

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

NucleoZOL is developed, designed, and sold for research purposes only. We offer a one year warranty from date of delivery that our products will conform to applicable specifications set forth in the product specifications if not sold to persons set forth in § 13 of German Civil Code. In such a case, the provisions of the German Civil Code shall be valid. This warranty does not cover defects in or damage to the products which are due to improper installation or maintenance, misuse, neglect or any use other than ordinary commercial application. The seller shall not be liable for the goods being fit for a particular purpose unless otherwise agreed upon, to which the buyer intends to put them. This warranty is strictly exclusive. 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; REPRODUCIBILITY; DURABILITY; FITNESS FOR A PARTICULAR PURPOSE OR USE; MERCHANTABILITY; CONDITION; OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS OR THE SALE OF MACHEREY-NAGEL PRODUCTS. If not superseded by these terms the current terms and conditions for the sale of goods (export version) are additionally valid.

Patents:

US Patents 7,794,932 and 8,367,817 B2 and international patent

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NucleoZOL

1 Contents

NucleoZOL		
REF	740404.6	740404.200
NucleoZOL reagent	6 mL	200 mL

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.

3 Storage conditions and preparation of working solutions

NucleoZOL can be stored at 15–25 °C and is stable for at least one year.


4 Safety instructions

NucleoZOL contains hazardous contents. Wear gloves and goggles!

GHS classification

Only harmful features must not 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
Inhalt	Gefahrstoff	GHS-Symbol	H-Sätze	P-Sätze
NucleoZOL	Phenol 30–60 % and guanidinium thiocyanate 30–60 % <i>Phenol 30–60 % und Guanidinthiocyanat 30–60 %</i> CAS 108-95-2, 593-84-0	 DANGER GEFAHR	301, 311, 314, 331, 341, 373, 412, EUH031	201, 202, 260, 273, 280, 301+310, 301+330+331, 302+352, 303+361+353, 304+340, 305+351+338, 308+313, 311, 361+364, 405, 501

Hazard phrases

H301	Toxic if swallowed. <i>Giftig bei Verschlucken.</i>
H311	Toxic in contact with skin. <i>Giftig bei Hautkontakt.</i>
H314	Causes severe skin burns and eye damage. <i>Verursacht schwere Verätzungen der Haut und schwere Augenschäden.</i>
H331	Toxic if inhaled. <i>Giftig bei Einatmen.</i>
H341	Suspected of causing genetic defects. <i>Kann vermutlich genetische Defekte verursachen.</i>
H373	May cause damage to organs through prolonged or repeated exposure. <i>Kann die Organe schädigen bei längerer oder wiederholter Exposition.</i>
H 412	Harmful to aquatic life with long lasting effects. <i>Schädlich für Wasserorganismen, mit langfristiger Wirkung.</i>
EUH031	Contact with acids liberates toxic gas. <i>Entwickelt bei Berührung mit Säure giftige Gase.</i>

Precaution phrases

P201	Obtain special instructions before use. <i>Vor Gebrauch besondere Anweisungen einholen.</i>
P202	Do not handle until all safety precautions have been read and understood. <i>Vor Gebrauch alle Sicherheitshinweise lesen und verstehen.</i>
P260	Do not breathe vapors. <i>Dampf nicht einatmen.</i>
P273	Avoid release to the environment. <i>Freisetzung in die Umwelt vermeiden</i>
P280	Wear protective gloves / eye protection. <i>Schutzhandschuhe / Augenschutz tragen.</i>
P301+310	IF SWALLOWED: Immediately call a POISON CENTER / doctor / ... <i>BEI VERSCHLUCKEN: Sofort GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.</i>
P301+330+331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. <i>BEI VERSCHLUCKEN: Mund ausspülen. KEIN Erbrechen herbeiführen.</i>
P302+352	IF ON SKIN: Wash with plenty of water / ... <i>BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser / ... waschen.</i>
P303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower. <i>BEI BERÜHRUNG MIT DER HAUT (oder dem Haar): Alle kontaminierten Kleidungsstücke sofort ausziehen. Haut mit Wasser abwaschen / duschen.</i>
P304+340	IF INHALED: Remove person to fresh air and keep comfortable for breathing. <i>BEI EINATMEN: Die Person an die frische Luft bringen und für ungehinderte Atmung sorgen.</i>
P305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. <i>BEI BERÜHRUNG MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen.</i>
P308+313	IF exposed or concerned: Get medical advice / attention. <i>BEI Exposition oder falls betroffen: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.</i>
P311	Call a POISONCENTER / doctor / ... <i>GIFTINFORMATIONSZENTRUM / Arzt / ... anrufen.</i>
P361+364	Take off immediately all contaminated clothing and wash it before reuse. <i>Alle kontaminierten Kleidungsstücke sofort ausziehen und vor erneutem Tragen waschen.</i>
P405	Store locked up. <i>Unter Verschluss aufbewahren.</i>
P501	Dispose of contents / container to ... <i>Inhalt / Behälter ... zuführen.</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).

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 **5x10⁶ 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 **5x10⁶ 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.