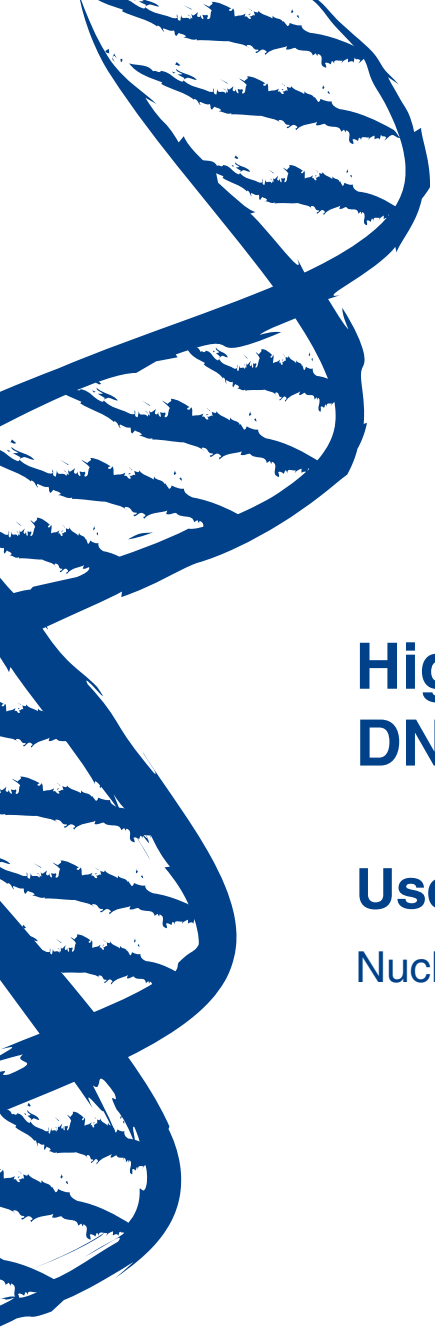


High molecular weight DNA

User manual

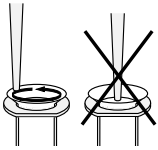
NucleoBond® HMW DNA

July 2018 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

High molecular weight DNA

Protocol at a glance (Rev. 01)

	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 5s 	5 mL H1 200 µL Proteinase K Vortex 5s 	4 mL H1 Vortex 5s 
3 Lysis	50 °C 30 min		
4 RNA digestion	100 µL RNase A RT5 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. 01)

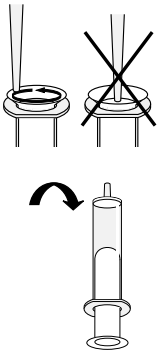
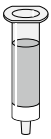
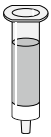
	NucleoBond® HMW DNA	
8 Flush filter column	6 mL H3	
9 Column wash	12 mL H4	
10 DNA elution	5 mL H5	
11 Precipitate nucleic acids	3.5 mL isopropanol mix 4,500 x *g* 10 min	
12 Wash DNA pellet	2 mL 70 % ethanol 4,500 x *g* 5 min	
13 Resuspend nucleic acids	150 µL HE	

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Table of contents

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
Manual	1

* 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)*
- *Optional: Enzymatic DNA Repair Kit to reduce amount of nicked DNA and to increase average read length in third generation sequencers*

Equipment

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

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. for third generation 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 gram positive bacteria, zymolyase for yeast, not supplied). Proteinase K will be active in Lysis Buffer H1, but other enzymes might be inactivated. 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.

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.

Alternatively, difficult to lyse samples like insects, yeast or bacteria can be homogenized by bead beating. Recommended in this case are the NucleoSpin® Bead Tubes Type A (not supplied, see ordering information, section 6.2). Mechanical disruption of cells using NucleoSpin® Bead Tubes Type A will decrease the mean fragment length and will result in maximum fragment lengths of below 100 kb with an average fragment length of about 50 kb. Nevertheless it is usually necessary to disrupt hard cell walls as this will result in a higher DNA yield, especially with yeast or bacteria. Furthermore bead beating it is more suitable to process many different samples at once in comparison to grinding.

Liquid samples like blood samples can be supplemented directly with the 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. 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 the Lysis Buffer H1 without mechanical disruption. Nevertheless the sample should be cut into small pieces. The smaller the tissue pieces are, 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.

Make sure to wash the filter as about 30% of the lysate volume will be trapped inside. 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 and other contaminants.

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. To ensure complete resuspenion, incubate the DNA pellets at least overnight in buffer HE.

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** kit 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** kit 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.

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
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 30 mg yeast or bacteria (ground under liquid nitrogen)
	Up to 300 mg yeast or bacteria (Lysed enzymatically or by bead beating)
	Up to 2 mL liquid sample, e. g., blood, body fluids or enzymatic reactions
Elution volume	50–250 µL
Preparation time	2 h/12 preps (including a 30 min lysis)
Typical yield	2–20 µg
Average fragment length	~ 150 kb (enzymatic lysis)
	~50 kb (mechanical lysis)

2.3 Working with high molecular weight DNA

DNA is easily sheared and fragmented by mechanical agitation. Be careful to handle isolated DNA with wide bore pipette tips and avoid shearing forces like vortexing or repeated pipetting steps if possible.

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

Depending on the lysis procedure 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.

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** and so on. 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. **Gram positive bacteria** might be lysed by **lysozyme** while **yeast** might be lysed by **lyticase** or **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.

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 **NucleoSpin® 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.

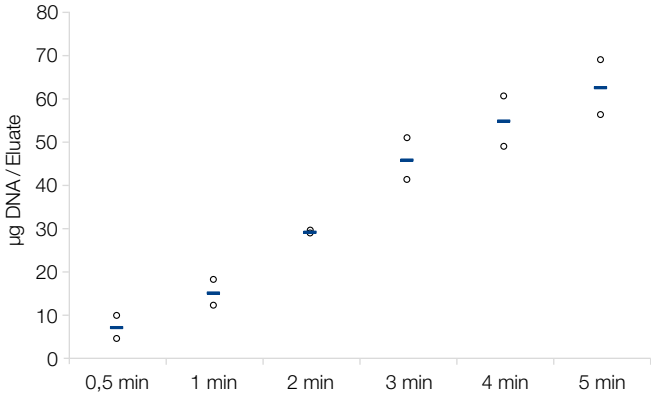


Figure 1 Total DNA yield isolated from 250 mg yeast cells by beat beating using a Retsch® Mill (MM 400) with NucleoSpin® Bead Tubes Type A. Longer bead beating times will increase the DNA yield (photometric quantification).

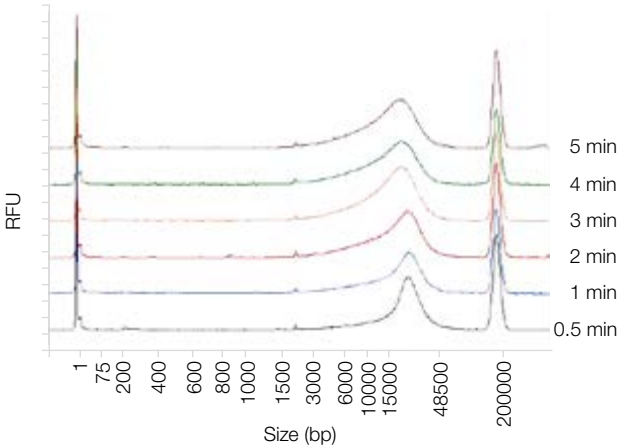


Figure 2 Size distribution of isolated DNA from figure 1. Increasing bead beating times will result in a higher percentage of fragmented DNA while maximal fragment length will not be influenced.

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.

2.5 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 room temperature (18–25 °C) and are stable for at least one year.
- **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

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS-Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
H2, H4, H5	ethanol 5–20 % *Ethanol 5–20 %* CAS 64-17-5d	 WARNING *ACHTUNG*	H226	P210

 The symbol shown on labels refers to further safety information in this section.
Das auf Etiketten dargestellte Symbol weist auf weitere Sicherheitsinformationen dieses Kapitels hin.

Hazard phrases

H 226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.

Precaution phrases

P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heissen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.

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**, transfer up to 300 mg into a 50 mL centrifuge tube (not supplied) and **lyse the cells with dedicated enzymes** (not supplied), e. g., use lysozyme for gram positive bacteria or zymolyase for yeast cells. Use the recommended reaction buffer and reaction conditions with a total volume of up to 2 mL.
- 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

5 mL H1

200 µL Proteinase K

Vortex 5s

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.

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

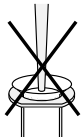
Add **100 µL Liquid RNase A** to each sample and mix by inverting the tube several times.

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 pre-wet.

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 sample

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.

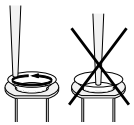
Discard flow through.

8 Flush filter column

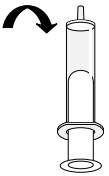
Add **6 mL Buffer H3** to the rim of the column filters and let the buffer pass the filter and the silica.

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.



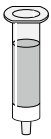
6 mL H3



9 Column wash

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

Discard flow through.

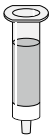


12 mL H4

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.

3.5 mL isopropanol

mix

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

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

4,500 x *g* 10 min

Carefully discard supernatant.

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***.

2 mL 70 % ethanol

4,500 x *g* 5 min

Carefully discard supernatant completely.

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

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

5 mL H1

200 µL Proteinase K

Vortex 5s

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.

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**.

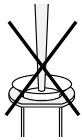
Proceed to the next step and equilibrate columns/ filters during the incubation time.

5 **Column equilibration**

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!



12 mL H2

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 pre-wet.

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.

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

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.

Load sample

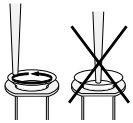
DNA and other polyanions will bind to the positively charged surface of the silica in this step.

Discard flow through.

8 **Flush filter column**

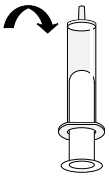
Add **6 mL Buffer H3** to the rim of the column filters and let the buffer pass the filter and the silica.

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



6 mL H3

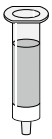
Discard flow through and column filter once the buffer has passed the silica.



9 **Column wash**

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

Discard flow through.

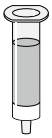


12 mL H4

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).*

3.5 mL isopropanol

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

mix

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

4,500 x *g*
10 min

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

Carefully discard supernatant.

12 Wash DNA pellet

2 mL
70 % ethanol

4,500 x *g*
5 min

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.

13 Resuspend nucleic acids

150 µL
HE

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.

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

add 300 mg sample

900µL H1

200 µL Proteinase K

homogenize

11,000 x *g* 2 min

Lysis of bacteria or yeast is enhanced by bead beating. For those sample types, bead beating is recommended. 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.

2 Add lysis buffer

4 mL H1

Vortex 5s

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.

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

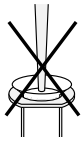
RT
5 min

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 pre-wet.

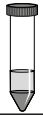
All steps involving the NucleoBond® HMW Column are performed with gravity flow. Do not use vacuum!

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**

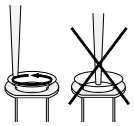
Load sample

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.

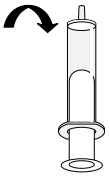
8 **Flush filter column**



6 mL H3

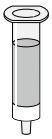
Add **6 mL Buffer H3** to the rim of the column filters and let the buffer pass the filter and the silica.

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.

9 **Column wash**

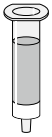


12 mL H4

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

Discard flow through.

10 **DNA elution**



5 mL H5

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

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

11 **Precipitate nucleic acids**

3.5 mL isopropanol

mix

*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.

4,500 x *g*
10 min

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

Carefully discard supernatant.

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.

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

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. *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 (18–25°C). 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 (18–25°C). 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.

6.2 Ordering information

Product	REF	Pack of
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
NucleoSpin® 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

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

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

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

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

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

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

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

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

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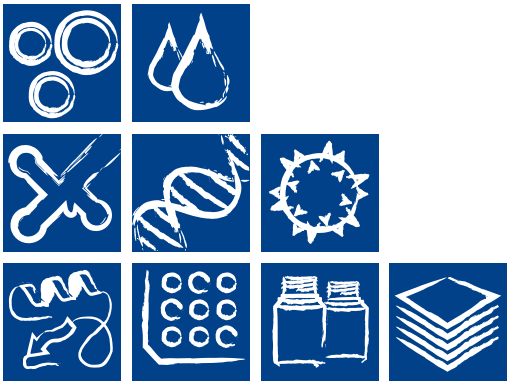

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