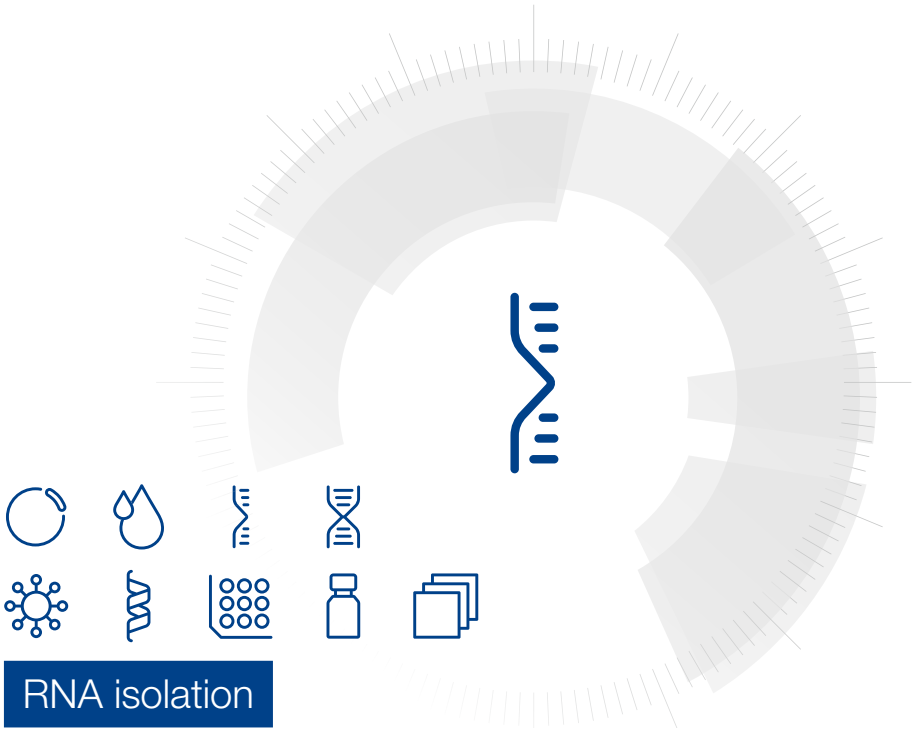
Bioanalysis

MACHEREY-NAGEL

User manual



RNA isolation

- NucleoZOL

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Table of contents

1 Components

1.1 Kit contents

	NucleoZOL
REF	**740404.200**
NucleoZOL reagent	200 mL

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

Reagents

- RNase-free water
- 75 % ethanol
- 70 % isopropanol
- 100 % isopropanol
- 100 % 4-bromoanisole (optional, see p. 11)

Consumables

- 1.5 mL, 2.0 mL or 15 mL centrifuge tubes (depending on the amount of sample to be processed per preparation)
- Sterile RNase-free tips

Equipment

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

1.3 RNase-free working environment

The reagent has been tested for functionality. However, an 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. Tubes for lysate preparation and RNA precipitation have to be supplied by the user.
- Keep all kit components sealed when not in use and all tubes tightly closed when possible.

1.4 About this user manual

Please read the detailed protocol if using NucleoZOL for the first time. Experienced users may refer to the short instruction manual.

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

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

2 Product description

2.1 The basic principle

NucleoZOL is designed for the isolation of total RNA (small and large RNA) in a single fraction or in separate fractions from a variety of sample materials, such as cells, tissue, and liquids from human or animal origin, plants, yeast, bacteria, viral materials, and other sources.

One of the most important factors during the isolation of RNA is to prevent degradation. First, cells and tissues are lysed and homogenized in NucleoZOL reagent based on guanidinium thiocyanate and phenol. Contaminating molecules such as DNA, polysaccharides, and proteins are precipitated by the addition of water and removed by centrifugation. The NucleoZOL procedure allows the separate isolation of small and large RNA by adding ethanol and isopropanol, respectively. RNA can be reconstituted by RNase-free water. A chloroform-induced phase separation is not necessary for high-quality RNA isolation.

The RNA is ready for use in qRT-PCR, microarrays, RNase protection assays, poly A+ isolation, blotting, and other applications.

2.2 Product specifications

Product specifications at a glance	
Technology	One-phase extraction
Sample material (per 500 µL NucleoZOL)*	< 1 × 106 cultured cells, bacteria, and yeast, < 50 mg human/animal/plant tissue, < 200 µL (viral) fluids
Fragment size	Small RNA (10 – 200 nt), large RNA (> 200 nt)
Typical yield (total RNA)	Liver: 6 – 8 µg/mg tissue Kidney, spleen: 3 – 4 µg/mg tissue Muscle, brain, lung: 0.5 – 1.5 µg/mg Cultured cells: 4 – 10 µg/10^6 cells
Typical yield (large RNA)	Liver: 5 – 7 µg/mg tissue Kidney, spleen: 3 – 4 µg/mg tissue Muscle, brain, lung: 0.5 – 1.5 µg/mg Cultured cells: 3 – 8 µg/10^6 cells
$A_{260/280}$ (total RNA)	1.8 – 2.1
Typical RIN (RNA integrity number)	> 9
Elution volume	flexible
Use	For research use only

* The standard protocols describe the procedure with 500 µL NucleoZOL. The procedure can be scaled up or down, dependent on the sample input.

2.3 Handling, preparation, and storage of starting materials

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 NucleoZOL after disruption at -20 °C to -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 NucleoZOL 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 the reagent.
- Samples can be submerged and stored in RNA stabilizing reagents such as RNA*later*®. Remove excess RNA*later*® solution from the tissue before processing the sample.

2.4 RNA reconstitution

The precipitated RNA can be dissolved in variable volumes of RNase-free water (see ordering information, section 6.3) to approach an RNA concentration of approximately 1 – 2 µg/µL for the large RNA fraction and approximately 0.1 µg/µL for the small RNA fraction.

3 Storage conditions and preparation of working solutions

Attention: NucleoZOL contains phenol (corrosive liquid/poison) and guanidium thiocyanate (irritant). Wear gloves and eye protection!

CAUTION: Read the warning note on the container and SDS. NucleoZOL contains phenol and guanidinium thiocyanate which CAUSES BURNS and can be fatal. When working with NucleoZOL, use gloves and eye protection (face shield, safety goggles). Do not get the reagent on skin or clothing. Avoid breathing fumes. In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and if necessary seek medical attention.

NucleoZOL can be stored at room temperature (18 – 25 °C) and is stable for at least one year.

4 Safety instructions

When working with the **NucleoZOL** wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at http://***www.mn-net.com/msds***).

Caution: guanidinium thiocyanate in **NucleoZOL** can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with **NucleoZOL** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 NucleoZOL protocols

5.1 Isolation of total RNA

Total RNA (including small RNA, e.g., miRNA) is isolated with the following protocol. This protocol describes the isolation of RNA with 500 µL NucleoZOL reagent. This allows for the processing of the whole procedure in microcentrifuge tubes. The procedure can be scaled up or down, dependent on the sample input.

1 Homogenization

Tissue

Homogenize tissue samples with a rotor-stator homogenizer or another mechanical disruption device using up to **50 mg of tissue per 500 µL NucleoZOL**. For tissues with high DNA content (e.g., spleen) it is recommended to use 25 mg of tissue / 500 µL reagent.

Cells

Cells grown in monolayer: Remove cell culture medium and lyse cells by addition of at least **1 mL of NucleoZOL** to the culture disk (diameter 3.5 cm, 10 cm^2). Ensure complete lysis by repeated pipetting. Calculate the amount of reagent based on culture dish area, not on cell number.

An insufficient volume of the reagent will lead to DNA contamination of the isolated RNA. Residual homogenate can be stored at -20 to -80 °C at least one year for later use.

Cells grown in suspension: Sediment cells and lyse directly by the addition of NuceoZOL. Add at least **500 µL NucleoZOL per 5 × 10^6 cells** and lyse cells by pipetting up and down several times.

Do not wash the cells before addition of NucleoZOL. Washing of cells might contribute to RNA degradation.

Liquid samples

Add **500 µL NucleoZOL per 200 µL liquid sample** for homogenization and lysis. For processing sample volumes smaller than 200 µL, add 500 µL of NucleoZOL and add water to a final volume of 700 µL.

Fatty samples

Homogenize lipid-rich samples as described above. Centrifuge the samples for **5 min** at **12,000 x *g***. After centrifugation, a fat layer appears on top of the sample. Pierce the upper layer with a syringe/pipette tip and transfer the supernatant into a new tube.

2 **Precipitate contaminants**

Add **200 µL RNase-free water per 500 µL NucleoZOL** to the lysate. **Shake** the sample vigorously for **15 s**. Incubate at **room temperature** for **5 min**.

For samples containing 50 mg tissue / 500 µL NucleoZOL, 15 min incubation at room temperature is recommended.

Centrifuge samples for **15 min** at **12,000 x *g*** at room temperature.

A semi-solid pellet containing DNA, proteins and polysaccharides forms at the bottom of the tube. The RNA is still solubilized in the supernatant.

Transfer **500 µL supernatant** to a fresh tube. Leave a layer of the supernatant above the DNA/protein pellet.

The pellet containing DNA, protein, and polysaccharides comprises approximately 10 % in volume of the total homogenate-water mix.

3 **Phase separation (optional)**

The basic protocol for total RNA isolation can be complemented by an optional phase separation. This is useful for samples with high DNA content and/or extracellular material.

Add **2.5 µL (0.5 % of supernatant volume) 100 % 4-bromoanisole** to **500 µL transferred supernatant**. **Mix** well for **15 s** and incubate at **room temperature** for **5 min**.

Do not substitute 4-bromoanisole with bromchloropropane or chloroform!

Centrifuge for **10 min** at **12,000 x *g*** at room temperature.

Residual DNA, proteins, and polysaccharides accumulate in the organic phase at the bottom of the tube. RNA is still solubilized in the supernatant.

4 **Precipitate total RNA**

Pipette RNA containing supernatant from step 2 or 3 into a fresh tube.

Add **500 µL of isopropanol** per **500 µL supernatant** in order to precipitate RNA. Incubate samples at **room temperature** for **10 min**.

Centrifuge samples for **10 min** at **12,000 x *g***.

Remove and discard supernatant.

Typically, RNA is obtained as a white pellet at the bottom of the tube. For spleen samples, RNA forms a gel-like membrane on the bottom of the tube. Upon washing with ethanol, the membrane becomes more visible.

5 Wash RNA

Use **500 µL 75 % ethanol** when precipitating in 1.5 mL tubes.

For larger tubes, add 500 µL 75 % ethanol per 1 mL supernatant.

Centrifuge the pellets for **3 min** at **8,000 x *g***. Remove ethanol from the pellet by pipetting. Repeat the ethanol washing step. Drying of the pellet is not necessary.

Drying the RNA pellet may lead to a decrease in solubility.

6 Reconstitute RNA

Dissolve the RNA pellet in RNase-free water to obtain an RNA concentration of 1 – 2 µg/µL. Vortex the sample **3 min** at **room temperature** for efficient solubilization.

For accurate determination of RNA concentration by OD measurement with a cuvette, dilute RNA in RNase-free water with a slightly alkaline pH or buffer with a pH > 8 (e.g., Elution Buffer AE, see ordering information, section 6.3). Distilled water typically has a pH < 7.

5.2 Isolation of small and large RNA in two separate fractions

Please note that RNA is separated in small RNA (10 – 200 nt) and large RNA (> 200 nt) in two fractions following this protocol.

1 Homogenization

Tissue

Homogenize tissue samples with a rotor-stator homogenizer or another mechanical disruption device using up to **50 mg of tissue per 500 µL NucleoZOL**. For tissues with high DNA content (e.g., spleen) it is recommended to use 25 mg of tissue / 500 µL reagent.

Cells

Cells grown in monolayer: Remove cell culture medium and lyse cells by addition of at least **1 mL of NucleoZOL** to the culture disk (diameter 3.5 cm, 10 cm^2). Ensure complete lysis by repeated pipetting. Calculate the amount of reagent based on culture dish area, not on cell number.

An insufficient volume of the reagent will lead to DNA contamination of the isolated RNA. Residual homogenate can be stored at -20 to -80 °C at least one year for later use.

Cells grown in suspension: Sediment cells and lyse directly by the addition of NuceoZOL. Add at least **500 µL NucleoZOL per 5 × 10^6 cells** and lyse cells by pipetting up and down several times.

Do not wash the cells before addition of NucleoZOL. Washing of cells might contribute to RNA degradation.

Liquid samples

Add **500 µL NucleoZOL per 200 µL liquid sample** for homogenization and lysis. For processing sample volumes smaller than 200 µL, add 500 µL of NucleoZOL and add water to a final volume of 700 µL.

Fatty samples

Homogenize lipid-rich samples as described above. Centrifuge the samples for **5 min** at **12,000 x *g***. After centrifugation, a fat layer appears on top of the sample. Pierce the upper layer with a syringe/pipette tip and transfer the supernatant into a new tube.

2 **Precipitate contaminants**

Add **200 µL RNase-free water per 500 µL NucleoZOL** to the lysate. **Shake** the sample vigorously for **15 s**. **Incubate** at **room temperature** for **5 min**.

For samples containing 50 mg tissue / 500 µL NucleoZOL, 15 min incubation at room temperature is recommended.

Centrifuge samples for **15 min** at **12,000 x *g*** at room temperature.

A semi-solid pellet containing DNA, proteins, and polysaccharides forms at the bottom of the tube. The RNA is still solubilized in the supernatant.

3 **Precipitate large RNA**

Pipette **500 µL** of the **supernatant** to a new tube. Leave a layer of the supernatant on top of the precipitate. Add **200 µL 75 % ethanol** to 500 µL supernatant for precipitation of the RNA.

Incubate samples at **room temperature** for **10 min**.

Centrifuge the samples for **8 min** at **12,000 x *g***. A white pellet containing the RNA will be formed at the bottom of the tube. Transfer the supernatant containing the small RNA to a new tube and store it at 4 °C or at -20 °C.

The small RNA containing supernatant can be stored at -20 °C for one year.

4 **Precipitate small RNA**

Add **500 µL of isopropanol** (~0.8 vol) to the supernatant obtained after precipitation of RNA (step 3). Incubate the samples for **30 min** at **4 °C.**

Centrifuge the samples for **15 min** at **12,000 x *g*** at room temperature.

Precipitated RNA will form a white pellet at the bottom of the tube.

Remove and discard supernatant.

5 Wash RNA

Large RNA

Add **500 µL 75 % ethanol** to the pellet.

For larger tubes, add 500 µL 75 % ethanol per 1 mL supernatant used for precipitation.

Centrifuge for **3 min** at **8,000 x *g***. Remove ethanol using a micropipette. Repeat washing step. Drying of the pellet is not necessary.

Small RNA

Add **500 µL 70 % isopropanol** to the pellet.

For larger tubes, add 500 µL 70 % isopropanol per 1 mL supernatant used for precipitation.

Centrifuge for **3 min** at **8,000 x *g***. Remove isopropanol using a micropipette. Repeat washing step. Drying of the pellet is not necessary.

Drying the RNA pellet may lead to a decrease in solubility.

6 Reconstitute RNA

Dissolve the RNA pellet in RNase-free water to obtain an RNA concentration of 1 – 2 µg/µL for the large RNA fraction and about 0.1 µg/µL for the small RNA fraction. Vortex the sample for **3 min** at **room temperature** for efficient solubilization.

For accurate determination of RNA concentration by OD measurement with a cuvette, dilute RNA in RNase-free water with a slightly alkaline pH, 1 mM NaOH or buffer with a pH > 8 (e.g., Elution Buffer AE, see ordering information 6.3). Distilled water typically has a pH < 7.

Note: The large RNA fraction contains RNA > 200 nt and contains 80 – 85 % of cellular RNA.

5.3 Isolation of total RNA in combination with NucleoSpin® RNA Set for NucleoZOL (REF 740406.50)

1 Homogenization

The homogenization procedure is identical to the standard protocols. For detailed information refer to section 5.2 or 5.1.

Tissue

Use up to **50 mg** tissue with **500 µL NucleoZOL**. Larger samples may exceed the RNA binding capacity of the NucleoSpin® RNA Binding Column.

Cells

Use up to **5 × 10^6 cells** with **500 µL NucleoZOL** per preparation.

Liquid samples

Use up to **200 µL liquid sample** with **500 µL NucleoZOL**.

2 Precipitate contaminants

Add **200 µL RNase-free water / 500 µL NucleoZOL** to the lysate.

Vortex the sample vigorously for **15 s** and incubate at **room temperature** for **5 min**.

For samples containing 50 mg tissue / 500 µL NucleoZOL, 15 min incubation at room temperature is recommended.

Centrifuge samples for **15 min** at **12,000 x *g*** at room temperature.

A semi-solid pellet containing DNA, proteins, and polysaccharides forms at the bottom of the tube. The RNA is still solubilized in the supernatant.

Transfer **500 – 600 µL supernatant** into a fresh tube (not provided). Do not disturb the DNA/protein pellet.

The pellet containing DNA, protein, and polysaccharides comprises approximately 10 % in volume of the total homogenate-water mix.

3 Adjust RNA binding conditions

Add **500 µL Buffer MX** to the transfered supernatant and **mix** by vortexing.

Add 1 vol Buffer MX per 1 mL NucleoZOL used for homogenization.

4 Bind RNA

For each preparation, take one **NucleoSpin® RNA Column** (light blue ring) placed in a Collection Tube and load 700 µL lysate. Centrifuge for **30 s** at **8,000 x *g***.

Discard flow-through and reuse Collection Tube. Load residual lysate to the column and centrifuge for 30 s at 8,000 x *g*.

Discard Collection Tube with flow-through and place the column in a new Collection Tube (provided).

Maximum loading capacity of NucleoSpin® RNA Columns is 700 µL. Repeat the procedure if larger volumes are processed.

5 Wash and dry silica membrane

1st wash

Add **700 µL Buffer RA3** to the NucleoSpin® RNA Column. Centrifuge for **30 s** at **8,000 x *g***. Discard flow-through and reuse Collection Tube.

2nd wash

Add **350 µL Buffer RA3** to the NucleoSpin® RNA Column. Centrifuge for **2 min** at **8,000 x *g***.

Place the NucleoSpin® RNA Column into an RNase-free Collection Tube (1.5 mL, supplied). Open the lid of the column and let the membrane **dry** for **3 min**.

If the liquid level in the Collection Tube has reached the NucleoSpin® RNA Column after centrifugation, discard flow-through and centrifuge again.

The procedure ensures complete removal of ethanol from the column.

6 Elute RNA

Add **60 µL RNase-free H_2O** onto the center of the membrane and centrifuge for **1 min** at **11,000 x *g***.

6 Appendix

6.1 Digestion of residual DNA in solution

NucleoZOL efficiently removes DNA when processing samples according to the standard protocol, resulting in minimal residual DNA in the purified RNA. Residual DNA will not be detectable in most downstream applications. If large samples or samples with high levels of DNA are processed, it may be difficult to remove all traces of DNA. The amount of residual DNA depends on the sample type, amount, DNA content and the detection sensitivity of the method used to analyze residual DNA. A typical example is an RT-PCR reaction in which the primer molecules do not differentiate between cDNA (derived from RNA) and contaminating genomic DNA. The effect is prominent if

- high copy number targets are analyzed (e.g., multi gene family, mitochondrial, plastidal 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. High quality, RNase-free, recombinant rDNase (REF 740963, see ordering information 6.3) facilitates such a digestion in solution in order to remove 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: NucleoSpin® RNA Clean-up, NucleoSpin® RNA Clean-up XS kits (see ordering information 6.3), 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 water.

6.2 Troubleshooting

Problem	Possible cause and suggestions
Insufficient yield	*Homogenization or sample lysis is incomplete* • Improve homogenization by testing more stringent conditions.
	Solubilization of the RNA pellet is incomplete. • Increase volume of RNase-free water for dissolving of RNA and prolong mixing for solubilization.
Ratio $A_{260/280}$ < 1.6	*Volume of NucleoZOL used for homogenization was too low* • Increase volume of NucleoZOL.
	Low pH during spectrophotometric quantification • Use Buffer AE (5 mM Tris pH 8.5) for sample dilution for spectrophotometric quantification.
	RNA pellet was only partly solubilized • Increase volume of RNase-free water for dissolving of RNA and prolong mixing for solubilization.
	Contamination of polysaccharide or proteoglycan • Perform phase separation as described in section 5.2.3.
Degraded RNA	*Inadequate tissue sampling* • Make sure to use fresh tissue or flash-freeze tissue immediately upon harvest.
	Inappropriate storage conditions • Store samples at -70 °C.
	Cell dissolution during trypsinization • Make sure cells stay intact during trypsinization.
	RNase contaminated solutions or tubes • Make sure to work in an RNase-free environment.

Problem	Possible cause and suggestions
Contamination with DNA	*Volume of NucleoZOL used for homogenization was too low* • Increase volume of NucleoZOL.
	Sample material contains strong buffers, organic solvents, alkaline solution, or salt • The precipitation of DNA (step 2) can be improved by the following modification: Increase incubation time to 15 min after addition of water (step 2). Centrifuge at 16,000 x *g*. • **Use support protocol 6.1 for subsequent rDNase digestion in solution**
Contamination with proteo-glycan, fat, or polysaccharide	*Inefficient precipitation of contaminants* • Centrifuge the initial crude homogenate (step 1) for phase separation in an additional step for 10 min at 12,000 x *g*.

6.3 Ordering information

Product	REF	Pack of
NucleoZOL	740404.200	200 mL
NucleoSpin® RNA Set for NucleoZOL	740406.50	50 preps
NucleoSpin® RNA Clean-up XS	740903.10 / 50 / 250	10 / 50 / 250 preps
NucleoSpin® RNA Clean-up	740948.10 / 50 / 250	10 / 50 / 250 preps
RNase-free Water	740378.1000	1000 mL
Elution Buffer AE	740917.1	1000 mL
rDNase Set	740963	One set

6.4 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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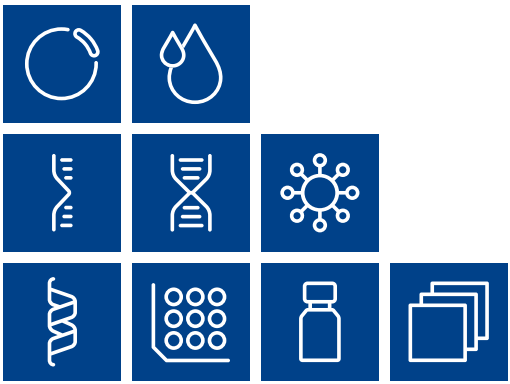
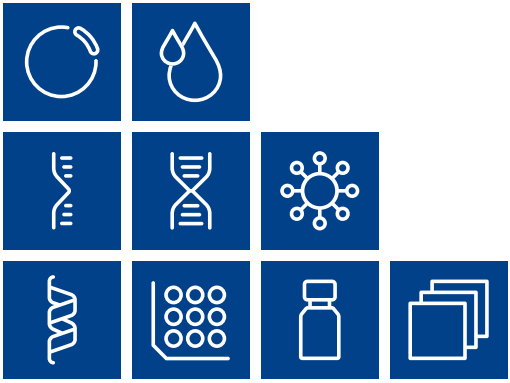


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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