

NucleoZOL

1. Contents

NucleoZOL reagent 740404.6 / .200

2. 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.

3. Storage conditions and preparation of working solutions

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

4. 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.

Disposal

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

5. 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.

5.1 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-stator-homogenizer.

Cells

Homogenize up to **5 × 10⁶ 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 x 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 x g**. Discard the supernatant.

Wash total RNA

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

Centrifuge for **3 min** at **8,000 x 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**.

5.2 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-stator-homogenizer.

Cells

Homogenize up to **5 × 10⁶ 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 x 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 x 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 x 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 x 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 x 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**.

6. Product use restrictions / 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 customer and are not a part of a contract of sale or of this warranty.

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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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