

## NucleoSpin® RNA Set for NucleoZOL

### 1 Contents

| NucleoSpin® RNA Set for NucleoZOL                                  |                       |                       |
|--|-----------------------|-----------------------|
| REF  | 10 preps<br>740406.10 | 50 preps<br>740406.50 |
| Binding Buffer MX  | 13 mL                 | 60 mL                 |
| Wash Buffer RA3 (concentrate)                                      | 6 mL                  | 12 mL                 |
| RNase-free H <sub>2</sub> O  | 13 mL                 | 13 mL                 |
| NucleoSpin® RNA Columns (light blue rings – plus Collection Tubes) | 10                    | 50                    |
| Collection Tubes (2 mL)  | 10                    | 50                    |
| Collection Tubes (1.5 mL)  | 10                    | 50                    |
| Leaflet  | 1                     | 1                     |

### 2 Product description

**NucleoSpin® RNA Set for NucleoZOL** is designed for combining the one-phase RNA isolation reagent NucleoZOL with silica spin columns. After lysing the sample in NucleoZOL and pelleting contaminating DNA and proteins, the supernatant is mixed with an optimized binding buffer for efficient binding of small and large RNA to the silica membrane. A convenient bind-wash-elute procedure allows highly efficient isolation of pure RNA (small and large RNA).

*Attention: NucleoSpin® RNA Set for NucleoZOL can only be used in combination with the NucleoZOL reagent (REF 740404.200).*

### 3 Storage conditions and preparation of working solutions

**NucleoSpin® RNA Set for NucleoZOL** can be stored at room temperature (15–25 °C) and is stable until: see package label.

**Wash Buffer RA3:** Add 24 mL of 96–100 % ethanol to 6 mL Wash Buffer RA3 Concentrate (740406.10) or add 48 mL of 96–100 % ethanol to 12 mL Wash Buffer RA3 Concentrate (740406.50).

### 4 Safety instructions

**NucleoSpin® RNA Set for 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   |
| MX        | 1,4-dioxane 90–100 %<br>1,4-Dioxan 90–100 %<br><br>CAS 123-91-1 | <br><br>GEFAHR<br>DANGER | H225,<br>H319,<br>H335,<br>H351 | P201, P210,<br>P261sh,<br>P280sh,<br>P403 + 233 |

**Hazard phrases**

- H225                    Highly flammable liquid and vapour.  
*Flüssigkeit und Dampf leicht entzündbar.*
- H319                    Causes serious eye irritation.  
*Verursacht schwere Augenreizung.*
- H335                    May cause respiratory irritation.  
*Kann die Atemwege reizen.*
- H351                    Suspected of causing cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard).  
*Kann vermutlich Krebs erzeugen (Expositionsweg angeben, sofern schlüssig belegt ist, dass diese Gefahr bei keinem anderen Expositionsweg besteht).*

**Precaution phrases**

- P201                    Obtain special instructions before use.  
*Vor Gebrauch besondere Anweisungen einholen.*
- P210                    Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.  
*Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
- P261sh                Avoid breathing dust/vapors.  
*Einatmen von Staub/Dampf vermeiden.*
- P280sh                Wear protective gloves / eye protection.  
*Schutzhandschuhe / Augenschutz tragen.*
- P403 + 233            Store in a well-ventilated place. Keep container tightly closed.  
*An einem gut belüfteten Ort aufbewahren. Behälter dicht verschlossen halten.*

*For further information please see Material Safety Data Sheets ([www.mn-net.com](http://www.mn-net.com)). Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ([www.mn-net.com](http://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 Isolation of total RNA**

This protocol is used for the isolation of total RNA, containing small RNA (< 200 nt) and large RNA (> 200 nt) in one fraction.

Homogenization / Lysis

For homogenization of the sample follow the instructions of the NucleoZOL user manual.

**Tissue**

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

**Cells**

Use up to **5 × 10<sup>6</sup> cells** with **500 µL NucleoZOL** per preparation.

**Liquid samples**

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

Precipitate contaminants

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

*Add 0.4 vol of RNase-free water/mL NucleoZOL used for homogenization.*

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

*Centrifugation can be performed at 4–28 °C. 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.*

#### Adjust RNA binding conditions

Add **500 µL Buffer MX** to the sample homogenate and **mix** by vortexing.

*Add 1 volume of Buffer MX per 1 mL NucleoZOL used for sample homogenization.*

#### Bind RNA

For each preparation, use one NucleoSpin® RNA Column 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 loading step if larger volumes are processed.*

#### Wash and dry silica membrane

##### **1<sup>st</sup> 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.

##### **2<sup>nd</sup> 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.*

#### Elute RNA

Add **60 µL RNase-free H<sub>2</sub>O** onto the center of the membrane and centrifuge for **1 min** at **11,000 x g**.

## **6 Product use restrictions / warranty**

**NucleoSpin® RNA Set for 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.