RNA

# User manuals

**NucleoZOL reagent** **740404.6 / .200**

Dear valued customer,

Thank you for choosing MACHEREY-NAGEL for your application.

This product is for research use only.

Important information regarding product components, specifications, safety instructions, and processing protocols, can all be found on the product website and accessed easily via the QR code.

QR-Code product website

*qr.mn-net.com/qr/(241)740404.200*

## Product description

NucleoZOL is a convenient reagent based on a combination of phenol with guanidinium thiocyanate which enables a quick isolation of total and small RNA from biological samples without liquid phase separation. NucleoZOL isolates pure and undegraded total RNA (small and large RNA) that is ready for downstream applications.

For research use only.

## Storage conditions and preparation of working solutions

NucleoZOL can be stored at 15 – 25 °C and is stable until: see package label.

## Safety instructions

When working with the NucleoZOL kit 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 *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 the 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 treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

QR-Code Safety Data Sheet

Please find a digital version of the safety data sheets by following the link below:

*www.mn-net.com/sds*

## Disposal

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

## Protocol

Please read the detailed protocol if using NucleoZOL for the first time. Download the detailed NucleoZOL user manual from the corresponding webpage *www.mn-net.com/NucleoZOL*. Experienced users may refer to this short instruction manual.

## Before starting the preparation:

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

- Reagents: RNase-free water, 75 % ethanol, 70 % isopropanol, 100 % isopropanol.
- Consumables: 1.5 mL, 2.0 mL, or 15 mL centrifuge tubes (depending on 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.

## Isolation of total RNA (small and large RNA in one fraction)

This protocol is used for the isolation of total RNA, containing small RNA (< 200 nt) and large RNA (> 200 nt) in one fraction. This protocol describes the isolation of RNA with 500 µL NucleoZOL. The procedure can be scaled up or down, dependent on the sample input.

## Lyse sample

### Tissue

Homogenize 50 mg tissue/500 µL NucleoZOL with e.g., a rotor-statorhomogenizer.

### Cells

Homogenize up to 5 × $10^6$ cells/500 µL NucleoZOL by pipetting.

### Liquids

Homogenize 200 µL liquid sample/500 µL NucleoZOL by pipetting.

### Precipitate contaminants

Add 200 µL water/500 µL NucleoZOL to the lysate.

Mix vigorously for 15 s and incubate 5 min at RT.

Centrifuge for 15 min at 12,000 × *g*.

### Precipitate total RNA

Transfer 500 µL of the supernatant to a new tube. Add 500 µL 100 % isopropanol. Incubate for 10 min at RT. Centrifuge for 10 min at 12,000 × *g*. Discard the supernatant.

### Wash total RNA

Add 500 µL 75 % ethanol to the pellet.

Centrifuge for 3 min at 8,000 × *g* and discard supernatant. Repeat washing. Drying of the pellet is not necessary.

### Reconstitute RNA

Add an appropriate volume of water to reconstitute the total RNA to approx. 1 µg/µL. Dissolve RNA pellets by vortexing at room temperature for 3 min.

### Isolation of small and large RNA in two separate fractions

This protocol is used for the isolation of small RNA (RNA < 200 nt) and large RNA (RNA > 200 nt) in two separate fractions. This protocol describes the isolation of RNA with 500 µL NucleoZOL. The procedure can be scaled up or down, dependent on the sample input.

## Lyse sample

### Tissue

Homogenize 50 mg tissue/500 µL NucleoZOL with e.g., a rotor-statorhomogenizer.

### Cells

Homogenize up to 5 × $10^6$ cells/µL NucleoZOL by pipetting.

### Liquids

Homogenize 200 µL liquid sample/500 µL NucleoZOL by pipetting.

### Precipitate contaminants

Add 200 µL water/500 µL NucleoZOL to the lysate.

Mix vigorously for 15 s and incubate 5 min at RT.

Centrifuge for 15 min at 12,000 × *g*.

### Precipitate large RNA

Transfer 500 µL supernatant to a new tube.

Add 200 µL 75 % ethanol. Incubate 10 min at RT. Centrifuge for 8 min at 12,000 × *g*.

Continue with the pellet for large RNA isolation. Transfer supernatant to a new tube for miRNA isolation.

### Precipitate small RNA

Add 500 µL of 100 % isopropanol (~0.8 vol) to the supernatant of the previous step. Incubate at 4 °C for 30 min.

Centrifuge for 15 min at 12,000 × *g*. Discard the supernatant.

### Wash large RNA

Add 500 µL 75 % ethanol to the pellet of the large RNA preparation.

Centrifuge for 3 min at 8,000 × *g* and discard supernatant. Repeat washing.

Drying of the pellet is not necessary.

### Wash small RNA

Add 500 µL 70 % isopropanol to the pellet of small RNA preparation.

Centrifuge for 3 min at 8,000 × *g* and discard supernatant. Repeat washing. Drying of the pellet is not necessary.

### Reconstitute RNA

Dissolve the RNA pellet in RNase-free water to obtain an RNA concentration of 1 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 RT.

## 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.

Last updated: 12/2023, Rev. 03

## Contact MN

### Germany and international

MACHEREY-NAGEL GmbH & Co. KG
Valencienner Str. 11 · 52355 Düren · Germany
Tel.: +49 24 21 969-0
Toll-free: 0800 26 16 000 (Germany only)
E-mail: info@mn-net.com

Technical support Bioanalysis

Tel.: +49 24 21 969-333
E-mail: support@mn-net.com

### USA

MACHEREY-NAGEL Inc.
924 Marcon Blvd. · Suite 102 · Allentown PA, 18109 · USA
Toll-free: 888 321 6224 (MACH)
E-mail: sales-us@mn-net.com

### France

MACHEREY-NAGEL SAS
1, rue Gutenberg – BP135 · 67720 Hoerdt Cedex · France
Tel.: +33 388 68 22 68
E-mail: sales-fr@mn-net.com

MACHEREY-NAGEL SAS (Société par Actions Simplifiée) au capital de 186600 €
Siret 379 859 531 00020 · RCS Strasbourg B379859531
N° intracommunautaire FR04 379 859 531

### Switzerland

MACHEREY-NAGEL AG
Hirsackerstr. 7 · 4702 Oensingen · Switzerland
Tel.: +41 62 388 55 00
E-mail: sales-ch@mn-net.com

A074779 / 0140.3