



Genomic DNA from lipid-rich tissue

User manual

NucleoSpin[®] DNA Lipid Tissue

This product distributed by
Takara Bio USA, Inc.

Ordering information
1.800.662.2566
ordersUS@takarabio.com

Technical support
1.800.662.2566
techUS@takarabio.com



takarabio.com

For research use only. Not for resale. Unless otherwise indicated, all trademarks are the property of Takara Bio Inc. ©2017

February 2016 / Rev. 01

Genomic DNA from lipid-rich tissue

Protocol-at-a-glance (Rev.01)

NucleoSpin® DNA Lipid Tissue


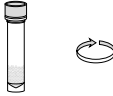
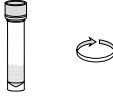
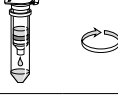
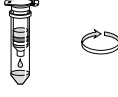
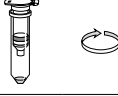

| | | |
|------------------------------------|--|---|
| 1 Prepare sample |  | < 40 mg lipid tissue (wet weight) 100 µL Buffer BE |
| 2 Lyse sample |  | 40 µL Buffer LT 10 µL Liquid Proteinase K Agitate on MN Bead Tube Holder for approx. 20 min 11,000 x g, 30 s |
| 3 Adjust binding conditions |  | 600 µL LT Mix 11,000 x g, 30 s |
| 4 Bind DNA |  | Load samples 11,000 x g, 30 s |
| 5 Wash silica membrane |  | 1st 500 µL BW 11,000 x g, 30 s 2nd 500 µL B5 11,000 x g, 30 s |
| 6 Dry silica membrane |  | 11,000 x g, 30 s |
| 7 Elute DNA |  | 100 µL BE RT, 1 min 11,000 x g, 30 s |

Table of contents

| | | |
|-------|--|----|
| 1 | Components | 4 |
| 1.1 | Kit contents | 4 |
| 1.2 | Reagents, consumables, and equipment to be supplied by user | 5 |
| 1.3 | About this user manual | 5 |
| 2 | Product description | 6 |
| 2.1 | The basic principle | 6 |
| 2.2 | Kit specifications | 6 |
| 2.3 | Handling, preparation, and storage of starting materials | 6 |
| 2.4 | Lysis and disruption of sample material | 7 |
| 2.4.1 | Disruption device | 7 |
| 2.4.2 | Type of bead tube | 7 |
| 2.4.3 | Time and frequency of disruption | 7 |
| 2.5 | Elution procedures | 9 |
| 3 | Storage conditions and preparation of working solutions | 9 |
| 4 | Safety instructions | 10 |
| 5 | Protocols | 12 |
| 5.1 | Protocol for fresh or frozen lipid-rich tissue samples | 12 |
| 5.2 | Protocol for purification of DNA from hard-to-lyse bacteria in (lipid-rich) tissue samples | 15 |
| 6 | Appendix | 16 |
| 6.1 | Troubleshooting | 16 |
| 6.2 | Ordering information | 17 |
| 6.3 | Product use restriction/warranty | 18 |

1 Components

1.1 Kit contents

| NucleoSpin® DNA Lipid Tissue | | |
|---|-----------------------|-----------------------|
| REF | 10 preps 740471.10 | 50 preps 740471.50 |
| Lysis Buffer LT | 10 mL | 38 mL |
| Wash Buffer BW | 6 mL | 30 mL |
| Wash Buffer B5 (Concentrate)* | 6 mL | 6 mL |
| Elution Buffer BE** | 13 mL | 30 mL |
| Liquid Proteinase K | 120 µL | 600 µL |
| NucleoSpin® Bead Tubes Type D | 10 | 50 |
| NucleoSpin® DNA Lipid Tissue Columns (light green rings) | 10 | 50 |
| Collection Tubes (2 mL) | 20 | 100 |
| User manual | 1 | 1 |

* For preparation of working solutions and storage, see section 3

** Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

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

Reagents

- 96–100 % ethanol (for preparation of Wash Buffer B5)

Consumables

- Disposable tips
- 1.5 mL or 2 mL microcentrifuge tubes for elution

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)
- Sample disruption device:
The MN Bead Tube Holder (REF 740469, see ordering information, section 6.2) is recommended to be used in combination with the Vortex-Genie® 2 for cost efficient and convenient disruption of lipid tissue samples. The Vortex Adapter (MoBio) for Vortex-Genie® 2 X is also suitable.

Alternatively, a swing mill can be used considering precautions of section 2.4.3 (e.g., mixer mill MM200, MM300, MM400 (Retsch®)).

WARNING: The use of other disruption devices like FastPrep® System (MP-Biomedicals), Precellys® (Bertin Technologies), MagNA™ Lyser (Roche), TissueLyser (QIAGEN), Bullet Blender® (Next Advance), Mini-Beadbeater™ (Biospec Products), Speed Mill (Analytik Jena), or similar devices might cause bead tube destruction. Such disruption devices can cause high mechanical stress on the bead tubes. Depending on bead tube type and content (beads like steel balls, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause destruction of the bead tubes. **If using such a disruption device, it is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes during the individual experimental setup (e.g., intensity of agitation).** See also section 2.4.3!

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the **NucleoSpin® DNA Lipid Tissue** kit before using this product. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available online at www.mn-net.com.

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

2 Product description

2.1 The basic principle

The **NucleoSpin® DNA Lipid Tissue** kit is designed for efficient isolation of genomic DNA from lipid-rich samples of human / animal origin. DNA can be isolated from a wide variety of fresh or frozen samples, such as brain, adipose tissue, and other tissue types that are rich in lipids, like fatty fish tissue.

Lipid-rich tissue can cause difficulties in DNA isolation due to lipids interfering with tissue disruption or by influencing the chemistry of the DNA isolation buffers.

The **NucleoSpin® DNA Lipid Tissue** kit combines enzymatic lysis and mechanical disruption of lipid-rich tissues with the NucleoSpin® Bead Tubes. The NucleoSpin® Bead Tubes can be used in combination with the MN Bead Tube Holder (REF 740469) and the Vortex-Genie® 2. They are also compatible with other disruption devices (see section 1.2 and section 2.4.1). High DNA yields can be obtained with the NucleoSpin® Bead Tubes from a large variety of sample types – enabling the procedure to be convenient, fast, and easy. Alternative bead types can be ordered separately for selected sample types (see section 2.4.3 for recommendations).

2.2 Kit specifications

Table 1: Kit specifications at a glance

| Parameter | NucleoSpin® DNA Lipid Tissue |
|---------------------|---|
| Technology | Silica-membrane technology |
| Format | Mini spin column |
| Sample material | Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue) |
| Sample amount | Up to approx. 40 mg (wet weight) |
| Typical yield | Varies by sample and disruption device. Up to 25 µg DNA can be obtained. |
| A_{260} / A_{280} | 1.7–1.9 |
| Elution volume | 25–200 µL |
| Preparation time | 35 min/6 preps |
| Binding capacity | 60 µg |

2.3 Handling, preparation, and storage of starting materials

Fresh or frozen tissue samples. Make sure not to use more than 40 mg starting material.

2.4 Lysis and disruption of sample material

In order to obtain optimal DNA yields, a complete disruption of the sample material is essential. The efficiency of sample disruption depends on the parameters listed below and suggestions for optimization are outlined in the subsequent sections.

2.4.1 Disruption device

The following devices are compatible with NucleoSpin® Bead Tubes. Please check whether NucleoSpin® Bead Tubes can be accommodated by the available tube adapters prior to starting the procedure.

- MN Bead Tube Holder in combination with the Vortex-Genie® 2 (recommended).
- Mixer mill MM200, MM300, MM400 (Retsch®) (suitable).

If other disruption devices (section 1.2) are intended to be used, consider section 2.4.2 and WARNING note in section 2.4.3!

2.4.2 Type of bead tube

Bead type, disruption time, and frequency / speed must be optimized for a given sample to obtain maximal DNA yield and quality.

Type of bead tube

- NucleoSpin® Bead Tubes Type D (3 mm steel balls)
Recommended for lipid-rich tissue samples (included in NucleoSpin® DNA Lipid Tissue kit).

Other types of bead tubes are available for other applications:

- NucleoSpin® Bead Tubes Type A (0.6–0.8 mm ceramic beads)
Recommended for soil and sediment (included in NucleoSpin® Soil, see ordering information, section 6.2).
- NucleoSpin® Bead Tubes Type B (40–400 µm glass beads)
Recommended for gram-positive and -negative bacteria (included in NucleoSpin® Microbial DNA, see ordering information, section 6.2).
- NucleoSpin® Bead Tubes Type C (1–3 mm corundum)
Recommended for yeast (see ordering information, section 6.2).
- NucleoSpin® Bead Tubes Type E (combination of 3 mm steel balls and 40–400 µm glass beads)
Recommended for difficult-to-lyse tissue containing gram-positive bacteria (see ordering information, section 6.2).

2.4.3 Time and frequency of disruption

The following recommendations have been established for the MN Bead Tube Holder in combination with a Vortex-Genie® 2 or a Retsch® Swingmill MM300 operating at highest frequency (30 Hertz). For using other disruption devices, and other sample materials, time and frequency have to be optimized.

| Sample material | Disruption device | Disruption time | Speed / intensity / frequency |
|--|---|-------------------------|-------------------------------|
| Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue) | MN Bead Tube Holder in conjunction with a Vortex-Genie® 2 | approximately 20 min | full speed |
| Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue) | Mixer mill (Retsch®) | approximately 0.5–5 min | 30 Hz |
| Fresh or frozen, lipid-rich tissues (e.g., brain, adipose tissue, fatty fish tissue) | other device | to be optimized by user | see recommendations below |

***Note:** Stability testing has been performed on the NucleoSpin® Bead Tubes Type D with the MN Bead Tube Holder on a Vortex-Genie® 2 and with a mixer mill MM300 (Retsch®) at highest frequency (30 Hertz). NucleoSpin® Bead Tubes Type D withstand shaking for several hours in the MN Bead Tube Holder on a Vortex-Genie® 2 and for up to 30 minutes on a mixer mill MM300 (Retsch®) at highest frequency (30 Hertz). For optimal sample processing, avoidance of DNA fragmentation, and highest DNA yield, see table above for recommendations of adequate disruption times. Other disruption devices (see section 2.4.1) will require different settings regarding frequency and duration for optimal performance with the selected sample material. Please note that the position of the tube within the machine (mixer mill, Retsch®) is important for optimal performance! Please refer to the user manual of the disruption device.*

WARNING: Many disruption devices (see section 1.2) can cause high mechanical stress on the bead tubes. Depending on bead tube type and content (beads, liquid volume, sample type), especially high frequency of shaking and/or long shaking duration can cause breaking-up of the bead tubes. **It is the responsibility of the user to perform initial stability tests to ensure stability of bead tubes during the individual experimental setup!**

These tests should be performed with water instead of lysis buffer in order to avoid spillage of chaotropic lysis buffer in case of tube breakage. Integrity and tightness of the tube need to be controlled after every run.

WARNING: In section 5 a certain liquid volume during disruption is recommended. The reduction of liquid content will severely increase the mechanical impact by the steel balls, and can result in damage of DNA and tube.

2.5 Elution procedures

In addition to the standard method, several modifications are possible to increase yield, concentration, and convenience.

- **Convenient elution (standard elution):** Elution can be performed by a single addition of 100 µL Elution Buffer onto the column.
- **High yield:** Elution can be performed in two serial elution steps of 100 µL each, resulting in a total volume of 200 µL.
- **High concentration:** Elution can be performed by application of 100 µL Elution Buffer, which is then re-used in a second elution step, resulting in 100 µL eluate with high DNA concentration. Alternatively, the elution volume can be reduced down to 25 µL.

3 Storage conditions and preparation of working solutions

Attention:

Lysis Buffer LT and Wash Buffer BW contain chaotropic salt! Wear gloves and goggles!

CAUTION: Buffers LT and BW contain chaotropic salts which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waster!

All kit components can be stored at room temperature (18–25 °C) and are stable for at least one year.

Before starting any **NucleoSpin® DNA Lipid Tissue** protocol, prepare the following:

- **Wash Buffer B5:** Add the indicated volume of ethanol (96–100 %) to **Wash Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer B5 can be stored at room temperature (18–25 °C) for at least one year.
- **Liquid Proteinase K** is ready to use. After first time use, store Liquid Proteinase K at 4 °C or – 20 °C.




| NucleoSpin® DNA Lipid Tissue | | |
|------------------------------|---------------------------|---------------------------|
| REF | 10 preps 740471.10 | 50 preps 740471.50 |
| Wash Buffer B5 (Concentrate) | 6 mL Add 24 mL ethanol | 6 mL Add 24 mL ethanol |

4 Safety instructions

The following components of the **NucleoSpin® DNA Lipid Tissue** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Only harmful features do not need to 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 |
| LT | Guanidinium thiocyanate 30–60 % <i>Guanidinthiocyanat 30–60 %</i> CAS 593-84-0 |  WARNING ACHTUNG | 302, 412, EUH031 | 260, 273, 301+312, 330 |
| BW | Guanidinium hydrochloride 36–50 % and 2-propanol 20–50 % <i>Guanidinhydrochlorid 36–50 % und 2-Propanol 20–50 %</i> CAS 50-01-1, 67-63-0 |  WARNING ACHTUNG | 226, 302, 319, 336 | 210, 233, 264, 280, 301+312, 305+351+338, 330, 337+313, 370+378, 403+235 |
| Liquid Proteinase K | Proteinase K, liquid 1–3 % <i>Proteinase K, flüssig 1–3 %</i> CAS 39450-01-6l |  WARNING ACHTUNG | 317 | 261, 272, 280, 302+352, 333+313, 363 |

Hazard phrases

| | |
|--------|---|
| H 226 | Flammable liquid and vapour. <i>Flüssigkeit und Dampf entzündbar.</i> |
| H 302 | Harmful if swallowed. <i>Gesundheitsschädlich bei Verschlucken.</i> |
| H 317 | May cause an allergic skin reaction. <i>Kann allergische Hautreaktionen verursachen.</i> |
| H 319 | Causes serious eye irritation. <i>Verursacht schwere Augenreizung.</i> |
| H 336 | May cause drowsiness or dizziness. <i>Kann Schläfrigkeit und Benommenheit verursachen.</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

- P 210 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.
- P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.
- P 260 Do not breathe dust/fume/gas/mist/vapours/spray.
Staub/Rauch/Gas/Nebel/Dampf/Aerosol nicht einatmen.
- P 261 Avoid breathing dust/fume/gas/mist/vapours/spray.
Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden.
- P 264 Wash ... thoroughly after handling.
Nach Handhabung ... gründlich waschen.
- P 272 Contaminated work clothing should not be allowed out of the workplace.
Kontaminierte Arbeitskleidung nicht außerhalb des Arbeitsplatzes tragen.
- P 273 Avoid release to the environment.
Freisetzung in die Umwelt vermeiden.
- P 280 Wear protective gloves/protective clothing/eye protection/face protection.
Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen.
- P 301+312 IF SWALLOWED: Call a POISON CENTER/ doctor/.../ if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM/Arzt/... anrufen.
- P 302+352 IF ON SKIN: Wash with plenty of water/...
BEI BERÜHRUNG MIT DER HAUT: Mit viel Wasser/... waschen.
- P 305+351+338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser ausspülen. Eventuell vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.
- P 330 Rinse mouth.
Mund ausspülen.
- P 333+313 If skin irritation or rash occurs: Get medical advice/attention.
Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.
- P 337+313 If eye irritation persists: Get medical advice/attention.
Bei anhaltender Augenreizung: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen.
- P 363 Wash contaminated clothing before reuse.
Kontaminierte Kleidung vor erneutem Tragen waschen.
- P 370+378 In case of fire: Use ... to extinguish.
Bei Brand: ... zum Löschen verwenden.
- P 403+235 Store in a well-ventilated place. Keep cool.
An einem gut belüfteten Ort aufbewahren. Kühl halten.

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (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 Protocols

5.1 Protocol for fresh or frozen lipid-rich tissue samples

Before starting the preparation:

- Check if Buffer B5 was prepared according to section 3.
- Check section 2.4 for lysis and disruption of sample material.

1 Prepare sample

Place the lipid-rich tissue sample in a **NucleoSpin® Bead Tube Type D** (provided).

Up to approximately 40 mg of wet weight tissue sample can be processed.

Add **100 µL Elution Buffer BE** to the sample.

Alternatively, molecular biology-grade water (not provided) can be used.



+ 100 µL BE

2 Lyse sample

Add **40 µL Buffer LT**. Then, add **10 µL Liquid Proteinase K** and close the tube.



+ 40 µL LT
+ 10 µL Liquid Proteinase K

Agitate the NucleoSpin® Bead Tube in the MN Bead Tube Holder on a Vortex-Genie® 2. Alternatively a swing mill (Retsch®) can be used (see section 2.4.3).

Agitate

***Note:** Optimal agitation duration, speed/frequency depends on the device used. For the MN Bead Tube Holder it is approximately 20 min; in a mixer mill MM200, MM300, MM400 (Retsch®), e.g., 0.5–5 min at maximal frequency (30 Hertz) is suitable (see section 2.4). On a swing mill, position of the tube in the mill can considerably influence the result. Please refer to the instruction manual of the device used. Respect warnings in section 1.2 and 2.4.3 if other devices are intended to be used!*

Centrifuge the NucleoSpin® Bead Tube **30 s** at **11,000 x g** to clean the lid.



**11,000 x g,
30 s**

Note: In this step foam is removed from the screw cap to allow clean opening of the tube.

Note: Depending on the fat amount of the sample, a fatty layer might form on top of the lysate. This layer does not have to be removed.

Attention: Do neither centrifuge at higher g-force, nor longer because this might damage the NucleoSpin® Bead Tubes.

3 Adjust DNA binding conditions

Add **600 µL Buffer LT** and **mix** (e.g., vortex for 3 s).



**+ 600 µL LT
Mix**

Note: Steel balls should be agitated in the tube; some residual pellet (cell debris) may remain on the bottom of the tube.

Centrifuge for **30 s** at **11,000 x g**.



**11,000 x g,
30 s**

Note: This centrifugation step is performed in order to clean the lid and sediment beads and cell debris. Depending on the fat amount of sample, fatty layer might form on top of the liquid. This fat layer is typically observed with adipose tissue but not with brain or fatty fish tissue.

Attention: Do neither centrifuge at higher g-force, nor longer because this might damage the NucleoSpin Bead Tube.

Alternatively, the lysate can be transferred without steel balls to a fresh and transparent centrifugation tube (not provided) before centrifugation in order to simplify supernatant transfer in step 5.

4 Bind DNA

Transfer the cleared liquid supernatant (~500–600 µL) onto the **NucleoSpin® DNA Lipid Tissue Column**, placed in a 2 mL Collection Tube (provided).

Note: Do not transfer the semi-liquid to soft-solid fatty layer typically observed with adipose tissue samples. Pierce the fatty layer with the pipet tip in order to aspirate the cleared liquid. The fatty matter can stay within the tube and/or will partially stick to the outside of the pipet tip.

Centrifuge for **30 s** at **11,000 x g**. Discard Collection Tube with flow through. Put column into a fresh Collection Tube (2 mL, provided).



Load samples



**11,000 x g,
30 s**

5 Wash silica membrane**1st wash**

Add **500 µL Buffer BW**. Centrifuge for **30 s** at **11,000 x g**. Discard flow-through and place the column back into the Collection Tube.

2nd wash

Add **500 µL Buffer B5** to the column and centrifuge for **30 s** at **11,000 x g**. Discard flow-through and place the column back into the Collection Tube.



+ 500 µL BW

**11,000 x g,
30 s**



+ 500 µL B5

**11,000 x g,
30 s**

6 Dry silica membrane

Centrifuge the column for **30 s** at **11,000 x g**.

Note: Residual wash buffer is removed in this step.



**11,000 x g,
30 s**

**7 Elute highly pure DNA**

Place the NucleoSpin® DNA Lipid Tissue Column into a 1.5 mL nuclease-free tube (not provided) and add **100 µL Buffer BE** onto the column. Incubate at room temperature for 1 min.

Centrifuge **30 s** at **11,000 x g**.

For alternative elution procedures see section 2.5.



**100 µL BE
RT, 1 min**



**11,000 x g,
30 s**

5.2 Protocol for purification of DNA from hard-to-lyse bacteria in (lipid-rich) tissue samples

The purification of DNA from hard-to-lyse bacteria (e.g., gram-positive bacteria) in tissue samples can be challenging as disruption of different organisms requires individual mechanical forces.

Therefore, MACHEREY-NAGEL has developed the NucleoSpin® Bead Tubes Type E, which contain 40–400 µm glass beads as well as 3 mm steel balls. NucleoSpin® Bead Tubes Type E can be used according to the protocol described for NucleoSpin® Bead Tubes Type D in section 5.1.

However, the use of NucleoSpin® Bead Tubes Type E is a very harsh method in terms of sample disruption. Please note that processing time on a selected disruption device (e.g., MN Bead Tube Holder or mixer mill (Retsch®)) has to be optimized by the user with regard to sample type, amount, and downstream application. Long disruption duration on high impact machines (e.g., mixer mill (Retsch®)) can cause total DNA loss due to massive DNA fragmentation. Please contact the technical service for further information. **Respect warnings in section 1.2 and 2.4.3 when using NucleoSpin® Bead Tubes Type E.**

6 Appendix

6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|----------------------|---|
| Damaged bead tubes | <i>Unsuitable disruption device or intensity</i> |
| | <ul style="list-style-type: none"> High force disruption devices can damage NucleoSpin® Bead Tubes Type D and E. Respect warnings in section 1.2 and 2.4.3. Use the recommended MN Bead Tube Holder. |
| | <i>Incomplete lysis</i> |
| No or poor DNA yield | <ul style="list-style-type: none"> Adjust lysis conditions (bead tube type, agitation device, duration, or frequency). |
| | <i>Reagents not applied properly</i> |
| | <ul style="list-style-type: none"> Prepare Buffer B5 according to instructions (section 3). |
| Poor DNA quality | <i>Suboptimal elution of DNA from the column</i> |
| | <ul style="list-style-type: none"> For certain sample types, preheat Buffer BE to 70 °C before elution. Apply Buffer BE directly onto the center of the silica membrane. Elution efficiencies decrease dramatically, if elution is done with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer BE (pH 8.5). Especially when expecting high yields from large amounts of material, we recommend elution with 200 µL Buffer BE and incubation of the closed columns in an incubator at 70 °C for 5 min before centrifugation. |
| | <i>High A_{260}/A_{280} ratio</i> |
| Poor DNA quality | <ul style="list-style-type: none"> Ratios > 1.9 can be caused by RNA contamination. Usually, such RNA contamination does not interfere with downstream applications. Depending on sample type, amount, and disruption procedure, preparations might contain small amounts of RNA. If it is necessary to reduce RNA contamination to the lowest possible level, incubate the lysate after the disruption step for 5 min at 70 °C in order to inactivate the Proteinase K. After cooling to room temperature, add 20 µL RNase A (20 mg/mL) and incubate 5 min. Continue with the application of the lysate onto the column. |
| | <i>Reagents not applied properly</i> |
| | <ul style="list-style-type: none"> Prepare Buffer B5 according to instructions (see section 3). |

| Problem | Possible cause and suggestions |
|--|---|
| | <i>Too much sample material used</i> |
| Clogged columns | <ul style="list-style-type: none"> Make sure to centrifuge the lysate after cell disruption in order to sediment beads and cell debris. Only apply cleared supernatant onto the column. |
| | <i>Carry-over of ethanol or salt</i> |
| Suboptimal performance of genomic DNA in enzymatic reactions | <ul style="list-style-type: none"> Make sure to centrifuge ≥ 1 min at 11,000 x g in order to remove all of ethanolic Buffer B5 before eluting the DNA. If, for any reason, the level of Buffer B5 has reached the column outlet after drying, repeat the centrifugation. Do not chill Buffer B5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer B5 to room temperature before use. |
| | <i>Contamination of DNA with inhibitory substances</i> |
| | <ul style="list-style-type: none"> Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer BE. |

6.2 Ordering information

| Product | REF | Pack of |
|--|------------------------|---------------------|
| NucleoSpin® DNA Lipid Tissue | 740471.10 / .50 | 10 / 50 preps |
| NucleoSpin® DNA Insect | 740470.10 / .50 | 10 / 50 preps |
| MN Bead Tube Holder | 740469 | 1 piece |
| NucleoSpin® Soil | 740780.10 / .50 / .250 | 10 / 50 / 250 preps |
| NucleoSpin® Bead Tubes Type A (0.6–0.8 mm ceramic beads recommended for soil and sediments) | 740786.50 | 50 pieces |
| NucleoSpin® Bead Tubes Type B (40–400 µm glass beads recommended for bacteria) | 740812.50 | 50 pieces |
| NucleoSpin® Bead Tubes Type C (1–3 mm corundum recommended for yeasts) | 740813.50 | 50 pieces |
| NucleoSpin® Bead Tubes Type D (3 mm steel balls recommended for insects) | 740814.50 | 50 pieces |

| Product | REF | Pack of |
|--|---------------------|-----------------|
| NucleoSpin® Bead Tubes Type E (40-400 µm glass and 3 mm steel balls recommended for hard-to-lyse bacteria within insect or tissue samples) | 740815.50 | 50 pieces |
| Buffer BE | 740306.100 | 125 mL |
| Buffer B5 Concentrate (for 125 mL Buffer B5) | 740921 | 25 mL |
| Buffer BW | 740922 | 100 mL |
| Liquid Proteinase K | 740396 | 5 mL |
| RNase A | 740505 740505.50 | 100 mg 50 mg |
| Collection Tubes (2 mL) | 740600 | 1000 |

Visit www.mn-net.com for more detailed product information.

6.3 Product use restriction/warranty

NucleoSpin® DNA Lipid Tissue kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for IN VITRO-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for IN VITRO-diagnostic use. Please pay attention to the package of the product. IN VITRO-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR IN VITRO-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

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, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL's sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agent 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 the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07 /2010, Rev. 03

Please contact:

MACHEREY-NAGEL GmbH & Co. KG

Tel.: +49 24 21 969-270

tech-bio@mn-net.com

Trademarks:

Retsch is a registered trademark of Retsch GmbH

FastPrep is a registered trademark of MP Biomedicals, LLC

Precellys is a registered trademark of Bertin Technologies

MagNA Lyser is a trademark of Roche Diagnostics GmbH

Bullet Blender is a registered trademark of Next Advance

Mini-Beadbeater is a trademark of Biospec Products

Vortex-Genie is a registered trademark of Scientific Industries

NucleoSpin is a registered trademark of MACHEREY-NAGEL GmbH & Co KG

All used names and denotations can be brands, trademarks, or registered labels of their respective owner – also if they are not special denotation. To mention products and brands is only a kind of information (i.e., it does not offend against trademarks and brands and can not be seen as a kind of recommendation or assessment). Regarding these products or services we can not grant any guarantees regarding selection, efficiency, or operation.



www.mn-net.com

MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6-8 · 52355 Düren · Germany

DE/International:

Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:

Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:

Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:

Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-mail: sales-us@mn-net.com

