | 12 mL Add 48 mL ethanol | 50 mL Add 200 mL ethanol |



- Binding Solution BS is ready to use.
- Reducing agent (e.g., β -mercaptoethanol) is not necessary for **NucleoSpin® RNA Plus** preparations.

4 Safety instructions

The following components of the **NucleoSpin® RNA Plus** kit 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> |
| LBP | Guanidinium thiocyanate 30–45 % <i>Guanidinthiocyanat 30–45 %</i> CAS 593-84-0 |  WARNING <i>ACHTUNG</i> | – | – |
| WB1 | Guanidinium hydrochloride 24–36 % and ethanol 20–35 % <i>Guanidinhydrochlorid 24–36 % und Ethanol 20–35 %</i> CAS 50-01-1, 64–17-5 |  WARNING <i>ACHTUNG</i> | – | – |

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

5 NucleoSpin® RNA Plus protocol

Before starting the preparation:

- Check if Wash Buffer WB2 was prepared according to section 3.

1 Homogenize and lyse sample

Add **350 µL Buffer LBP** per sample and disrupt the sample according to one of the methods described in section 2.3.

Note: Lysis tube is not included in the kit. Addition of reducing agent (e.g. β -mercaptoethanol, DTT, or TCEP) is not necessary. The sample material should be broken up and lysed as completely as possible.

Note: If considerable amounts of non-lysed sample material is still visible after sample disruption (which might occur with certain plant material), briefly centrifuge the lysate (approx. 3 s at 2,000 x g) in order to sediment the sample debris. If the sediment represents e.g., > 20 % of the total lysate volume, do not transfer the sediment onto the NucleoSpin® gDNA Removal Column.



+ 350 µL LBP

For certain sample types (e.g., some plant types) it can be beneficial to use 500 µL lysis buffer instead of 350 µL, especially, if a lot of sample debris remains are observed after sample disintegration.

Note: Use of increased volumes of Buffer LBP require additional volumes of Binding Solution BS (not provided in the kit).

2 Remove gDNA and filtrate lysate

Place NucleoSpin® gDNA Removal Column (yellow ring) in a Collection Tube (2 mL; provided), transfer the homogenized lysate to the NucleoSpin® gDNA Removal Column, and centrifuge for 30 s at 11,000 x g.

Discard the column and **continue with the flowthrough**.

Note: Ensure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

If (in rare cases) the flowthrough contains obvious undissolved sediment, recover flowthrough without sediment. Optimize mechanical sample disruption for subsequent preparations.



11,000 x g,
30 s

3 Adjust RNA binding conditions

Add **100 µL Binding Solution BS** to the flowthrough and mix well by moderate vortexing or by pipetting up and down several times.

! *Note: If mixing is done by vortexing, be careful in order to avoid spilling, because the Collection Tube does not contain a lid.*

Note: If another volume than 350 µL of Lysis Buffer LBP has been used for lysis (e.g., 500 µL), make sure to add approximately 0.3 volume of Binding Buffer BS to the cleared lysate in order to adjust RNA binding conditions.



**+ 100 µL BS
Mix**

Note: Use of increased volumes of Buffer LBP require additional volumes of Binding Solution BS (not provided in the kit).

After addition of Binding Solution BS a stringy precipitate may become visible which will not affect the RNA isolation. Be sure to disaggregate any precipitate by mixing and load all of the precipitate on the column as described in the following step. Do not centrifuge the lysate after addition of Binding Solution before loading it onto the column in order to avoid pelleting the precipitate.

4 Bind RNA

Transfer the whole lysate (~ 450 µL) to the NucleoSpin® RNA Plus Column (light blue ring) preassembled with a Collection Tube. Centrifuge for **15 s** at **11,000 x g**.



Load lysate

Note: Flowthrough (~ 450 µL) may stay in the Collection Tube. Alternatively, discard the flowthrough and reuse collection tube or place the column into a new 2 mL collection tube (not provided).



**1,000 x g,
15 s**

5 Wash and dry silica membrane**1st wash**

Add **200 µL Buffer WB1** to the NucleoSpin® RNA Plus Column. Centrifuge for **15 s** at **11,000 x g**. Discard the flowthrough with collection tube and place the column into a new 2 mL Collection Tube (provided).



+ 200 µL WB1

11,000 x g,
15 s**2nd wash**

Add **600 µL Buffer WB2** to the NucleoSpin® RNA Plus Column. Centrifuge for 15 s at 11,000 x g. Discard flowthrough and place the column back into the Collection Tube.



+ 600 µL WB2

11,000 x g,
15 s

Note: Make sure that residual buffer from the previous steps is washed away with Buffer WB2 especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer WB2.

3rd wash

Add **250 µL Buffer WB2** to the NucleoSpin® RNA Plus Column. Centrifuge for **2 min** at **11,000 x g** to dry the membrane completely. Place the column into a nuclease-free Collection Tube (1.5 mL, provided).



+ 250 µL WB2

11,000 x g,
2 min

If for any reason, the liquid level in the Collection Tube has reached the NucleoSpin® RNA Plus Column after centrifugation, discard flowthrough, and centrifuge again.

6 Elute RNA

Add **30 µL RNase-free H₂O** and centrifuge at **11,000 x g** for 1 min.

+ 30 µL RNase-free
H₂O11,000 x g,
1 min

Add again **30 µL RNase-free H₂O** and centrifuge at **11,000 x g** for 1 min.

+ 30 µL RNase-free
H₂O11,000 x g,
1 min

Alternatively, elution can be performed with 1 x 60 µL, but yield can be slightly reduced compared to elution with 2 x 30 µL. For alternative elution procedures see section 2.4.

6 Appendix

6.1 rDNase digestion in solution

The passage of the lysed sample through NucleoSpin® gDNA Removal Column according to the standard protocol is very efficient in DNA binding resulting in minimal residual DNA in the purified RNA. Residual DNA will not be detectable in most downstream applications. Despite this, there are still certain applications which require even lower contents of residual DNA. However, removal of DNA to a completely undetectable level is challenging and the efficiency of the NucleoSpin® gDNA Removal Column is sometimes not sufficient for downstream applications requiring lowest residual content of DNA. This can be especially the case if a large amount of sample or a sample containing much DNA is processed with the NucleoSpin® gDNA Removal Column. The amount of residual DNA detected depends on sample type, amount and its DNA content and the detection sensitivity of the method used to analyse residual DNA.

A typical example for such a demanding application is an RT-PCR reaction in which the primer molecules do not differentiate between cDNA (derived from RNA) and contaminating genomic DNA. Especially, if

- high copy number targets are analyzed (e.g., multi gene family, mitochondrial, plasmid or plasmid targets (from transfections))
- the target gene is of a very low expression level
- the amplicon is relatively small (< 200 bp).

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase and digested DNA) are usually required.

The high quality, recombinant, RNase-free rDNase (REF 740963) facilitates such a digestion in solution in order to remove even traces of contaminating DNA.

A Digest DNA (Reaction setup)

Add **6 µL Reaction Buffer for rDNase** and **0.6 µL rDNase** to **60 µL eluted RNA**.

(Alternatively premix 100 µL Reaction Buffer for rDNase and 10 µL rDNase and add 1/10 volume to one volume of RNA eluate).

Gently swirl the tube in order to mix the solution. Spin down gently (approx. 1 s at 1,000 x g) to collect every droplet of the solution at the bottom of the tube.

B Incubate sample

Incubate for **10 min** at **37 °C**.

C Repurify RNA

Repurify RNA with a suitable RNA cleanup procedure, for example by use of the NucleoSpin® RNA Clean-up, NucleoSpin® RNA Clean-up XS kits (see ordering information), or by ethanol precipitation.

Ethanol precipitation, exemplary:

Add **0.1 volume** of **3 M sodium acetate, pH 5.2** and **2.5 volumes** of **96–100 % ethanol** to **one volume of sample**. Mix thoroughly.

Incubate **several minutes** to **several hours** at **-20 °C** or **4 °C**.

Note: Choose long incubation times if the sample contains low RNA concentration. Short incubation times are sufficient if the sample contains high RNA concentration.

Centrifuge for **10 min** at **maximum speed**.

Wash RNA pellet with **70 % ethanol**.

Dry RNA pellet and resuspend RNA in RNase-free H₂O.

6.2 Troubleshooting

| Problem | Possible cause and suggestions |
|----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| RNA is degraded/ no RNA obtained | <i>RNase contamination</i> <ul style="list-style-type: none">• Create an RNase free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use. |
| | <i>Insufficient sample quality</i> <ul style="list-style-type: none">• Control sample harvest, storage and lysis. Make sure that samples are harvested, stored and lysed adequately in order to preserve RNA integrity. |
| Poor RNA quality or yield | <i>Reagents not applied or restored properly</i> <ul style="list-style-type: none">• Reagents not properly restored. Add the indicated volume of 96 % ethanol to Buffer WB2 Concentrate and mix.• Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.• No Binding Solution BS has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of Binding Solution. |
| | <i>Kit storage</i> <ul style="list-style-type: none">• Store kit components at room temperature. Storage at low temperatures may cause salt precipitation.• Keep bottles tightly closed in order to prevent evaporation or contamination. |
| | |

Ionic strength and pH influence A_{260} absorption as well as ratio A_{260}/A_{280}

Poor RNA
quality or yield
(continued)

- For adsorption measurement, use 5 mM Tris pH 8.5 as diluent. Please see also:
 - Manchester, K L. 1995. Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. *Biotechniques* 19, 208–209.
 - Wilfinger, W W, Mackey, K and Chomczynski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* 22, 474–481.

Sample material

- Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid N₂. Samples should always be kept at -70 °C. Never allow tissues to thaw before addition of Buffer LBP. Perform disruption of samples in liquid N₂.
- Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption.

Carry-over of guanidinium thiocyanate

Low A_{260}/A_{230}
ratio

- Carefully load the lysate to the NucleoSpin® RNA Plus Column and try to avoid a contamination of the upper part of the column and the column lid.
- Make sure that a sufficient amount / concentration of RNA is used for quantification so that the A_{230} value is significantly higher than the background level.
- Measurement of low amount / concentration of RNA will cause unstable A_{260}/A_{230} ratio values.

Sample material

Clogged
NucleoSpin®
Column/
Poor RNA
quality
or yield

- Too much starting material used. Overloading may lead to decreased overall yield. Reduce amount of sample material or use larger volume of lysis buffer.
 - Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use NucleoSpin® gDNA Removal Column for DNA removal and for easy homogenization of disrupted starting material.
 - Increase g-force and centrifugation time if necessary.
-

Too much cell material used

- Reduce quantity of cells or tissue used.

DNA detection system too sensitive

The amount of DNA contamination is effectively reduced by the NucleoSpin® gDNA Removal Column. However, it can not be guaranteed that the purified RNA is 100 % free of DNA, therefore in very sensitive applications it might still be possible to detect DNA. The probability of DNA detection with PCR increases with:

Contamination of RNA with genomic DNA

- the number of DNA copies per preparation: single copy target < plastidial/mitochondrial target < plasmid transfected into cells
- decreasing of PCR amplicon size.
- Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible.
- **Use support protocol 6.1 for subsequent rDNase digestion in solution.**

Carry-over of ethanol or salt

Suboptimal performance of RNA in downstream experiments

- Do not let the flowthrough touch the column outlet after the second Buffer WB2 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer WB2 completely.
- Check if Buffer WB2 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer WB2.

Store isolated RNA properly

- Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at -70 °C.
-

6.3 Ordering information

| Product | REF | Pack of |
|-------------------------------|--------------------|-----------------|
| NucleoSpin® RNA Plus | 740984.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA | 740955.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA Midi | 740962.20 | 20 |
| NucleoSpin® miRNA | 740971.10/.50/.250 | 10/50/250 preps |
| NucleoSpin® RNA/Protein | 740933.10/.50/.250 | 10/50/250 |
| NucleoSpin® TriPrep | 740966.10/.50/.250 | 10/50/250 |
| NucleoSpin® totalRNA FFPE XS | 740969.10/.50/.250 | 10/50/250 |
| NucleoSpin® totalRNA FFPE | 740982.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA Blood | 740200.10/.50 | 10/50 |
| NucleoSpin® RNA Blood Midi | 740210.20 | 20 |
| NucleoSpin® 8 RNA Blood | 740220/.5 | 12 x 8 / 60 x 8 |
| NucleoSpin® 96 RNA Blood | 740225.2/.4 | 2 x 96 / 4 x 96 |
| NucleoSpin® RNA Clean-up | 740948.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA XS | 740902.10/.50/.250 | 10/50/250 |
| NucleoSpin® RNA Clean-up XS | 740903.10/.50/.250 | 10/50/250 |
| rDNase Set | 740963 | 1 |
| Collection Tubes (2 mL) | 740600 | 1000 |
| NucleoSpin® Bead Tubes Type B | 740812.50 | 50 |
| NucleoSpin® Bead Tubes Type C | 740813.50 | 50 |

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

6.4 Product use restriction / warranty

NucleoSpin® RNA Plus 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.

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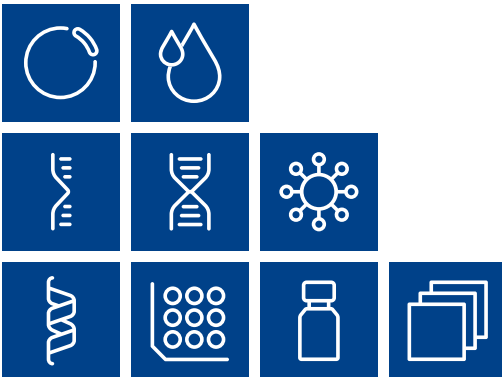
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Dispomix[®] (e.g. Xiril / VWR)

gentleMACSTM Dissotiator (Miltenyi Biotec)

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Plasmid DNA

Clean up

RNA

DNA

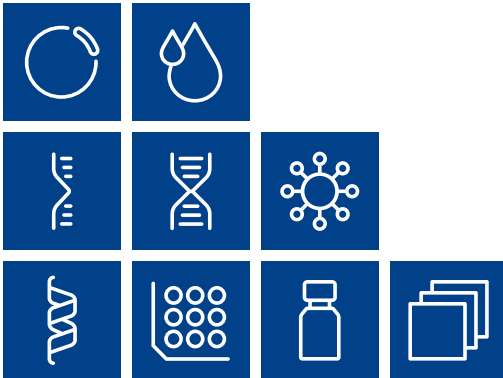
Viral RNA and DNA

Protein

High throughput

Accessories

Auxiliary tools



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