MACHEREY-NAGEL

User manual

Bioanalysis

High molecular weight DNA

- NucleoBond® HMW DNA

November 2022 / Rev. 07

MACHEREY-NAGEL
www.mn-net.com

High molecular weight DNA

Protocol at a glance (Rev. 07)

	NucleoBond® HMW DNA		
	Enzymatic lysis	**Lysis with liquid nitrogen and mortar / pestle**	**Bead beating based cell lysis**
1 Prepare sample	Prepare samples	Homo-genize samples	900 µL H1 200 µL Proteinase K homogenize 11,000 x *g* 2 min Transfer supernatant
2 Add lysis buffer	5 mL H1 200 µL Proteinase K Vortex 5 s	5 mL H1 200 µL Proteinase K Vortex 5 s	4 mL H1 Vortex 5 s
3 Lysis	50 °C 30 min		
4 RNA digestion	100 µL RNase A RT 5 min		
5 Column equilibration	12 mL H2		
6 Adjust binding conditions	10 mL H2 mix		
7 Bind DNA	Load sample		

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High molecular weight DNA

Protocol at a glance (Rev. 07)

	NucleoBond® HMW DNA
8 **Flush filter column**	6 mL H3
9 **Column wash**	12 mL H4
10 **DNA elution**	5 mL H5
11 **Precipitate nucleic acids**	3.5 mL isopropanol mix 4,500 x *g* 10 min
12 **Wash DNA pellet**	2 mL 70 % ethanol 4,500 x *g* 5 min
13 **Resuspend nucleic acids**	150 µL HE

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1 Components

1.1 Kit contents

NucleoBond® HMW DNA	
REF	**20 preps** **740160.20**
Lysis Buffer H1	125 mL
Binding Buffer H2	500 mL
Wash Buffer H3	125 mL
Wash Buffer H4	250 mL
Elution Buffer H5	125 mL
Resuspension Buffer HE*	13 mL
Liquid Proteinase K	3 x 1.5 mL
Liquid RNase A	4 x 0.6 mL
NucleoBond® HMW Columns + Column Filter	20
Plastic Washer	10
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* Composition of Resuspension Buffer HE: 5 mM Tris-Cl, pH 8.5

1.2 Reagents, consumables and equipment to be supplied by user

Reagents

- Isopropanol
- 70 % ethanol

Consumables

- 50 mL centrifuge tubes
- Nuclease-free pipette tips
- Wide bore pipette tips (for handling of eluted DNA)
- *Optional: Dedicated lytic enzymes and reaction buffers (e.g., lyticase, zymolyase, lysozyme, cellulase, chitinase, pectinase, driselase and others)*
- *Optional: Enzymatic DNA Repair Kit to reduce amount of nicked DNA and to increase average read length in third generation sequencers (e. g. NEBNext FFPE DNA Repair Mix)*

Equipment

- Incubator or water bath
- Centrifuge for 50 mL tubes, capable of reaching 4,500 x *g*
- Recommended: mortar and pestle plus liquid nitrogen
- *Optional: MN Bead Tubes (recommended: MN Bead Tubes Type A, see ordering information, section 6.2)*

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the NucleoBond® HMW DNA 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.

2 Product description

2.1 Basic principle

The **NucleoBond® HMW DNA Kit** is designed for the purification of ultra pure high molecular weight DNA, e.g., long range single molecule sequencing.

The most gentle and thus recommended lysis procedure is to lyse the sample enzymatically. Therefore Proteinase K is included in the kit. Nevertheless, some sample types require a more dedicated lytic enzyme (e.g., lysozyme for bacteria, zymolyase for yeast, not supplied). The before mentioned enzymes will be active in Lysis Buffer H1, but other enzymes might be inactivated. When using a specialized enzyme it is recommended to perform a small scale experimental test to check if this enzyme is active in Lysis Buffer H1 or requires a dedicated reaction buffer.

In case of the use of a dedicated lytic enzyme, use the reaction buffer supplied or recommended by the supplier of the enzyme and add Lysis Buffer H1 and Proteinase K after the enzymatic digestion is complete.

Please keep in mind that excess substrate concentrations (i.e., too much sample) might decrease the enzymatic reaction speed. The optimal ratio of enzyme to substrate (sample) and necessary cofactors must be determined empirically or according to instructions provided by the supplier of the enzyme.

Make sure the contact surface area between reaction buffer and sample is a high as possible and agitate the reaction mixture continuously if possible.

Cells expressing thick cell walls like plant leaf cells cannot be completely lysed enzymatically. It is recommended to grind the sample material under liquid nitrogen with mortar and pestle in this case. Always use precooled material and do not let the sample thaw in between. Grinding of sample material with mortar and pestle under liquid nitrogen will result in a typical mean fragment length distribution starting at about 15 kb with a maximum at about 150 kb and above. When grinding bacteria with mortar and pestle under liquid nitrogen make sure not to overload the purification columns later on. Intact cells will clog the silica matrix and result in very slow flow rates. It is preferred to lyse bacteria and yeast enzymatically or by bead beating.

Recommended in this case are the MN Bead Tubes Type A (not supplied, see ordering information, section 6.2). Bead Beating is a universally applicable method and is especially useful for lysing large amount of cells and obtaining high yields without establishing an optimized enzymatic lysis protocol. Mechanical disruption of cells using MN Bead Tubes will result in an average fragement length of 50 kb to 100 kb. Furthermore, bead beating it is more suitable to process many different samples at once in comparison to grinding (less cross contamination) and less expensive than large scale enzymatic reactions.

Liquid samples like blood samples can be supplemented directly with Lysis Buffer H1. This results in a dilution of chemical agents within the Lysis Buffers H1 and Binding Buffer H2 which will reduce lysis efficiency if the sample volume exceeds 2 mL. For nucleated blood and other liquid samples with high DNA content it is recommended to reduce the sample volume to 250 µL. Otherwise, the high molecular weight DNA will increase the sample viscosity and reduce the column flow rate, resulting in long preparation times. If larger sample volumes need to be processed, the volumes of buffers H1 and H2 must be increased proportionally.

Solid tissues like biopsy samples can be lysed directly in Lysis Buffer H1 without mechanical disruption. Nevertheless, the sample should be cut into small pieces. The smaller the tissue pieces, the faster a complete cell lysis will occur and the less influence DNases / oxygen radicals will have.

An incubation of the sample in Lysis Buffer H1, supplemented with Proteinase K, results in the release of DNA into the lysis mixture where DNA is stabilized. Independent of the chosen sample homogenization and lysis procedure, an incubation time of about 30 minutes at 50 °C is sufficient for most sample types. **If needed, the incubation time can be increased up to an overnight incubation** without negative effects on DNA integrity and molecular weight. This might be relevant for larger solid tissue biopsy blocks to ensure complete sample lysis.

After complete lysis, residual RNA is digested by RNase A. The lysed sample is mixed with Binding Buffer H2 afterwards and poured onto the equilibrated NucleoBond® HMW column, supplemented with a cellulose column filter. The column filter removes undigested sample material and is washed in a first washing step with Wash Buffer H3 to flush the dead volume. After the first washing step with Wash Buffer H3, the filter is discarded. DNA in the filter flowthrough will bind to the chemically modified silica matrix by ionic interaction.

The silica matrix is washed with Wash Buffer H4 which removes proteins, polysaccharides, polyphenoles, low molecular weight nucleic acids below 10 kb, and other contaminants. The removal of low molecular weight nucleic acids depends on fragment size and is more efficient the smaller nucleic acids are. As a result, part of the low molecular weight DNA is removed deliberately, reducing the total yield as compared to other purification methods. Instead the focus is on long fragments above 10 kb.

High molecular weight DNA is eluted using the Elution Buffer H5. Desalting and concentration of DNA is performed by a standard and gentle isopropanol precipitation. The precipitated DNA is washed, dryed and resuspended in 50–250 µL of resuspension buffer HE, resulting in highly pure and concentrated DNA. Other customer supplied resuspension buffers like TE buffer or pure water can be also used. **Complete resuspension with careful agitation might take quite a long time, depending on the DNA yield**. Resuspend DNA by pipetting up and down with wide bore pipette tips or careful vortexing at low speed. The higher the molecular weight, the longer it will take to bring DNA back into solution again.

If working with small DNA pellets in a large reaction tube during the isopropanol precipitation is too cumbersome or error prone, there are additional desalting methods available.

The **NucleoSnap® Finisher** will use vacuum to filtrate precipitated DNA onto a small filter surface. After a washing and drying step the DNA can simply be eluted in a minispin centrifuge. This kit requires some additional equipment for processing nevertheless. Besides a vacuum source and vacuum tubing (customer supplied) a vacuum manifold, valves and a vacuum regulator are required (see ordering information, section 6.2).

The **NucleoSpin® Finisher** is the centrifuge based version of the NucleoSnap® Finisher design but might reduce the fragment length due to shearing forces that occur during centrifugation. No additional equipment is required using the NucleoSpin® Finisher.

Both Finisher versions will reduce the handling time for the precipitation to a few minutes and don't bear the risk of a lost DNA pellet. Nevertheless the elution should be performed several times as large DNA molecules need to be transported from the upper filter layers through the filters and into the eluates. Repeat the elution step at least 2 to 3 times. The previous eluates should be reloaded in order to prevent a decrease in total DNA concentration.

For more information visit our website: https://***www.mn-net.com/de/culturedmicroorganism***

2.2 Kit specifications

The **NucleoBond® HMW DNA** Kit is designed for the isolation of pure high molecular weight DNA from cultured cells, tissue, and plant samples.

Kit specifications at a glance	
Parameter	**NucleoBond® HMW DNA**
Format	Anion exchange chromatography
Processing	Manual, Gravity flow
Sample material	Up to 1.5 g plant leaves (ground under liquid nitrogen)
	Up to 10^7 cultured cells (enzymatic lysis)
	Up to 300 mg solid tissue (cut into small pieces and lysed enzymatically with increased lysis time, ground under liquid nitrogen or lysed by bead beating)
	Up to 300 mg yeast or bacteria (Lysed by bead beating)
	Up to 100 mg bacteria or 250 mg yeast (enzymatic lysis)
	Up to 30 mg yeast or bacteria (ground under liquid nitrogen)
	Up to 2 mL liquid sample, e.g., blood, body fluids or enzymatic reactions
	Up to 250 µL liquid sample (DNA rich samples such as nucleated blood or highly concentrated reactions)
Elution volume	50–250 µL
Preparation time	2 h/12 preps (including a 30 min lysis)
Typical yield	Depending on sample amount and type
Average fragment length	~150 kb (enzymatic lysis)
	~50 kb (mechanical lysis)

2.3 Working with high molecular weight DNA

Make sure to handle isolated DNA with wide bore pipette tips and minimize shearing forces such as vigorous vortexing or repeated pipetting steps wherever possible.

DNases will be removed by the preparation. Take care to use certified DNase-free centrifuge tubes and pipette tips.

The eluted DNA might be nicked, resulting in shorter average read lengths in single molecule sequencing reactions. Use of repair kits could reduce the amount of nicks and increase average reading lengths. Suitable kit recommendations might be given by the supplier of the sequencing machine.

Very high molecular weight DNA will increase the viscosity of the sample lysates, especially when using a gentle enzymatic lysis which returns the highest molecular weight. In combination with a large sample input amount this high viscosity might lead to low column flow rates or even column clogging. Add additional buffer H1 and H2 in a 1:2 ratio if column clogging is observed. Reduce sample input amount to 25–50 % of the maximum amount when testing an unknown sample for the first time.

2.4 Alternative lysis procedures

The most gentle way to lyse cells is an enzymatic lysis, assisted by detergents. Proteinase K is included in the kit and will work for many sample types like **body fluids, tissue, cultured cells** etc.. Nevertheless, many organisms develop a strong and stable cell wall which is difficult to lyse with Proteinase K and detergents alone.

If available, it is preferred to use dedicated enzymes to specifically digest cell walls. **Bacteria** might be lysed by **lysozyme** while **yeast** might be lysed by **zymolyase**. These enzymes are not included in the kit and are supplied by the user. **Please refer to the protocol supplied by the enzyme manufacturer / vendor** or contact MACHEREY-NAGEL (tech-bio@mn-net.com) if unsure how to use specialized lytic enzymes.

Please keep in mind that excess substrate concentrations (i.e., too much sample) might decrease the enzymatic reaction speed. The optimal ratio of enzyme to substrate (sample) and necessary cofactors must be determined empirically or according to instructions provided by the supplier of the enzyme.

Make sure the contact surface area between reaction buffer and sample is a high as possible and agitate the reaction mixture continuously if possible.

Buffer H1 will denature many proteins and reduce the reaction speed up to complete inhibition. Nevertheless the reaction speed was found to be sufficiently high for proteinase K, RNase A, zymolyase and lysozyme in Buffer H1. When using a different enzyme, always use a reaction buffer recommended for this enzyme. Refer to manufacturers' recommendations or literature for buffer composition and necessary sample to enzyme ratios as well as incubation conditions. Do not combine other enzymes with proteinase K. To prevent denaturation or degradation of those enzymes by the components of Buffer H1 (detergents, Proteinase K) it is necessary to perform the lysis in the reaction buffer supplied or recommended by the manufacturer before the addition of Buffer H1 and Proteinase K. Do not exceed a reaction volume larger than 2 mL and proceed with the protocol according to enzymatic lysis (protocol 5.1) after the lysis. **Enzymatic lysis** will yield average fragment lengths around **150 kb**, depending on the integrity of the sample material.

Plant cells (e.g., leaves or roots) express a strong cell wall which might be additionally coated by wax or lignin. If no specialized lytic enzymes are available, it is recommended to **grind the cells with mortar and pestle under liquid nitrogen**. For more convenient processing of multiple samples, bead beating with **MN Bead Tubes Type A** (not supplied, see ordering information, section 6.2) is recommended. Bead beating might also be an option for **bacteria** or **yeast cells**. Average fragment length might decrease by using a mechanical cell disruption down to around **50 kb**, but total yield will greatly increase.

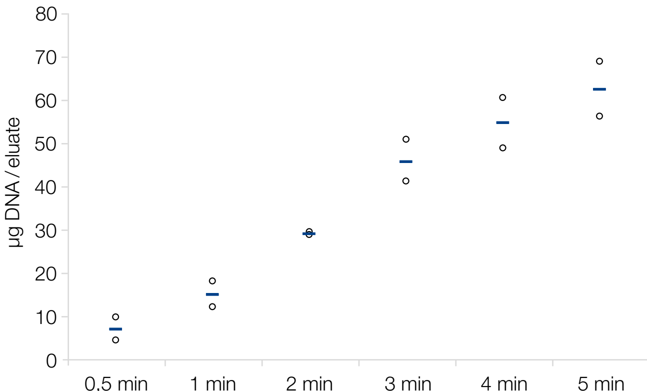

Figure 1 Total DNA yield isolated from 250 mg yeast cells by beat beating using a Retsch® Mill (MM 400) with MN Bead Tubes Type A. Longer bead beating times will increase the DNA yield (photometric quantification).

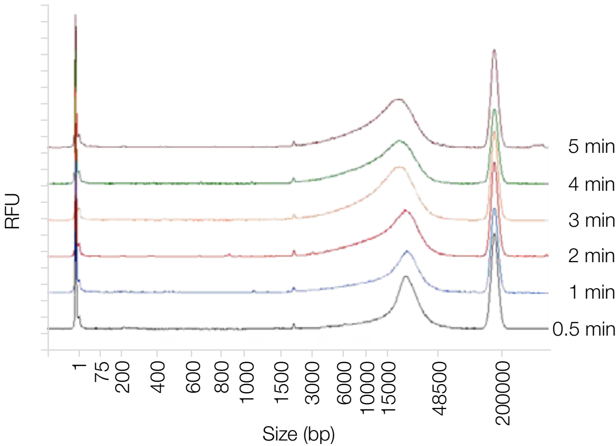

Figure 2 Size distribution of isolated DNA from figure 1. Increasing bead beating times will result in a higher percentage of fragmented DNA while maximal fragment length will not be influenced.

Make sure to lyse the cells completely as a large amount of unlysed cells will clog the columns, resulting in low flow rates and long preparation times.

Ultrasonic or high pressure devices for cell lysis will also result in decreased fragment lengths and are not recommended.

2.4.1 Lysis of bacteria with lysozyme

Preparation of lysozyme stock solution:

Weigh 100 mg of lysozyme (1 mg enzyme should correspond to 50–100 kU) into a DNase-free centrifuge tube and add 1 mL of Resuspension Buffer HE. Mix until the lysozyme is resuspended completely.

Note: 1 mL stock solution is sufficient for 10 reactions with approximately 20 mg bacterial wet weight per reaction each.

Store the prepared stock solution at -20°C for up to 6 months. Use of aliquots is recommended.

Enzymatic lysis:

Pellet bacteria, discard supernatant and determine the pellet wet weight.

Note: Bacteria grown overnight (~ 16 hours) in a complex medium under standard conditions reach approximately 5 mg wet weight in 1 mL culture volume.

Resuspend up to 20 mg bacteria in 1 mL of Lysis Buffer H1 (Volume of Lysis Buffer H1 can be adjusted proportionally to the pellet wet weight, e. g., resuspend a bacterial pellet of 30 mg wet weight in 1.5 mL Lysis Buffer H1).

Add 100 µL lysozyme stock solution for each mL of Lysis Buffer H1 (Volume of lysozyme stock solution can be adjusted proportionally to volume of buffer H1, e. g., add 150 µL lysozyme stock solution to 1.5 mL Lysis Buffer H1 with 30 mg bacterial cells).

Vortex well (5 s).

Incubate at 37 °C for 1 h, preferably under constant agitation (e. g., 1400 rpm on a thermoshaker).

Add fresh Lysis Buffer H1 to a total volume of 5 mL. Add 200 µL liquid Proteinase K and mix well.

Proceed with step 3 of the protocol.

2.4.2 Lysis of yeast with zymolyase

Preparation of zymolyase stock solution:

Weigh 100 mg of zymolyase (1 mg enzyme should correspond to 20–100 U) into a DNase-free centrifuge tube and add 1 mL of Resuspension Buffer HE. Mix until the zymolyase is resuspended completely.

Note: 1 mL stock solution is sufficient for 10 reactions with approximately 50 mg yeast wet weight per reaction each.

Store the prepared stock solution at -20°C for up to 6 months. Use of aliquots is recommended.

Enzymatic lysis:

Determine yeast wet weight.

Resuspend up to 50 mg yeast in 1 mL of Lysis Buffer H1 (volume of Lysis Buffer H1 can be adjusted proportionally to the pellet wet weight, e. g., resuspend a yeast pellet of 25 mg wet weight in 0.5 mL Lysis Buffer H1).

Add 100 µL zymolyase stock solution for each mL of Lysis Buffer H1 (volume of zymolyase stock solution can be adjusted proportionally to the pellet wet weight, e. g., add 50 µL zymolyase stock solution to 0.5 mL Lysis Buffer H1 with 25 mg yeast cells).

Vortex well (5 s).

Incubate at 37 °C for 2 h, preferably under constant agitation (e. g., 1400 rpm on a thermoshaker).

Add fresh Lysis Buffer H1 to a total volume of 5 mL. Add 200 µL liquid Proteinase K and mix well.

Proceed with step 3 of the protocol.

2.5 DNA clean up with Finisher columns

Anion exchange chromatography is a well established method to gain very pure DNA, free of contaminating macromolecules and with a very limited amount of shearing forces. Nevertheless, anion exchange chromatography requires large elution volumes with high salt elution buffers. As a result, the eluted DNA must be precipitated to desalt and concentrate the nucleic acids.

Isopropanol precipitation is the standard method, although it is labor and time intensive and there is a chance that the DNA pellet is lost during the washing step.

As an alternative the **NucleoSnap® Finisher** (vacuum based) or the **NucleoSpin® Finisher** (centrifuge based) can be used to capture precipitated DNA on a depth filter where it can easily be washed and eluted from in less than 5 minutes total clean up time. Use an elution volume of at least 100 µL and reload the eluates into the Finisher columns for a second and third round of elution.

3 Storage conditions and preparation of working solutions

- All kit components can be stored at 15–25 °C and are stable until: see package label.
- Once the enzymes have been opened it is recommended to store them at 4 °C.
- **CTAB in Buffer H1 may precipitate if stored at temperatures below 20 °C**. If a precipitate is observed in Buffer H1, incubate bottle at 50 °C for several minutes and mix well.

4 Safety instructions

When working with the **NucleoBond® HMW DNA** 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***).

The waste generated with the **NucleoBond® HMW DNA** 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 Protocol

5.1 Enzymatic lysis

Before starting the preparation:

- Check Lysis Buffer H1 for precipitated CTAB according to section 3.

---

1 Prepare sample

Prepare samples

- Pellet up to 10^7 cultured cells in a clean 50 mL centrifuge tube (not supplied).
- Cut **solid tissue** (e.g., biopsy blocks) into small pieces and transfer up to **300 mg** into a clean 50 mL centrifuge tube (not supplied).
- Pellet bacteria or yeast and determine wet weight.
- Use up to 20 mg bacteria per 1 mL of Lysis Buffer H1 (reaction volume can be adjusted) and add lysozyme (50–100 kU / mg) to a working concentration of 10 mg/mL.
  Incubate at 37 °C for 1 h (recommended: constant agitation)
  See chapter 2.4.1 for detailed information
  Use up to 50 mg yeast per 1 mL of Lysis Buffer H1 (reaction volume can be adjusted) and add zymolyase (20–100 U / mg) to a working concentration of 10 mg/mL.
  Incubate at 37 °C for 2 h (recommended: constant agitation)
  See chapter 2.4.2 for detailed information
- Add up to **2 mL liquid sample** (e.g., EDTA full blood or enzymatic reaction mixture) into a clean 50 mL centrifuge tube (not supplied).

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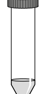
2 Add lysis buffer

Add **5 mL Lysis Buffer H1** and **200 µL Liquid Proteinase K** to each sample. In case of reactions with lysozyme or zymolyase, add Lysis Buffer H1 to a final volume of 5 mL).



5 mL H1

200 µL Proteinase K

Vortex 5s

If more sample material needs to be processed (e.g., due to a low DNA content of the samples), increase volumes proportionally in this step and step 6.

Vortex Tubes for **5 s**.

Take care that no large amounts of sample stick to the inner wall of the centrifuge tube.

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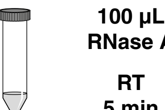
3 Lysis

50 °C
30 min

Incubate for **30 min** at **50 °C**.

Continuous careful agitation of the lysate is advantageous, but not necessary. Mix lysates by inversion or slow rotation from time to time.

4 RNA digestion



100 µL
RNase A

RT
5 min

Add **100 µL Liquid RNase A** to each sample and mix by inverting the tube several times.

Incubate for **5 min** at **room temperature**.

Proceed to the next step and equilibrate columns / filters during the incubation time.

5 Column equilibration

12 mL
H2

For each sample, combine a **NucleoBond® HMW Column** (including a filter) with a **Plastic Washer** and arrange the combination on a 50 mL tube or laboratory flask (not supplied).

For more convenient processing, use the NucleoBond® Xtra Rack or the NucleoBond® Smart Rack (not supplied, see ordering information).

Take care that the Plastic Washer does not seal the waste vessel air tight. If the connection is air tight an increasing positive pressure in the waste vessel might reduce the flow-rate dramatically. Discard waste flow through regularly!

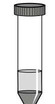
Equilibrate filters and columns with **12 mL Buffer H2**.

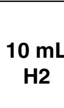
Add Buffer H2 on the upper rim of the column filters and make sure the complete filter and the silica matrix are prewet.

All steps involving the NucleoBond® HMW Column are performed with gravity flow. Do not use vacuum!

Discard flow through.

6 Adjust binding conditions





10 mL
H2

mix

Add **10 mL Buffer H2** to each sample and **mix by inverting** the tube.

Increase volume proportionally if lysis volume was increased in step 2.

7 Bind DNA

Load samples with any debris into the center of the column filters and let the lysate pass the silica matrix by gravity flow.

DNA and other polyanions will bind to the positively charged surface of the silica in this step.

Discard flow through.

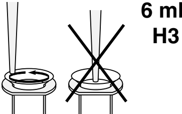
Load sample

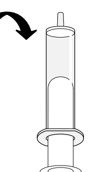
8 Flush filter column

Add **6 mL Buffer H3** to the rim of the column filters and let the buffer pass the filter and the silica.

Lysate which was trapped in the dead volume of the filter column is flushed onto the silica in this step.

Discard flow through and column filter once the buffer has passed the silica.



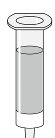


6 mL H3

9 Column wash

Wash the NucleoBond® HMW Column without filter with **12 mL Buffer H4**.

Discard flow through.

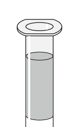


12 mL H4

10 DNA elution

Place the NucleoBond HMW column on top of a fresh 50 mL tube (not supplied).

Elute DNA with **5 mL Buffer H5**.



5 mL H5

11 Precipitate nucleic acids

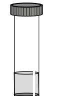
*Precipitation can be assisted by the **NucleoSnap®** (Vacuum based) or **NucleoSpin®** (centrifuge based) **Finisher** kits (see ordering information, section 6.2).*

Add **3.5 mL isopropanol** (not supplied) to each eluate.

Mix by **inverting the tube** 10 times.

Centrifuge at room temperature for **10 min** at **> 4,500 x *g***.

Carefully discard supernatant.



3.5 mL isopropanol

mix

4,500 x *g* 10 min

12 Wash DNA pellet

Add **2 mL 70 % ethanol** (not supplied) to each DNA pellet.

Centrifuge at room temperature for **5 min** at **> 4,500 x *g***.

Carefully discard supernatant completely.

Dry the pellet by incubation at room temperature until no ethanolic drops are visible anymore.

Do not over dry the pellet, as this might lead to difficulties in resuspension.

Avoid the use of vacuum concentrators as these may lead to over drying.

2 mL
70 % ethanol

4,500 x *g*
5 min

13 Resuspend nucleic acids

Add **150 µL Resuspension Buffer HE** to the DNA pellet und carefully resuspend the DNA by slow agitation. For complete resuspension, incubate samples in Buffer HE overnight.

Use wide bore pipette tips for all further downstream applications when handling the DNA.

Depending on the fragment length a complete resuspension will take some time.

The volume of Resuspension Buffer HE is variable and may be adapted.

150 µL
HE

5.2 Lysis with liquid nitrogen and mortar / pestle

Before starting the preparation:

- Check Lysis Buffer H1 for precipitated CTAB according to section 3.

---

1 Prepare sample

Homogenize samples

- Grind **plant leaves** under liquid nitrogen with mortar and pestle and transfer up to **1.5 g** to a clean, precooled 50 mL centrifuge tube (not supplied).
- Cut **solid tissue** (e.g., biopsy blocks) into small pieces, lyse by grinding under liquid nitrogen and transfer up to **300 mg** into a clean 50 mL centrifuge tube (not supplied).
- Pellet **bacteria or yeast** and grind the pellet under liquid nitrogen with mortar and pestle. Transfer up to **30 mg** into a clean 50 mL centrifuge tube (not supplied).
- Add up to **2 mL liquid sample** (e.g., EDTA full blood or enzymatic reaction mixture) into a clean 50 mL centrifuge tube (not supplied).

---

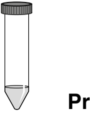
2 Add lysis buffer

Add **5 mL Lysis Buffer H1** and **200 µL Liquid Proteinase K** to each sample.

If more sample material needs to be processed (e.g., due to a low DNA content of the samples), increase volumes proportionally in this step and step 6.

Vortex Tubes for **5 s**.

Take care that no large amounts of sample stick to the inner wall of the centrifuge tube.



5 mL H1

200 µL Proteinase K

Vortex 5s

---

3 Lysis

Incubate for **30 min** at **50 °C**.

Continuous careful agitation of the lysate is advantageous, but not necessary. Mix lysates by inversion or slow rotation from time to time.

50 °C 30 min

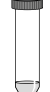
---

4 RNA digestion

Add **100 µL Liquid RNase A** to each sample and mix by inverting the tube several times.

Incubate for **5 min** at **room temperature**.

Proceed to the next step and equilibrate columns / filters during the incubation time.

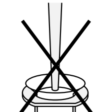


100 µL RNase A

RT 5 min

5 Column equilibration

For each sample, combine a **NucleoBond® HMW Column** (including a filter) with a **Plastic Washer** and arrange the combination on a 50 mL tube or laboratory flask (not supplied).



12 mL H2

For more convenient processing, use the NucleoBond® Xtra Rack or the NucleoBond® Smart Rack (not supplied, see ordering information).

Take care that the Plastic Washer does not seal the waste vessel air tight. If the connection is air tight an increasing positive pressure in the waste vessel might reduce the flow-rate dramatically. Discard waste flow through regularly!

Equilibrate filters and columns with **12 mL Buffer H2**.

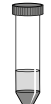
Add Buffer H2 on the upper rim of the column filters and make sure the complete filter and the silica matrix are prewet.

All steps involving the NucleoBond® HMW Column are performed with gravity flow. Do not use vacuum!

Discard flow through.

6 Adjust binding conditions

Add **10 mL Buffer H2** to each sample and **mix by inverting** the tube.



10 mL H2

mix

Increase volume proportionally if lysis volume was increased in step 2.

7 Bind DNA

Load samples with any debris into the center of the column filters and let the lysate pass the silica matrix by gravity flow.

Load sample

DNA and other polyanions will bind to the positively charged surface of the silica in this step.

Discard flow through.

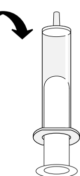
8 Flush filter column

Add **6 mL Buffer H3** to the rim of the column filters and let the buffer pass the filter and the silica.

Lysate which was trapped in the dead volume of the filter column is flushed onto the silica in this step.

Discard flow through and column filter once the buffer has passed the silica.

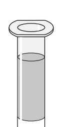
6 mL H3



9 Column wash

Wash the NucleoBond® HMW Column without filter with **12 mL Buffer H4**.

Discard flow through.

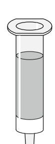


12 mL H4

10 DNA elution

Place the HMW column on top of a fresh 50 mL tube (not supplied).

Elute DNA with **5 mL Buffer H5**.



5 mL H5

11 Precipitate nucleic acids

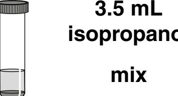
*Precipitation can be assisted by the **NucleoSnap®** (Vacuum based) or **NucleoSpin®** (centrifuge based) **Finisher** kits (see ordering information, section 6.2).*

Add **3.5 mL isopropanol** (not supplied) to each eluate.

Mix by **inverting the tube** 10 times.

Centrifuge at room temperature for **10 min** at **> 4,500 x *g***.

Carefully discard supernatant.



3.5 mL isopropanol

mix

4,500 x *g* 10 min

12 Wash DNA pellet

Add **2 mL 70 % ethanol** (not supplied) to each DNA pellet.

Centrifuge at room temperature for **5 min** at **> 4,500 x *g***.

Carefully discard supernatant completely.

Dry the pellet by incubation at room temperature until no ethanolic drops are visible anymore.

Do not over dry the pellet, as this might lead to difficulties in resuspension.

Avoid the use of vacuum concentrators as these may lead to over drying.

2 mL
70 % ethanol

4,500 x *g*
5 min

13 Resuspend nucleic acids

Add **150 µL Resuspension Buffer HE** to the DNA pellet und carefully resuspend the DNA by slow agitation. To increase the rate of DNA resuspension mix the sample by gently pipetting up and down using wide bore pipette tips.

Use wide bore pipette tips for all further downstream applications when handling the DNA.

Depending on the fragment length a complete resuspension will take some time.

The volume of Resuspension Buffer HE is variable and may be adapted.

Avoid exceeding a final DNA concentration of 200 ng/µL, as this can significantly increase the viscosity of the solution and lead to incomplete resuspension of the pellet-

150 µL
HE

5.3 Bead beating based cell lysis

Before starting the preparation:

- Check Lysis Buffer H1 for precipitated CTAB according to section 3.

---

1 Prepare sample by bead beating

Lysis of bacteria or yeast is enhanced by bead beating. For those sample types, bead beating is recommended. MN Bead Tubes Type A are recommended and need to be ordered separately (see ordering information, section 6.2). Bead beating will reduce the average fragment length but will increase the DNA yield.

Fill up to **300 mg** of sample material (e.g., yeast or bacteria) into a **Bead Tube Type A**.

Add **900 µL Lysis Buffer H1**.

Add **200 µL Liquid Proteinase K** and vortex briefly.

Connect the Bead Tube with a compatible shaker and shake for

- **5 min** at **30 Hz** or
- **30 s** at **5 m/s** or
- **5 min** at **full speed** horizontally on a flat bed vortexer

Optimal speed and times should be identified for each specific sample type separately with the above values as starting point.

The Vortex Genie 2 (Scientific Industries) can be combined with the MN Vortex Adapter (see ordering information).

Centrifuge Bead Tube for **2 min** at **11,000 x *g*** and **transfer supernatant into a clean 50 mL centrifuge tube** (not supplied). Use wide bore pipette tips for the transfer.

add 300 mg sample

900µL H1

200 µL Proteinase K

homogenize

11,000 x *g* 2 min

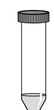
---

2 Add lysis buffer

Add **4 mL Lysis Buffer H1** to each sample.

Vortex Tubes for **5 s**.

Take care that no large amounts of sample stick to the inner wall of the centrifuge tube.



4 mL H1

Vortex 5s

3 Lysis

Incubate for **30 min** at **50 °C**.

Continuous careful agitation of the lysate is advantageous, but not necessary. Mix lysates by inversion or slow rotation from time to time.

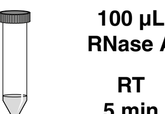
50 °C
30 min

4 RNA digestion

Add **100 µL Liquid RNase A** to each sample and mix by inverting the tube several times.

Incubate for **5 min** at **room temperature**.

Proceed to the next step and equilibrate columns / filters during the incubation time.



100 µL
RNase A

RT
5 min

5 Column equilibration

For each sample, combine a **NucleoBond® HMW Column** (including a filter) with a **Plastic Washer** and arrange the combination on a 50 mL tube or laboratory flask (not supplied).

For more convenient processing, use the NucleoBond® Xtra or NucleoBond® Smart Rack (not supplied).

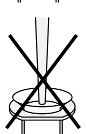
Take care that the Plastic Washer does not seal the waste vessel air tight. If the connection is air tight an increasing positive pressure in the waste vessel might reduce the flow-rate dramatically. Discard waste flow through regularly!

Equilibrate filters and columns with **12 mL Buffer H2**.

Add Buffer H2 on the upper rim of the column filters and make sure the complete filter and the silica matrix are prewet.

All steps involving the NucleoBond® HMW Column are performed with gravity flow. Do not use vacuum!

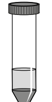
Discard flow through.



12 mL
H2

6 Adjust binding conditions

Add **10 mL Buffer H2** to each sample and **mix by inverting** the tube.



10 mL
H2

mix

7 Bind DNA

Load samples with any debris into the center of the column filters and let the lysate pass the silica matrix by gravity flow.

DNA and other polyanions will bind to the positively charged surface of the silica in this step.

Discard flow through.

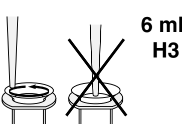
Load sample

8 Flush filter column

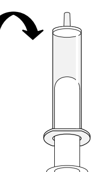
Add **6 mL Buffer H3** to the rim of the column filters and let the buffer pass the filter and the silica.

Lysate which was trapped in the dead volume of the filter column is flushed onto the silica in this step.

Discard flow through and column filter once the buffer has passed the silica.



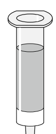
6 mL H3



9 Column wash

Wash the NucleoBond® HMW Column without filter with **12 mL Buffer H4**.

Discard flow through.

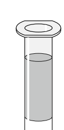


12 mL H4

10 DNA elution

Place the HMW column on top of a fresh 50 mL tube (not supplied).

Elute DNA with **5 mL Buffer H5**.



5 mL H5

11 Precipitate nucleic acids

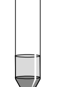
*Precipitation can be assisted by the **NucleoSnap®** (Vacuum based) or **NucleoSpin®** (centrifuge based) **Finisher** kits (see ordering information, section6.2).*

Add **3.5 mL isopropanol** (not supplied) to each eluate.

Mix by **inverting the tube** 10 times.

Centrifuge at room temperature for 10 min at **> 4,500 x *g***.

Carefully discard supernatant.



3.5 mL isopropanol

mix

4,500 x *g* 10 min

12	**Wash DNA pellet** Add **2 mL 70 % ethanol** (not supplied) to each pellet. Centrifuge at room temperature for **5 min at > 4,500 x *g***. Carefully discard supernatant completely. Dry the pellet by incubation at room temperature until no ethanolic drops are visible anymore. **Do not over dry the pellet, as this might lead to difficulties in resuspension.** Avoid the use of vacuum concentrators as these may lead to over drying.	**2 mL** **70 % ethanol** **4,500 x *g*** **5 min**
13	**Resuspend nucleic acids** Add **150 µL Resuspension Buffer HE** to the DNA pellet and carefully resuspend by pipetting up and down with wide bore pipette tips or careful vortexing at low speed. The higher the molecular weight, the longer it will take to bring DNA back into solution again. *Use wide bore pipette tips for all further downstream applications when handling the DNA.* **Depending on the fragment length a complete resuspension will take some time.** **The volume of Resuspension Buffer HE is variable and may be adapted.**	**150 µL** **HE**

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or low nucleic acid yield	*Sample material contained low amounts of DNA* • Plant material: use fresh, growing leaves, if available. Increase sample mass and volume of H1, Proteinase K and H2 proportionally. • Cultured cells: lyse cells quickly after removal of culture medium. Avoid long time periods with dry cell pellets *Too much sample input* • Depending on the sample the lysis buffer might be overloaded with the maximum amount of sample input. Use less sample material. • Take care that all of the sample is accessible to the lysis buffer. Agitate samples regularly and mix by inverting from time to time. Cut samples into as small pieces as possible and resuspend cells completely. Use of 2 mL reaction tubes is preferred over 1.5 mL reaction tubes and 50 mL reaction tubes are preferred over 15 mL reaction tubes. *Precipitated CTAB in buffer H1* • Check buffer H1 for precipitated CTAB prior to use. Incubate at 50°C for several minutes and mix well prior to use. • Mind the correct volume ratio of buffer H1 to H2 (1:2). *Elution insufficient* • All steps of the preparation should be performed at room temperature. Different ambient temperatures might alter the pH-value of the buffered solutions and influence the elution efficiency. *DNA was lost during isopropanol precipitation* • The DNA pellet is usually quite small. Take care not to discard the DNA pellet by accident when removing the supernatant after the centrifugation steps. Use the NucleoSpin® or NucleoSnap® Finisher to prevent loss of DNA pellets.

Problem	Possible cause and suggestions
Fragmented DNA	*Storage of sample material* • Use the sample material as freshly as possible. Freeze in liquid nitrogen and keep frozen at all times if sample acquisition and preparation cannot be perfomed succeedingly. • Do not let frozen samples thaw. Use prechilled materials for weighing the sample. • Add buffer H1 and Liquid Proteinase K as quickly as possible to the sample.
	DNase contamination • Always use material like pipette tips and centrifuge tubes that are certified free of DNase. • When using a customer supplied resuspension buffer, take appropriate measures to prevent introduction of DNases. • All steps of the preparation should be performed at room temperature. Different ambient temperatures might alter the pH-value of the buffered solutions and influence the washing efficiency.
	Treatment of resuspended DNA • Always use wide bore pipette tips for working with high molecular weight DNA solutions. Avoid repeated pipetting steps, avoid vortexing, avoid repeated freeze-thaw cycles, avoid exposure to UV light. Keep on ice but not frozen.
Slow/incomplete resuspension of the pellet	*Over drying of the pellet* • Avoid drying the pellet for too long. Remove as much supernatant as possible from the walls of the tube by pipetting. Avoid the use of vacuum concentrators *Resuspension too viscous* • Avoid exceeding a final DNA concentration of 200 ng/µL, as this can significantly increase the viscosity of the solution and lead to incomplete resuspension of the pellet.

Problem	Possible cause and suggestions
Ineffective enzymatic lysis of microbial samples	*Improper reaction buffer* • Use only the reaction buffer recommended by the supplier of the enzyme. Do not carry out enzymatic digestion in Lysis Buffer H1 because the components of the buffer will interfere with enzymatic activity. *Cell wall not susceptible to enzyme applied* • Make sure that your organism of interest can be efficiently lysed with the enzyme that you are applying. *Enzyme inactive* • Make sure that the enzyme is not expired and has been stored according to the recommendations of the supplier. *Wrong protocol for enzymatic digestion* • Make sure to use the protocol as suggested by the supplier of the enzyme *reaction buffer overloaded* • The reaction speed of enzymes depends on the substrate concentration. If the substrate concentration is too high (too much sample) the reaction speed is decreased. • Released high molecular weight DNA contributes to the sample viscosity. If the sample viscosity is too high the lysis efficiency drops as the distribution of the enzymes becomes inhomogeneous.
Inconsistent photometric readings	*Incomplete resuspension of the DNA pellet* • Dilute the sample.

6.2 Ordering information

Product	REF	Pack of
NucleoBond® HMW DNA	740160.20	20 preps
NucleoBond® Xtra Combi Rack	740415	1
NucleoBond® Smart Rack	740413	1
NucleoSnap® Finisher (Vacuum processing)	740434.10 / .50	10 / 50 preps
NucleoSpin® Finisher (Centrifuge processing)	740439.10 / .50	10 / 50 preps
Liquid RNase A	740397	500 preps
MN Bead Tubes Type A (0.6–0.8 mm ceramic beads)	740786.50	50
MN Bead Tube Holder	740469	1
NucleoVac 24 Vacuum Manifold	740299	1
Vacuum Regulator	740641	1
NucleoVac Valves	740298.24	24

6.3 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.

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Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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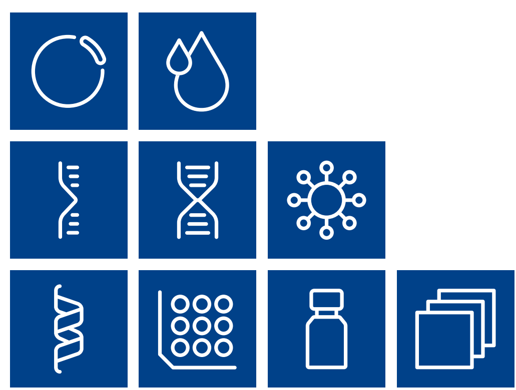

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