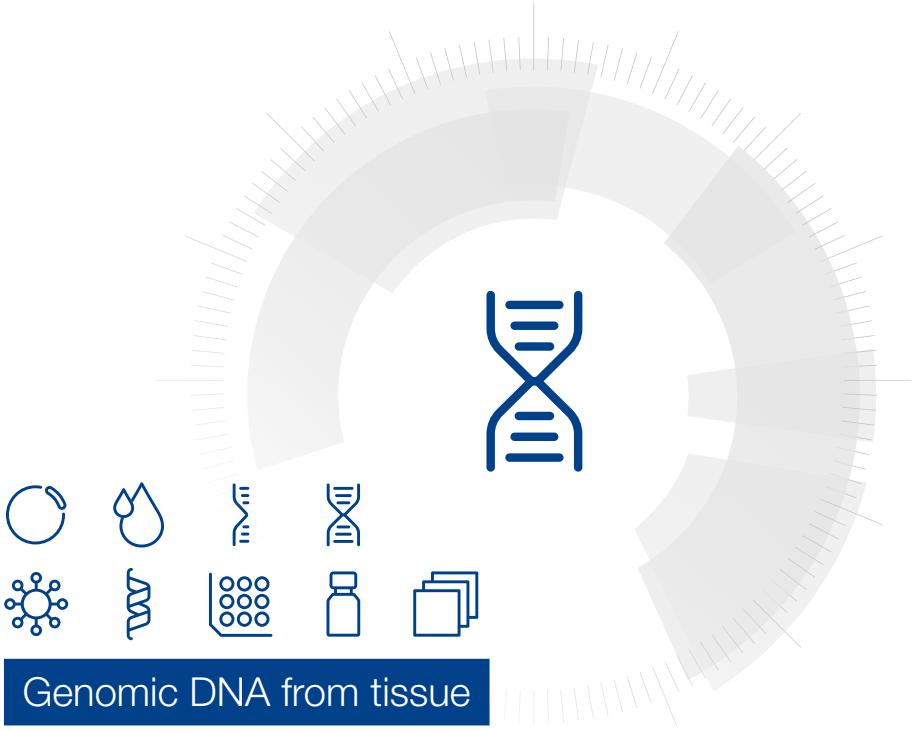
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from tissue

■ NucleoSpin® Tissue

January 2023 / Rev. 20

Genomic DNA from tissue

Protocol at a glance (Rev. 20)

NucleoSpin® Tissue

1	**Prepare sample**		Cut 25 mg into small pieces
2	**Pre-lyse sample**		180 µL T1 25 µL Proteinase K 56 °C, 1–3 h
3	**Lyse sample**		200 µL B3 70 °C, 10 min
4	**Adjust DNA binding conditions**		210 µL 96–100 % ethanol
5	**Bind DNA**	 	Load all 11,000 x *g*, 1 min
6	**Wash silica membrane**	 	1st wash 500 µL BW 2nd wash 600 µL B5
		1st and 2nd 	11,000 x *g*, 1 min
7	**Dry silica membrane**		11,000 x *g*, 1 min
8	**Elute highly pure DNA**	 	100 µL BE RT, 1 min 11,000 x *g*, 1 min

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1 Components

1.1 Kit contents

	NucleoSpin® Tissue		
REF	**10 preps 740952.10**	**50 preps 740952.50**	**250 preps 740952.250**
Lysis Buffer T1	5 mL	20 mL	100 mL
Lysis Buffer B3	10 mL	15 mL	75 mL
Wash Buffer BW	6 mL	30 mL	150 mL
Wash Buffer B5 (Concentrate)*	6 mL	12 mL	50 mL
Elution Buffer BE*	13 mL	13 mL	60 mL
Proteinase K (lyophilized)**	6 mg	30 mg	2 × 75 mg
Proteinase Buffer PB	1.8 mL	1.8 mL	8 mL
NucleoSpin® Tissue Columns (light green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
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* Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

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

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

Reagents

- 96 – 100 % ethanol

Consumables

- 1.5 mL microcentrifuge tubes for sample lysis and DNA elution
- Disposable tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

For support protocols (see section 6) additional material might be required -see individual support protocols for further information.

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Tissue** kit is used for the first time. 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 on the internet at *www.mn-net.com*.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions. Note: Buffer B3 is delivered premixed now.

2 Product description

2.1 The basic principle

With the NucleoSpin® Tissue method genomic DNA can be prepared from tissue, cells (e.g., bacteria), and many other sources. Lysis is achieved by incubation of the sample material in a proteinase K/SDS solution. Appropriate conditions for DNA binding to the silica membrane in the **NucleoSpin® Tissue Columns** are achieved by the addition of chaotropic salts and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by subsequent washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin® Tissue** is designed for the rapid, small-scale preparation of highly pure genomic DNA from any tissue, cells, bacteria, yeast, forensic samples, serum, plasma, or other body fluids. It is also suitable for preparation of DNA from human or animal blood. The purified DNA can be used directly for PCR, Southern blotting, or any kind of enzymatic reactions.
- The kit allows purification of up to 35 µg of pure genomic DNA with an A_{260}/A_{280} ratio between 1.7 and 1.9. The **NucleoSpin® Tissue Column** is capable of binding up to 60 µg of genomic DNA.
- For lysis of certain bacterial and yeast strains, additional enzymes may be necessary which are not part of this kit. See the relevant support protocol for details.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Tissue
Technology	Silica membrane technology
Format	Mini spin column
Sample material	< 25 mg tissue 10^2 – ^{107} cultured cells
Typical yield	20 – 35 µg
Elution volume	60 – 100 µL
Preparation time	20 min/prep (excluding lysis)
Binding capacity	60 µg
Use	For research use only

- **Forensic quality product:**
  NucleoSpin® Tissue is certified as forensic quality product. Consumables used in forensics need to be treated carefully to prevent DNA contamination. MACHEREY-NAGEL therefore has a stringently controlled production process to avoid DNA contamination of consumables. Further, MACHEREY-NAGEL uses ethylene oxide (EO) treatment to remove amplifiable DNA, which might still be introduced during the manufacturing process. MACHEREY-NAGEL products carrying the forensic quality seal, contain plastic materials that are EO treated. This means, DNA of any kind, which might still be introduced into plastic consumables during the production process, is inactivated by means of the treatment with ethylene oxide, in order to prevent the generation of accidental human profile by PCR amplification. Ethylene oxide treatment has been shown to be the method of choice to prevent DNA profiles due to DNA contamination (Shaw et al. 2008; Figure 1).

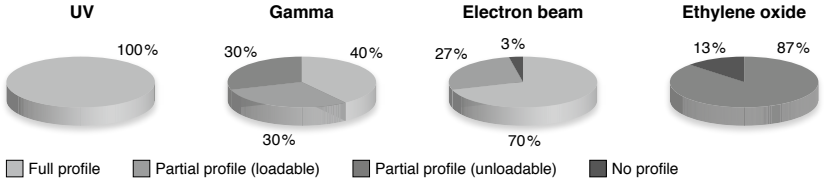

Figure 1 According to Shaw et al., 2008, Comparison of the effects of sterilization techniques on subsequent DNA profiling. Int J Legal Med 122: 29 – 33.

2.3 Elution procedures

In addition to the standard method (recovery rate about 70 – 90 %), several modifications are possible to increase yield, concentration, and convenience. Use elution buffer for one of the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90 – 100 % of bound nucleic acid can be eluted.
- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be approximately 30 % higher than with standard elution. The yield of eluted nucleic acid will be about 80 %.
- **High yield and high concentration:** Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85 – 100 % of bound nucleic acid is eluted in the standard elution volume at a high concentration.
- **Elution at 70 °C:** For certain sample types, heating the elution buffer to 70 °C increases the DNA yield.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi use storage at 4 °C

or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications. Note: Elution Buffer BE (5 mM Tris/HCl, pH 8.5) provided with the kit does not contain EDTA.

For optimal performance of isolated DNA in downstream applications, we recommend eluting with the supplied elution buffer and storage, especially long term, at -20 °C. Freeze-thaw cycles will have no effect on most downstream applications. Possible exceptions are detection of trace amounts of DNA or long-range PCR (e.g., > 10 kbp). Multiple freeze-thaw cycles or storing DNA at 4 °C or room temperature may influence detection sensitivities or reaction efficiencies due to DNA shearing or adsorption to surfaces.

3 Storage conditions and preparation of working solutions

Attention: Buffers B3 and BW contain chaotropic salt! Wear gloves and goggles!

CAUTION: Buffers B3 and BW contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components can be stored at 15 – 25 °C and are stable until: see package label.
- During storage, especially at low temperatures, a white precipitate may form in Buffer T1 or B3. Such precipitates can be easily dissolved by incubating the bottle at 50 – 70 °C before use.

Before starting any **NucleoSpin® 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 15 – 25 °C for at least one year.
- **Proteinase K:** Add the indicated volume of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for at least 6 months.

	NucleoSpin® Tissue		
	10 preps	**50 preps**	**250 preps**
REF	**740952.10**	**740952.50**	**740952.250**
Wash Buffer B5 (Concentrate)	6 mL Add 24 mL ethanol	12 mL Add 48 mL ethanol	50 mL Add 200 mL ethanol
Proteinase K	6 mg Add 260 µL Proteinase Buffer	30 mg Add 1.35 mL Proteinase Buffer	2 × 75 mg Add 3.35 mL Proteinase Buffer to each vial

4 Safety instructions

When working with the **NucleoSpin® Tissue** 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 *http://www.mn-net.com/msds*).

Caution: Guanidine hydrochloride in buffer B3 and buffer BW 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 **NucleoSpin® Tissue** 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 and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

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

5 Standard protocol for human or animal tissue and cultured cells

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Tissue

Cut 25 mg human or animal tissue into small pieces. Place the sample in a microcentrifuge tube (not provided). Proceed with step 2.

Samples that are difficult to lyse can be ground under liquid nitrogen or may be treated in a mechanical homogenizer (Polytron®, Ultra-Turrax®): Add 25 mg of tissue to a 1.5 mL microcentrifuge tube (not provided), add 50 – 75 µL phosphate buffered saline (PBS) and homogenize.

Cultured cells

Resuspend up to **10^7 cells** in a final volume of **200 µL Buffer T1**. Add **25 µL Proteinase K** solution and **200 µL Buffer B3**. Vortex to mix and incubate the sample at 70 °C for 10 – 15 min. **Proceed with step 4.**

2 Pre-lyse sample

Add **180 µL Buffer T1** and **25 µL Proteinase K** solution. Vortex to mix. Be sure that the samples are completely covered with lysis solution.

+180 µL T1

+25 µL Proteinase K

Mix

If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do not mix Buffer T1 and Proteinase K more than 10 – 15 min before addition to the sample: Proteinase K tends to self-digest in Buffer T1 without substrate.

Incubate at **56 °C** until complete lysis is obtained **(at least 1 – 3 h)**. Vortex occasionally during incubation or use a shaking incubator.

Samples can be incubated overnight as well. If RNA-free DNA is crucial for downstream applications, a RNase digest may be performed: Add 20 µL RNase A (10 mg/mL) solution (not included; see ordering information) and incubate for an additional 5 min at room temperature.

56 °C, 1 – 3 h

or

56 °C, overnight

3 Lyse sample

Vortex the samples. Add **200 µL Buffer B3**, vortex vigorously and incubate at **70 °C** for **10 min**. Vortex briefly.

If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a new microcentrifuge tube (not provided).

+200 µL B3

70 °C, 10 min

4 Adjust DNA binding conditions

Add **210 µL ethanol (96 – 100 %)** to the sample and vortex vigorously.

After addition of ethanol a stringy precipitate may become visible. This will not affect the DNA isolation. Be sure to load all of the precipitate on the column in the following step.

+210 µL ethanol

Vortex

5 Bind DNA

For each sample, place one **NucleoSpin® Tissue Column** into a Collection Tube. Apply the sample to the column. Centrifuge for **1 min** at **11,000 x *g***. Discard Collection Tube with flowthrough and place the column in a new Collection Tube (povided).

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

Load samples

11,000 x *g*, 1 min

6 Wash silica membrane

1 st wash

Add **500 µL Buffer BW**. Centrifuge for **1 min** at **11,000 x *g***. Discard flowthrough and place the column back into the Collection Tube.

+500 µL BW

11,000 x *g*, 1 min

2nd wash

Add **600 µL Buffer B5** to the column and centrifuge for **1 min** at **11,000 x *g***. Discard flowthrough and place the column back into the Collection Tube.

+600 µL B5

11,000 x *g*, 1 min

7 Dry silica membrane

Centrifuge the column for **1 min** at **11,000 x *g***.

Residual ethanol is removed during this step.

11,000 x *g*, 1 min

8 Elute highly pure DNA

Place the NucleoSpin® Tissue Column into a 1.5 mL microcentrifuge tube (not provided) and add **100 µL Buffer BE**. Incubate at room temperature for 1 min. Centrifuge **1 min** at **11,000 x *g***.

For alternative elution procedures see section 2.3.

+100 µL BE

RT, 1 min

11,000 x *g*, 1 min

6 Support protocols

6.1 Support protocol for mouse or rat tails

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Cut two 0.6 cm-pieces of mouse tail and place them in a 1.5 mL centrifuge tube (not provided).

If processing rat tails, one 0.6 cm-piece is sufficient.

2 Pre-lyse sample

Add 180 µL Buffer T1 and 25 µL Proteinase K and vortex. Incubate at 56 °C overnight or until complete lysis is obtained. Lysis time can substantially be reduced down to approximately one hour if the tissue is broken up mechanically (e.g., if the tissue is cut into very small pieces before lysis).

Vortex occasionally during incubation or use a shaking water bath. To remove residual bones or hair, centrifuge for 5 min at high speed (e.g., 11,000 x *g*). Transfer 200 µL supernatant to a new tube.

If processing several samples, Proteinase K and Buffer T1 may be premixed directly before use. Do never mix Buffer T1 and Proteinase K more than 10 – 15 min before addition to the sample: Proteinase K tends to self-digest in Buffer T1 without substrate.

3 Lyse sample

Add 200 µL Buffer B3 to the lysate and vortex vigorously.

Buffer B3 and ethanol (see step 4) can be premixed before addition to the lysate.

Adjust DNA binding conditions

Add 210 µL ethanol to the lysate and vortex vigorously.

Proceed with step 5 of the standard protocol (see section 5).

6.2 Support protocol for bacteria

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Up to 1 mL of bacterial culture can be used for the preparation depending on, for example, density of culture, culture medium, and bacterial strain.

Centrifuge up to **1 mL culture** for **5 min** at **8,000 x *g***. Remove supernatant.

2 Pre-lyse sample

Resuspend the pellet in **180 µL Buffer T1** by pipetting up and down. Add **25 µL Proteinase K**. Vortex vigorously and incubate at 56 °C until complete lysis is obtained (at least 1 – 3 h). Vortex occasionally during incubation or use a shaking incubator.

Samples can be incubated overnight as well.

If RNA-free DNA is crucial for downstream applications, an RNase digest may be performed: Add 20 µL RNase A (20 mg/mL) solution (not included; see ordering information) and incubate for an additional 5 min at room temperature.

Hard-to-lyse bacteria: *Some strains, especially Gram-positive bacteria, are more difficult to lyse. In such cases, a preincubation with a lytic enzyme is necessary: Resuspend the pelleted cells in 20 mM Tris/HCl; 2 mM EDTA; 1 % Triton X-100; pH 8 (instead of Buffer T1) supplemented with a final concentration of 20 mg/mL lysozyme or 0.2 mg/mL lysostaphin and incubate for 30 – 60 min at 37 °C. Add 25 µL Proteinase K, incubate at 56 °C until complete lysis is obtained.*

Proceed with step 3 of the standard protocol (see section 5).

6.3 Support protocol for yeast

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check that sorbitol buffer and lyticase or zymolase (not provided with the kit) is available for sample pre-lysis.
- Set an incubator or water bath to 30 °C and 56 °C.

1 Prepare sample

Harvest **3 mL YPD yeast culture** ($OD_{600} \leq 10$) by centrifugation for **10 min** at **5,000 x *g***. Wash the cells once with **1 mL 10 mM EDTA, pH 8**. Remove the supernatant and pellet the cells by centrifugation **(5,000 x *g*, 10 min)**.

2 Pre-lyse sample

Resuspend the pellet in **600 µL sorbitol buffer** (1.2 M sorbitol; 10 mM CaCl2; 0.1 M Tris/HCl pH 7.5; 35 mM ß-mercaptoethanol). Add **50 U lyticase or zymolase***. Incubate at **30 °C** for **30 min**. This step degrades the yeast cell wall creating spheroplasts. Spheroplast formation may be checked microscopically. Centrifuge the mixture for **10 min** at **2,000 x *g*** remove supernatant and resuspend the pelleted spheroplasts in **180 µL Buffer T1**. Add **25 µL Proteinase K** solution and vortex vigorously. Incubate at **56 °C** until complete lysis is obtained (at least 1 – 3 h). Vortex occasionally during incubation or use a shaking water bath.

Samples can be incubated overnight as well.

If RNA-free DNA is crucial for downstream applications, an RNase digest may be performed: Add 20 µL RNase A (20 mg/mL) solution (not included; see ordering information) and incubate for an additional 5 min at room temperature.

Proceed with step 3 of the standard protocol (see section 5).

* Other protocols use 5 – 200 U lyticase or zymolase depending on enzyme quality or brand. Increasing the enzyme concentration may be required if spheroplasts are not formed.

6.4 Support protocol for dried blood spots (e.g., NucleoCard®s, FTA® cards, Guthrie cards)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Cut out one or two **dried blood spots** as accurately as possible. Cut spots into small pieces and place them in a 1.5 mL microcentrifuge tube (not provided).

The area of the dried blood spots should be between 15 and 30 mm^2.

2 Pre-lyse sample

Add **180 µL Buffer T1** and mix by vortexing. Place the samples in a water bath or heating block and heat for **10 min** at **94 °C**. Let the sample cool down. Add **25 µL Proteinase K** solution. Spin the samples briefly, vortex and incubate at **56 °C** for **1 h**. Vortex occasionally during incubation or use a shaking water bath.

Make sure that the samples are completely covered with lysis buffer during incubation.

3 Lyse sample

Add **200 µL Buffer B3**, vortex vigorously to mix and incubate at **56 °C** for **10 min**.

Proceed with step 4 of the standard protocol (see section 5).

6.5 Support protocol for genomic DNA and viral DNA from blood samples

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.

1	**Prepare sample** Not necessary
2	**Pre-lyse sample** Not necessary
3	**Lyse blood sample** Pipette **25 µL Proteinase K** and up to **200 µL blood**, buffy coat, or body fluid sample (equilibrated to room temperature) into 1.5 mL microcentrifuge tubes (not provided). *For sample volumes less than 200 µL, add PBS to adjust the volume to 200 µL. If purifying DNA viruses, we recommend starting with 200 µL serum or plasma. If cultured cells are used, resuspend up to 5 × 10^6 cells in a final volume of 200 µL PBS.* Add **200 µL Buffer B3** to the samples and vortex the mixture vigorously (10 – 20 s). Incubate samples at **room temperature** for **5 min**. Mix. Incubate samples at **70 °C** for **10 – 15 min**. *The lysate should become brownish during incubation with Buffer B3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.*
4	**Adjust DNA binding conditions** Add **210 µL ethanol (96 – 100 %)** to each sample and vortex again.
5	**Bind DNA** For each preparation, take one **NucleoSpin® Tissue Column** placed in a Collection Tube and load the sample. Centrifuge **1 min** at **11,000 x *g***. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000 x *g*). Discard Collection Tube with flowthrough and place the column in a new Collection Tube (provided).
	Proceed with step 6 (Wash silica membrane) of the standard protocol (see section 5).

6.6 Support protocol for hair roots

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Cut off the hair roots from the hair sample (up to 100) and collect them in a 1.5 mL microcentrifuge tube (not provided).

2 Pre-lyse sample

Add **180 µL Buffer T1** to the hair roots and freeze the samples in liquid nitrogen. Thaw samples in a **56 °C water bath**. Repeat this procedure 4 times. Add 25 µL Proteinase K solution, mix by vortexing, and incubate **6 – 8 h** or **overnight** at **56 °C**. Use a shaking water bath or vortex occasionally.

Proceed with step 3 of the standard protocol (see section 5).

6.7 Support protocol for paraffin-embedded tissue

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check if xylene or n-octane is available.
- Set an incubator or water bath to 37 °C and 56 °C.

1 Prepare sample

Prepare small sections (up to 25 mg) from blocks of fixed, embedded tissue. If possible, trim excess paraffin from the block before slicing. Handle the sections with tweezers or toothpicks and place the samples into microcentrifuge tubes.

Add **1 mL n-octane or xylene** to each tube. Vortex vigorously and incubate at room temperature for about 30 min. Vortex occasionally.

Centrifuge at **11,000 x *g*** for 3 min. Pipette off supernatant.

Add **1 mL ethanol (96 – 100 %)** to each tube. Close and mix by inverting several times. Centrifuge at **11,000 x g** for **3 min**. Pipette off supernatant.

Repeat the ethanol washing step. Pipette off as much of the ethanol as possible.

Incubate the open tube at **37 °C** until the ethanol has evaporated **(~ 15 min)**.

Proceed with step 2 of the standard protocol (see section 5).

6.8 Support protocol for genomic DNA from stool

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check if TE buffer is available.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Add **250 mg feces** to 1 mL TE buffer (10 mM Tris/Cl; 1 mM EDTA, pH 8). Resuspend the sample by vigorous vortexing **(30 s)**.

Centrifuge the sample for **15 min** at **4,000 x *g***. Discard supernatant.

Resuspend the pellet in **0.2 – 1 mL Buffer T1**. Use as much buffer as necessary for good resuspension of the sample.

The prepared pellet contains, among other constituents, cells from the digestive tract and bacteria.

Transfer **200 µL** of the **resuspended sample** to a new microcentrifuge tube.

Proceed with the addition of 25 µL Proteinase K in step 2 of the standard protocol (see section 5).

Human cells, bacterial cells, and cells of pathogens in the stool lyse during the incubation step at 56 °C with Proteinase K with different efficiency. For the detection of cells that are difficult to lyse (e.g., some bacteria and parasites) it can be beneficial to perform an additional incubation at increased incubation temperature (up to 95 °C; 5 – 10 min). DNA yield will often be higher with such an additional incubation step at high temperature. However, note that the ratio of human to non-human DNA will typically change due to the increased release of bacterial/pathogen DNA.

6.9 Support protocol for viral DNA (e.g., CMV) from stool

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Prepare 0.9 % NaCl.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Suspend the stool sample in **0.9 % NaCl** (ca. 0.5 g in max. 4 mL).

Centrifuge aliquots of the stool sample for **5 min** at **800 x *g*** at **RT** (e.g., 4 mL: 4 × 1 mL in a 1.5 mL microcentrifuge tube). Carefully reunite supernatant (do not touch the pellet).

Filtrate supernatant through 0.22 – 0.45 µm sterile filter. Fractionate the filtrate and centrifuge for **1 min** at **11,000 x *g***.

2 Pre-lyse sample

Carefully remove the supernatant by decanting. Add **400 µL Buffer T1** and **35 µL Proteinase K** and mix by vortexing.

3 Lyse sample

Add **400 µL Buffer B3** and mix by vortexing. Incubate for at least **30 min** at **70 °C**.

4 Adjust DNA binding conditions

Add **420 µL ethanol (96 – 100 %)** and mix by vortexing.

5 Bind DNA

For each sample, place one **NucleoSpin® Tissue Column** into a Collection Tube. Load the NucleoSpin® Tissue Column successively. Centrifuge for **1 min** at **4,500 x *g***. Discard the flowthrough and place the column back into the Collection Tube.

If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

6 Wash silica membrane

1 st wash

Add **600 µL Buffer BW**. Centrifuge for **1 min** at **4,500 x *g*.** Discard flowthrough and place the column back into the Collection Tube.

2nd wash

Add **600 µL Buffer B5** to the column and centrifuge for **1 min** at **4,500 x g**. Discard flowthrough and place the column back into the Collection Tube.

3rd wash

Add **600 µL Buffer B5** to the column and centrifuge for **2 min** at **11,000 x *g***. Discard flowthrough.

7 Dry silica membrane

Place the NucleoSpin® Tissue Column into a new Collection Tube and incubate with open lid for **1 – 2 min** at **70 °C**.

Residual ethanol is removed during this step.

8 Elute highly pure DNA

Place the NucleoSpin® Tissue Column into a 1.5 mL microcentrifuge tube (not provided) and add **100 µL Buffer BE**. Incubate with closed lid for **3 – 5 min**. Centrifuge for **1 min** at **4,500 x *g***.

For alternative elution procedures see section 2.3.

Use 10 µL DNA extract for a 20 µL PCR reaction mix.

Add inhibition control mix (10 µL DNA extract with human DNA) and amplify with for example actin-/ß-globin-/ or other human specific primer.

6.10 Support protocol for detection of *Mycobacterium tuberculosis or Legionella pneumophila* in sputum or bronchoalveolar lavage

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.
- Prepare N-acetyl cystein/NaOH (2 g NaOH; 1.45 g sodium citrate; 0.5 g N-acetyl cystein. Add water to 100 mL).

1 Prepare sample

Add **200 – 500 µL** sputum or bronchoalveolar lavage to an **equal volume N-acetyl cystein/NaOH**. Vortex gently to mix.

Incubate the mixture for **25 min** at **room temperature** with shaking.

Adjust the **volume to 25 mL** with **sterile water**.

Centrifuge for **30 min** at **4,000 x *g***. Discard the supernatant.

Resuspend the pellet in **0.5 – 1 mL Buffer T1** (depending on sample viscosity).

Transfer 2**00 µL of the resuspended sample** to a new microcentrifuge tube (not provided).

Proceed with step 2 of the standard protocol, see section 5 (addition of Proteinase K and incubation).

6.11 Support protocol for detection of EHEC bacteria in food (e.g., fresh cows' milk)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Prepare mTSB and 3.2 M sodium acetate.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

To a sterile 1 Liter flask, add **25 mL milk** and **225 mL prewarmed (37 °C) mTSB medium** (supplied with Novobiocin). Incubate the mixture in a shaking water bath for **5 – 6 h** or overnight at 37 °C.

Preparation of mTSB medium: 30 g Tryptic Soy Broth (Gibco), 1.5 g bile salts No. 3 (Oxoid), 1.5 g KH2PO4. Add 900 mL H_2O. Filter the medium and adjust the pH with 2 M NaOH to 7.4. Add water to 1 Liter and autoclave for 15 min at 121 °C.

Centrifuge **100 mL culture** for **40 min** at **6,000 x *g***.

Gently pour off the supernatant and resuspend the pellet in 2 mL sterile water. Centrifuge for **10 min** at **10,000 x *g***.

2 Pre-lyse sample

Resuspend the pellet in **180 µL Buffer T1** and add **25 µL Proteinase K** solution.

Carry out the standard protocol, beginning with step 3 (see section 5).

After elution of the DNA, proceed with the following step.

Precipitate the DNA by adding **20 µL 3.2 M sodium acetate** and **400 µL ethanol** to **200 µL eluate**. Centrifuge for **30 min** at **11,000 x *g***. Discard supernatant and wash the pellet with **1 mL 70 % ethanol** and resuspend in **10 µL sterile water**.

6.12 Support protocol for purification of bacterial DNA (e.g., *Chlamydia trachomatis*) from cultures, biological fluids, or clinical specimens

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Isolation of bacterial DNA from **bacterial cultures or biological fluids**: Pellet bacteria by centrifugation for **5 min** at **13,000 x *G*** and proceed with step 2 of the standard protocol, see section 5.

Isolation of bacterial DNA from **eye, nasal or pharyngeal swabs**: Collect samples, add 2 mL PBS containing a common fungicide, and incubate for **several hours** at **room temperature**. Pellet bacteria by centrifugation for **5 min** at **13,000 x *g***. Discard supernatant.

Proceed with step 2 of the standard protocol (see section 5).

6.13 Support protocol for purification of bacterial DNA (e.g., *Borrelia burgdorferi*) from urine

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Centrifuge **1 mL urine** sample at **13,000 x *g*** for **30 min**. Discard supernatant, add again **1 mL urine** sample to the pellet and centrifuge at **13,000 x *g*** for **30 min**. This step a can be repeated up to three times.

The sample material should be fresh. Storage at - 20 °C to - 80 °C is only recommended for a couple of days. After thawing incubate the sample at 40 °C until all precipitates are dissolved. Urine tends to form precipitates when stored at low temperatures.

Proceed with step 2 of the standard protocol (see section 5).

6.14 Support protocol for purification of viral DNA (e.g., CMV) from urine

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 70 °C.

1 Prepare sample

Centrifuge aliquots of the urine sample for **10 min** at **full speed** (e.g., 4 mL: 4 × 1 mL in a 1.5 mL microcentrifuge tube). Carefully decant supernatant.

If frozen urine samples are used precipitates may appear after thawing, which must be dissolved before the centrifugation step. This can be done by a 30 min incubation step at 37 – 40 °C. If a complete solution is not achieved let the precipitate sediment and proceed with step 1 of the support protocol using only the supernatant.

2 Pre-lyse sample

Resuspend the pellet in **180 µL Buffer T1** and **25 µL Proteinase K**.

Resuspend the first pellet in 180 µL Buffer T1 and 25 µL Proteinase K. Transfer the resuspended solution of the first tube to the second tube and the resuspended solution of the second tube to the third tube and so on. Finally continue with step 3.

3 Lyse sample

Add **200 µL Buffer B3**, vortex and incubate at least for **20 min** at **70 °C**.

4 Adjust DNA binding conditions

Add **210 µL ethanol (96 – 100 %)** to the sample and vortex vigorously.

5 Bind DNA

For each sample, place one **NucleoSpin® Tissue Column** into a Collection Tube. Apply the sample to the column. Centrifuge for **1 min** at **4,500 x *g*.** Discard the flowthrough and place the column back into the Collection Tube.

6 Wash silica membrane

1 st wash

Add 500 µL **Buffer BW**. Centrifuge for **1 min** at **4,500 x *g***. Discard flowthrough and place the column back into the Collection Tube.

2nd wash

Add **600 µL Buffer B5** to the column and centrifuge for **2 min** at **11,000 x *g***. Discard flowthrough and place the column back into the Collection Tube.

7 Dry silica membrane

Incubate with open lid for **1 – 2 min** at **70 °C**.

Residual ethanol is removed during this step.

8 Elute highly pure DNA

Add **70 µL Buffer BE**, close the lid and incubate for further **3 – 5 min**. Centrifuge for **1 min** at **4,500 x *g***.

6.15 Support protocol for purification of genomic DNA from insects

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Homogenize not **more than 50 mg insects** under liquid nitrogen and transfer the powder into a 1.5 mL microcentrifuge tube (not provided).

Proceed with step 2 of the standard protocol (see section 5) with addition of Proteinase K.

6.16 Support protocol for purification of genomic DNA from dental swabs

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Place swab material (paper, cotton, brushes, plastic) in a 1.5 mL microcentrifuge tube (not provided).

2 Pre-lyse sample

Add **180 µL Buffer T1** and **25 µL Proteinase K** to each sample. Close the microcentrifuge tube and spin briefly for **15 s** at **1,500 x *g*** in order to submerge the swab material completely. Incubate at room temperature for 5 min. Vortex the tube vigorously for **15 s** and spin briefly for **15 s** at **1,500 x *g***.

Incubate the tubes at **70 °C** in an incubator for 10 min. Place a weight on top of the tube in order to prevent the caps from popping off. Shift the temperature to **95 °C** for **5 min**. Spin briefly for **15 s** at **1,500 x *g*** to collect any sample from the lids. Open the microcentrifuge tubes.

Skip incubation at 95 °C, depending on the bacterial strain to be detected.

2a Separate lysis solution from dental swabs

Alternative A:

Transfer the swab tip into a **NucleoSpin® Forensic Filter** (not provided; see ordering information). Cut off swab shaft. Centrifuge the NucleoSpin® Forensic Filter for **1 min** at **11,000 x *g***. Discard the NucleoSpin® Forensic Filter. Continue with flowthrough.

Alternative B:

Place a **NucleoSpin® Filter** (not provided; see ordering information) into a Collection Tube (2 mL). Transfer the swab tip (cut off swab shaft) and the remaining solution onto the NucleoSpin® Filter. Centrifuge for **1 min** at **11,000 x *G***. Discard the NucleoSpin® Filter. Continue with flowthrough.

Alternative C:

Transfer as much as possible of the lysate solution to a 1.5 mL microcentrifuge tube (not provided). Discard swab and continue with recovered solution.

Proceed with step 3 of the standard protocol (see section 5).

6.17 Support protocol for purification of genomic DNA from buccal swabs

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Check if PBS is available.
- Set an incubator or water bath to 56 °C.

1 Prepare sample

Collect the samples with cotton, dacron® (Daigger), or C.E.P. swabs (Gibco BRL). Scrape firmly against the inside of each cheek several times and let the swabs air dry.

The respective individual should not have consumed food or drink within 30 min before collection of the sample.

2 Pre-lyse sample

Place the dry swab material in 2 mL microcentrifuge tubes (not provided). Add **400 – 600 µL PBS** and **25 µL Proteinase K** solution to the swabs.

The volume of PBS is depending on the type of swab used: for cotton and dacron swabs, 400 µL are sufficient; for C.E.P. swabs, 600 µL are necessary.

Mix by vortexing 2 × 5 s and incubate **10 min** at **56 °C**.

2a Separate lysis solution from buccal swabs

Alternative A:

Transfer the swab tip into a **NucleoSpin® Forensic Filter** (not provided; see ordering information). Cut off swab shaft. Centrifuge the NucleoSpin® Forensic Filter for **1 min** at **11,000 x *g***. Discard the **NucleoSpin® Forensic Filter**. Continue with flowthrough.

Alternative B:

Place a **NucleoSpin® Filter** (not provided; see ordering information) into a Collection Tube (2 mL). Transfer the swab tip (cut off swab shaft) and the remaining solution onto the NucleoSpin® Filter. Centrifuge for **1 min** at **11,000 x *g***. Discard the NucleoSpin® Filter. Continue with flowthrough.

Alternative C:

Transfer as much as possible of the lysate solution to a 1.5 mL microcentrifuge tube (not provided). Discard swab and continue with recovered solution.

3 Lyse sample

Add **one volume Buffer B3** (400 or 600 µL; depending on the swab type/volume of PBS buffer used) and vortex vigorously. Incubate the samples at **70 °C** for **10 min**.

Note: Depending on the number of preparations, additional Buffer B3 might be needed (see ordering information).

4 Adjust DNA binding conditions

Add **one volume 96 – 100 % ethanol** (400 or 600 µL, depending on the swab type) to each sample and mix by vortexing.

5 Bind DNA

Transfer **600 µL of the samples** from the 2 mL microcentrifuge tubes into **NucleoSpin® Tissue Columns**. Centrifuge at **11,000 x *g*** for **1 min**. If the samples are not drawn through completely, repeat the centrifugation. Discard flowthrough.

Place the columns back into the Collection Tubes and repeat step 5 once or twice, depending on the lysis volume.

When all of the lysate has been applied to the columns, discard Collection Tube and place the column in a new Collection Tube. Proceed with step 6 of the standard protocol (section 5).

7 Appendix

7.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	*Incomplete lysis* • Sample not thoroughly homogenized and mixed with Buffer T1/ Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer T1. • Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months. *Reagents not applied properly* • Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add ethanol to the lysates before loading them onto the columns. *Suboptimal elution of DNA from the column* • 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.
Poor DNA quality	*Incomplete lysis* • Sample not thoroughly homogenized and mixed with Buffer T1 / Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer T1. • Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Problem	Possible cause and suggestions
Poor DNA quality *(continued)*	*Reagents not applied properly* • Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add ethanol to the lysates before loading them on the columns. *RNA in sample* • If RNA-free DNA is desired, add 20 µL of RNase A solution (10 mg/mL; not supplied with the kit) before addition of Buffer B3 and incubate at 37 °C for 5 min.
Clogged columns	*Too much sample material used* • Do not use more sample material than recommended (25 mg for most tissue types). If insoluble material like bones or hair remains in the lysate, spin down the debris and transfer the clear supernatant to a new microcentrifuge tube before proceeding with addition of Buffer B3 and ethanol. The use of the NucleoSpin® Filter prior column loading prevents column clogging. *Incomplete lysis* • Sample not thoroughly homogenized and mixed with Buffer T1/Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer T1. • Decreased Proteinase K activity: Store dissolved Proteinase K at -20 °C for 6 months. *Reagents not applied properly* • Prepare Buffer B5 and Proteinase K solution according to instructions (see section 3). Add ethanol to the lysates before loading them on the columns.
Suboptimal performance of genomic DNA in enzymatic reactions	*Carry-over of ethanol or salt* • 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.

Problem	Possible cause and suggestions
Suboptimal performance of genomic DNA in enzymatic reactions *(continued)*	*Contamination of DNA with inhibitory substances* • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer BE. • If the A_{260}/A_{280} ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume Buffer B3 plus 1 volume ethanol (96 – 100 %) to the eluate. Load the mixture onto a NucleoSpin® Tissue Column and proceed with step 5 of the standard protocol (see section 5).

7.2 Ordering information

Product	REF	Pack of
NucleoSpin® Tissue	740952.10/.50/.250	10/50/250 preps
NucleoSpin® Tissue XS	740901.10/.50/.250	10/50/250 preps
NucleoSpin® DNA RapidLyse	740100.10/.50/.250	10/50/250 preps
NucleoSpin® Blood	740951.10/.50/.250	10/50/250 preps
Buffer T1	740940.25	50 mL
Buffer B3	740920	100 mL
Buffer B5 Concentrate(for 100 mL Buffer B5)	740921	20 mL
Buffer BW	740922	100 mL
Proteinase K	740506	100 mg
RNase A	740505.50 740505	50 mg 100 mg
NucleoSpin® Microbial DNA	740235.10/.50	10/50 preps
MN Bead Tube Holder	740469	1 piece
MN Bead Tubes Type A (0.6 – 0.8 mm ceramic beads; recommended for soil and sediments)	740786.50	50 pieces

Product	REF	Pack of
MN Bead Tubes Type B (40 – 400 µm glass beads; recommended for bacteria)	740812.50	50 pieces
MN Bead Tubes Type C (1 – 3 mm corundum; recommended for yeasts)	740813.50	50 pieces
MN Bead Tubes Type D (3 mm steel balls; recommended for insects)	740814.50	50 pieces
MN Bead Tubes Type E (40 – 400 µm glass beads and 3 mm steel balls; recommended for hard-tolyse bacteria within insect or tissue samples)	740815.50	50 pieces
MN Bead Tubes Type F (1 – 3 mm corundum +3 mm steel balls; use only with MN Bead Tube Holder!)	740816.50	50 pieces
NucleoSpin® DNA FFPE XS	740980.10/.50/.250	10/50/250 preps
NucleoSpin® Forensic Filters	740988.10/.50/.250	10/50/250 pieces
NucleoSpin® Forensic Filters (Bulk)	740988.50B/.250B / 1000B	50/250 / 1000 pieces
Collection Tubes (2 mL)	740600	1000
NucleoSpin® Filters	740606	50
NucleoCard®	740403.10/.100	10/100 cards

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

7.3 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-NAGELS 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.

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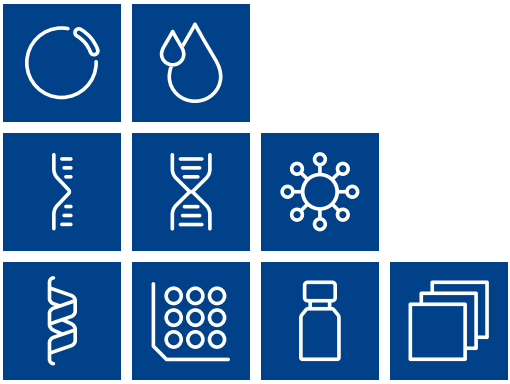
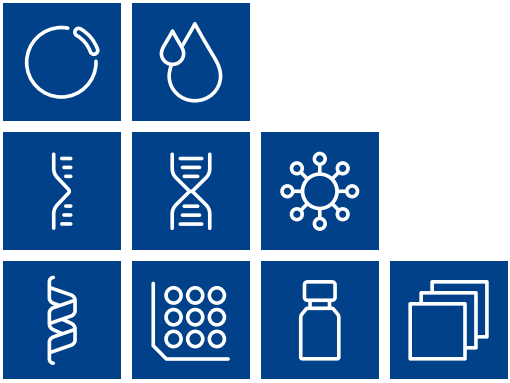


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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