Bioanalysis

MACHEREY-NAGEL

NucleoBond® HMW DNA

Ultrapure, high molecular weight DNA

- High quality, high molecular weight (HMW) DNA up to 200 kbp
- Minimized DNA shearing due to established anion exchange technology
- Validated with diverse samples and sequencing platforms

High molecular weight DNA isolation from various samples

Isolation of high molecular weight DNA from various samples

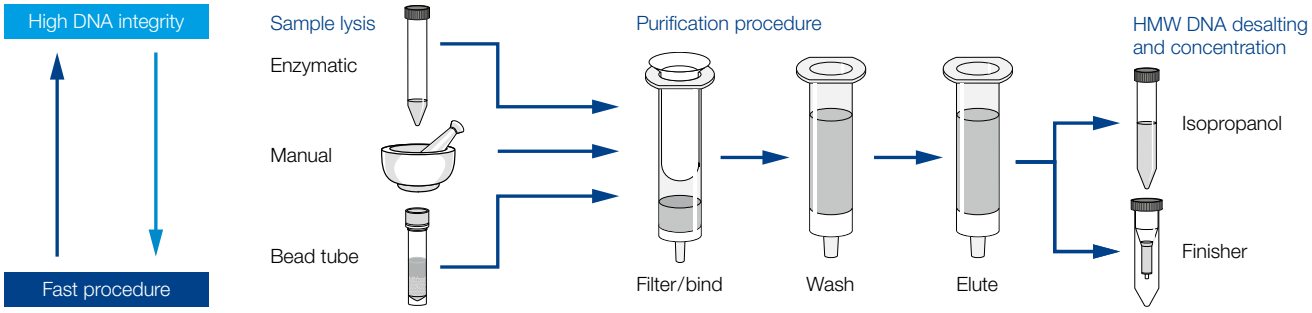
The new NucleoBond® HMW DNA kit enables isolation of large amounts of high quality DNA from up to 300 mg of microbial samples (gram positive and gram negative bacteria, yeast), 1.5 g of plant material, 300 mg of mammalian tissue, up to two mL of blood as well as other samples such as insects. The NucleoBond® gravity flow procedure minimizes shearing forces, preserving the high integrity of the DNA. Furthermore, the anion exchange technology results in high purity and inhibitor removal. This makes NucleoBond® HMW DNA ideal for the commonly used long-read sequencing technologies such as SMRT and Nanopore as well as long-range short-read methods such as Next GEM (10X Genomics).

The kit can be combined with NucleoSpin® Bead Tubes for fast homogenization of difficult to lyse samples. Alternatively, various commercially available enzymes can be used for prelysis. Apart from DNA precipitation using isopropanol which ensures optimal DNA integrity, NucleoSnap® Finishers or NucleoSpin® Finishers can be used for faster DNA desalting and concentration. The Finishers are applied on a vacum manifold or centrifuge, respectively.

Product at a glance

	Midi NucleoBond® HMW DNA
Technology	Anion exchange chromatography
Format	Midi gravity flow columns
Fragment size	Up to 200 kbp with > 90 % depletion of fragments < 10 kbp
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)
Typical yield	Depending on the sample amount and type
Elution volume	50–250 µL
Processing time	2 h/12 preps (including 30 min lysis)

Procedure



Universal core procedure

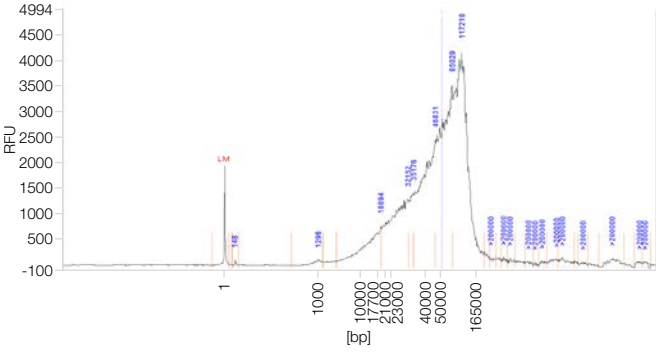
The NucleoBond® HMW DNA kit allows for diverse, sample specific lysis protocols. Following the lysis step, the lysate is loaded onto the NucleoBond® HMW DNA Column. The lysate is cleared in parallel with the binding thanks to the specially designed, disposable column filters. Following the sample binding step and discarding of the filter, a wash procedure ensures efficient removal of inhibitors and contaminants while avoiding any shearing of the HMW DNA. After elution, the DNA is desalted and concentrated following one of the several procedures that are compatible with the kit.

Custom protocol optimization

Depending on priorities, there are several options for workflow optimization. For high integrity and length of DNA, it is recommended to perform enzymatic prelysis of bacterial and yeast samples. For a faster procedure with intermediate DNA integrity, many samples can be homogenized in bead tubes (e.g., NucleoSpin® Bead Tubes) prior to lysis. Some samples, such as plant tissues, can also be homogenized with a mortar and pestle. Following the wash and elution steps, the user can choose a faster procedure for DNA desalting on a vacuum manifold (NucleoSnap® Finishers) or using a centrifuge (NucleoSpin® Finishers). Alternatively, for optimal DNA integrity, isopropanol precipitation is recommended.

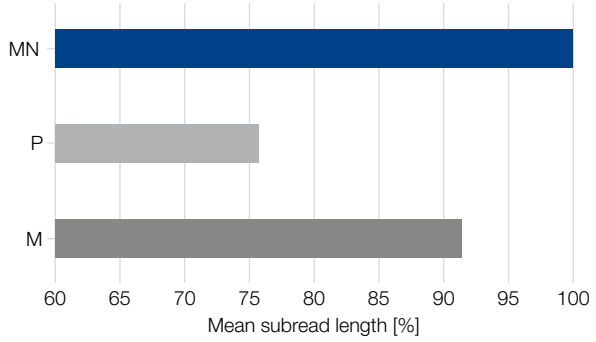
High molecular weight DNA isolation from various samples

Application data



Large fragments of DNA

DNA was purified from *Brassica sp.*, a plant species, by mechanical lysis. The purified DNA was then analyzed on a Femto Pulse System. Fragments up to 200000 bp could be detected with a peak at 117218 bp, demonstrating the ability of the NucleoBond® HMW DNA kit to extract and purify high molecular weight DNA.



High quality with market-leading results

DNA was purified from HeLa cells by enzymatic lysis and analyzed on a Single Molecule Real Time (SMRT) sequencing platform (PacBio RS II). The subread length of NucleoBond® HMW DNA (MN) was compared with two commonly used competitor products (kit P and kit M both from competitor Q). The use of NucleoBond® technology results in superior mean subread lengths, enabling longer reads and better sequencing results.

"For long read sequencing pipelines, the purity of genomic DNA is key to success. For Pacific Biosciences and Oxford Nanopore Technology, the commercially available NucleoBond® HMW DNA Kit from MACHEREY-NAGEL was very well suited to get this pure and long gDNAs which resulted in high gigabase yields when sequencing shotgun genomic libraries on the GridION, Nanopore or the Sequel, Pacific Biosciences."

(Dr. B. Hüttel, Max-Planck-Genome-centre Cologne)

Read length [kb]	Number of reads	Accumulative read length [bp]
Total	130.591	1.096.733.169
> 1 kb	75.906	1.066.775.188
> 10 kb	36.755	925.569.331
> 20 kb	21.874	705.439.290
> 30 kb	10.918	435.266.462
> 40 kb	4.361	209.403.539
> 50 kb	1.328	75.094.595
> 60 kb	276	18.362.150
> 70 kb	52	4.041.525
> 80 kb	11	1.019.289
> 90 kb	6	597.205
> 100 kb	2	215.960

Optimal procedure for longer reads

DNA was purified from *Brassica sp.* by mechanical lysis. The purified DNA was sequenced on a MinION® device (Oxford Nanopore). More than 1600 reads longer than 50 kbp, inlcuding some reads longer than 100 kbp, were sequenced, indicating the excellent integrity and quality of the high molecular weight DNA extracted with the kit.

Parameters	Liver	Blood	*B. subtilis*	Yeast	*A. thaliana*
µg DNA/eluate	45.9	59.4	205.2	39.8	51.8
A_{260}/A_{230}	2.31	2.39	2.21	2.43	2.14
A_{260}/A_{280}	1.86	1.86	1.82	1.76	1.80
ng/µL	176.0	253.4	547.2	254.1	146.9

Optimal results from various samples using NucleoBond® HMW DNA

In addition to molecular weight and integrity, purity and yield are critical for good results with HMW DNA sequencing and are often an issue with the common methods for HMW DNA extraction. Diverse samples were extracted using the NucleoBond® HMW DNA kit. All extractions yielded sufficient amounts of DNA for sequencing experiments. Absorbance ratios were $A_{260}/A_{280} > 1.75$ and $A_{260}/A_{230} > 2.00$, indicating high purity of the samples.

High molecular weight DNA isolation from various samples

Ordering information

Product	Specifications	Preps / Pack of	REF
NucleoBond® HMW DNA	NucleoBond® HMW Columns with inserted column filters, plastic washers, buffers, Liquid Proteinase K, Liquid RNase	2 / 20 preps	740160.2 / .20
NucleoSnap® Finisher Midi	For purification of eluates from NucleoBond® preparations: NucleoSnap® Finisher Columns, buffers, Collection Tubes	10 / 50 preps	740434.10 / .50
NucleoSpin® Finisher Midi	For purification of eluates from NucleoBond® preparations: NucleoSpin® Finisher Columns, buffers, Collection Tubes	10 / 50 preps	740439.10 / .50
MN Bead Tube Holder	To house up to 12 x 2 mL bead tubes in combination with a Vortex-Genie® instrument	1	740469
NucleoSpin® Bead Tubes Type A–G	Check *www.mn-net.com/beadtubes* for more information		
NucleoVac 24 Vacuum Manifold	Vacuum manifold for processing NucleoSnap® or NucleoSpin® columns	1	740299
NucleoVac Mini Adapter	Disposable adapters for processing NucleoSpin® or NucleoSnap® columns on the NucleoVac 24 Vacuum Manifold	100	740297.100
NucleoVac Valves (24)	Stop-cocks for processing samples with different flow rates on the NucleoVac 24 Vacuum Manifold	24	740298.24

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