



Genomic DNA from Tissue

User manual

NucleoSpin[®] 96 Tissue HC

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MACHERY-NAGEL



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1 Kit contents

Cat.No.	NucleoSpin® 96 Tissue HC	
	4 x 96 preps	24 x 96 preps
	740 750.4	740 750.24
Buffer T1	200 ml	2 x 600 ml
Buffer BQ1 ¹	100 ml	2 x 300 ml
Buffer B5 (concentrate) ¹	2 x 100 ml	6 x 200 ml
Buffer BW	2 x 125 ml	2 x 750 ml
Buffer BE	100 ml	6 x 100 ml
Proteinase K (lyophilized) ¹	4 x 75 mg	24 x 75 mg
Proteinase Buffer	15 ml	3 x 35 ml
NucleoSpin® Tissue HC Binding Plates (green)	4	24
Round-well Blocks ²	1	4
Adhesive Aluminum Foil	4	24
MN Square-well Blocks	8	48
Round-well Blocks (low)	8	48
Self-adhering Foil	4	24
Cap Strips	48	2 x 144
Protocol	1	1

¹ For preparation of working solutions and storage conditions see section 3.

² For prewarming elution buffer BE

2 Product description

2.1 The basic principle

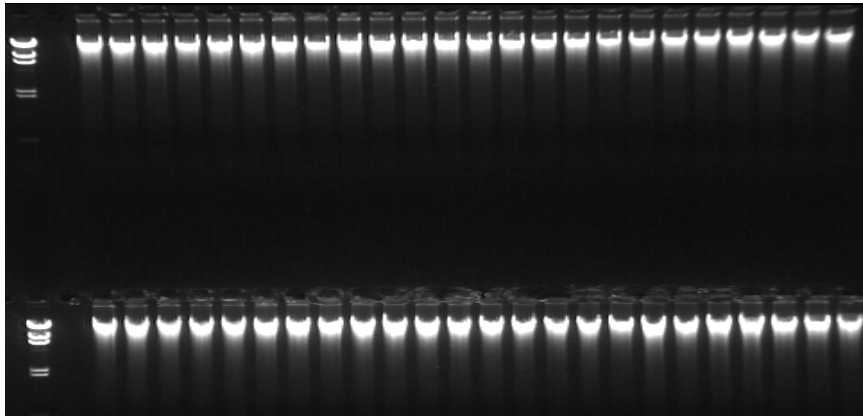
With the NucleoSpin[®] 96 Tissue HC method, lysis is achieved by incubation of the samples in a solution containing SDS and Proteinase K. Appropriate conditions for binding of DNA to the silica membrane in the NucleoSpin[®] 96 Tissue HC columns are created by addition of large amounts of chaotropic salt (buffer BQ1) and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- **NucleoSpin[®] 96 Tissue HC** is designed for the automated simultaneous preparation of highly pure genomic DNA from tissue samples, e.g. mouse and rat tails. The obtained DNA can be used directly as template for PCR, blotting, or any kind of enzymatic reactions.
- This kit provides reagents and consumables for purification of up to 40 µg (average 20 µg) of pure genomic DNA from up to 20 mg tissue samples with an $A_{260/280}$ ratio between 1.80 and 1.90 and a typical concentration of 100-200 ng/µl.
- From up to two 0.6 cm long³ mouse tail tip section (age of mice: 4 – 6 weeks), up to 35 µg of pure genomic DNA can be prepared (typical yields: 15 – 25 µg).
- **NucleoSpin[®] 96 Tissue HC** can be processed completely under centrifugation on suitable liquid handling instruments with centrifuge option.

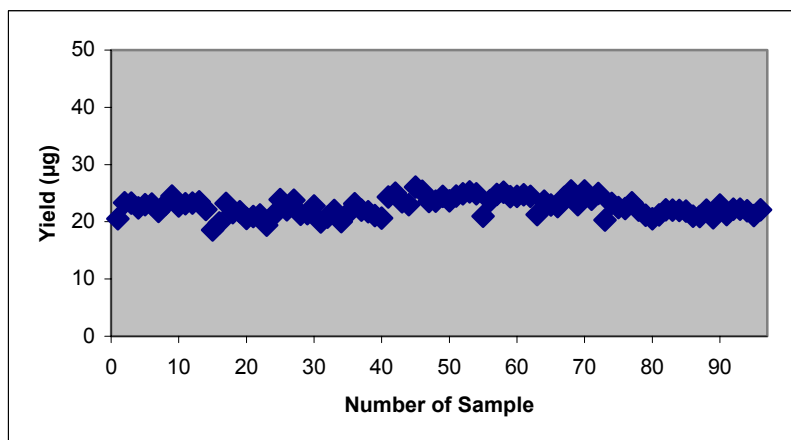
³ Please note that due to the legal situation in your country the size of tip section to be taken from one mouse might be limited to a smaller amount, e.g. 0.4 cm.

Kit specifications at a glance	
NucleoSpin® 96 Tissue HC	
Sample size	up to 20 mg
Average yield	20 µg
Binding capacity	40 µg
Elution volume	100 µl
Processing time for 2 plates	2 h (after lysis)



Genomic DNA has been isolated from 96 mouse tails (20 mg each) under centrifugation. Average yield is $22.61 \pm 1.58 \mu\text{g}$ (CV: 7%). 8 µl out of 200 µl eluate from 48 samples are shown on a 0.7% agarose gel.

Marker: λ HindIII



2.3 Elution procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 70 - 90 %) there are several modifications possible. Use elution buffer preheated to 70°C for one of the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90-100% of bound nucleic acid can be eluted.
- **High concentration:** Perform one elution step with 60% of the volume indicated in the individual protocol. Concentration of DNA will be ca. 30% higher than with standard elution. Maximal yield of bound nucleic acid is about 80%.
- **High yield and high concentration:** Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85-100% of bound nucleic acid is eluted in the standard elution volume at a high concentration.
- **Convenient elution:** For convenience, elution buffer of ambient temperature may be used. This will result in a somewhat lower yield (approximately 20%) compared to elution with heated elution buffer.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi-use storage at 4°C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in downstream applications we recommend elution with the supplied elution buffer and storage, especially long term, at -20°C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g. > 10kb) or detection sensitivity of trace amount of DNA species might be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at 4°C or room temperature due to shearing of DNA or adsorption to surfaces.

2.5 Automation

NucleoSpin® 96 Tissue HC is designed for fully automated purification of gDNA on laboratory workstations with integrated centrifuge (e.g. the PerkinElmer MultiProbe II HT EX with Gripper Integration Platform and integrated robotic centrifuge Hettich Rotanta 46 RSC). For the availability of scripts and general considerations about adapting **NucleoSpin® 96 Tissue HC** please contact MN.

Visit MN on the internet at www.mn-net.com or contact your local MACHEREY-NAGEL distributor for technical support regarding hardware, software, setup instructions and selection of the protocol.

3 Storage conditions and preparation of working solutions

Attention:

Buffers BQ1 and BW contain guanidinium hydrochloride! Wear gloves and goggles!

- All kit components can be stored at room temperature (20-25°C) and are stable up to one year.

Before starting any **NucleoSpin® 96 Tissue HC** protocol prepare the following:

- Add the indicated volume of 96 – 100 % ethanol to buffer B5 concentrate.
- Before first use of the kit, add the indicated volume of **Proteinase Buffer** to dissolve lyophilized **Proteinase K**. **Proteinase K solution is stable at +4°C for up to 6 months**. Storage at -20°C is recommended if the solution will not be used up during this period.

NucleoSpin® 96 Tissue HC		
	4 x 96 preps	24 x 96 preps
Cat. No.	740 750.4	740 750.24
Buffer B5 (concentrate)	2 x 100 ml add 400 ml ethanol to each bottle	6 x 200 ml add 800 ml ethanol to each bottle
Proteinase K (lyophilized)	4 x 75 mg add to each vial 2.6 ml Proteinase Buffer	24 x 75 mg add to each vial 2.6 ml Proteinase Buffer

4 Safety instructions – risk and safety phrases

The following components of the NucleoSpin® 96 Tissue HC kits contain hazardous contents.

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

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
BQ1	guanidine hydrochloride	Xⁿ Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	S 22
BW	guanidine hydrochloride	Xⁿ Xn*	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	S 22
Proteinase K	Proteinase K, lyophilized	Xⁿ Xn*	Irritating to eyes, respiratory system and skin, may cause sensitization by inhalation	R 36/37/38-42	S 22-24-26-36/37

Risk Phrases

- R 22 Harmful if swallowed
- R 36/37/38 Irritating to eyes, respiratory system and skin
- R 36/38 Irritating to eyes and skin
- R 42 May cause sensitisation by inhalation

Safety Phrases

- S 22 Do not breathe dust
- S 24 Avoid contact with the skin
- S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
- S 36/37 Wear suitable protective clothing and gloves

* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

5 General Procedure NucleoSpin® 96 Tissue HC

1 Lyse samples

2 × 0.6 cm mouse tail⁴
180 µl T1
(optional: 400 µl)

25 µl Proteinase K,
mix,
seal plate (aluminium foil
or cap strips)

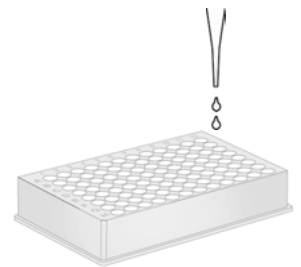
56°C, ≥ 6 h



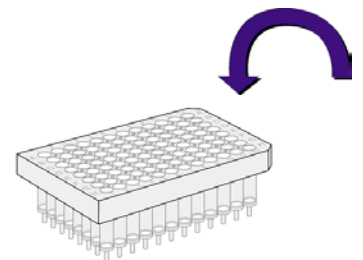
2 Adjust DNA binding conditions

200 µl lysate

200 µl BQ1
200 µl ethanol
mix

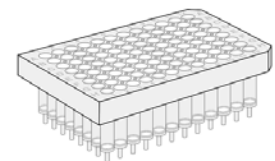


3 Transfer lysate to NucleoSpin®
Tissue HC Binding Plate (green)



4 Bind DNA to silica membrane

5,600 × g, 10 min



⁴ Please note that due to the legal situation in your country the size of tip section to be taken from one mouse might be limited to a smaller amount, e.g. 0.4 cm.

5 Wash silica membrane

500 µl BW
5,600 × g, 2 min

700 µl B5
5,600 × g, 2 min

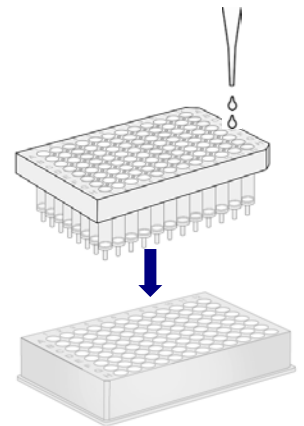
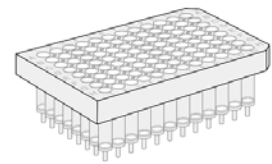
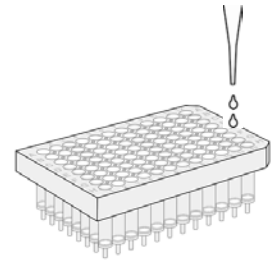
700 µl B5
5,600 × g, 2 min

Dry silica membrane

5,600 x g, 10 min

6 Elute highly pure DNA

100 µl BE, 70°C
5,600 × g, 2 min



5.1 Standard protocol for the purification of genomic DNA

Before starting with the preparation, set incubator or oven to 56°C. Equilibrate buffer BE to 70°C. Prepare buffer B5, and Proteinase K solution (see section 3).

For each preparation, cut up to two 0.6 cm-pieces⁵ (20 mg) of mouse tail into one well of a Round-well Block (low).

If preparing DNA from rat tails, one 0.6 cm-piece³ is sufficient.

1 Lyse samples

Prepare a Proteinase K working solution: For each sample, mix 25 µl Proteinase K with 180 µl T1 and vortex. Transfer 200 µl of the resulting solution to each well of the Round-well Block (low) containing the mouse tail sections. Close the individual wells with Cap Strips (supplied). Mix by vigorous shaking for 10 – 15 s. Spin briefly (15 s; 1,500 × g) to collect any sample at the bottom of the wells.

The mouse tails must be submerged in the solution. Never prepare the Proteinase K working solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in T1 without substrate.

Alternatively, seal the Round-well Block (low) after cutting the mouse tails into the wells with Adhesive Aluminium Foil (supplied with the kit). Dispense T1/ Proteinase K mix through the aluminium foil using steel needles. To compensate for evaporation during the subsequent incubation step increase volume of buffer T1 from 180 µl to 400 µl – 500 µl (Proteinase K solution is still 25 µl). The increased volume of buffer T1 is required to compensate for evaporation during the 6 h to overnight incubation step.

Incubate the Round-well Block (low) containing the samples at 56°C for at least 6 h or overnight until the mouse tails are completely lysed. For optimal lysis, mix occasionally during incubation.

After lysis, set the incubator to 70°C.

Centrifuge the Round-well Block (low) (15 s; 1,500 × g) to collect any condensate from the Cap Strips/Adhesive Aluminium Foil. Remove Cap Strips. Aluminium foil may also be pierced with tips during the subsequent step.

Residual hair and/or bones in the lysate can be settled by centrifugation (5 min; 5,600 – 6,000 × g)

Place a NucleoSpin® Tissue HC Binding Plate on an MN Square-well Block. If using more than one plate, label the plates for later identification.

⁵ Please note that due to the legal situation in your country the size of tip section to be taken from one mouse might be limited to a smaller amount, e.g. 0.4 cm.

2 Adjust DNA binding conditions

Mix 200 µl binding buffer BQ1 and 200 µl 96 – 100 % ethanol with 200 µl lysate of each sample. Binding buffer, ethanol and lysate are ideally aspirated consecutively with same 1 ml disposable tip before dispensing the mixture to the binding plate.

Ethanol and BQ1 can be premixed before addition to the samples, if the mixture is to be used up during the next 3 months.

If consecutive aspiration is not possible binding buffer, ethanol and sample can be premixed in a deep-well block (not included in kit).

3 Transfer lysate to NucleoSpin® Tissue HC Binding Plate

Dispense binding buffer, ethanol and lysate into the wells of the NucleoSpin® Tissue HC Binding Plate. Mix at least 3 times with 500 µl after dispensing the mixture to the NucleoSpin® Tissue HC Binding Plate.

4 Bind DNA to silica membrane

Place the NucleoSpin® Tissue HC Binding Plate on an MN Square-well Block and place both in the rotor buckets. Centrifuge at 5,600 – 6,000 × *g* for 10 min.

Typically, the lysates will have passed through the silica membrane within a few minutes. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.

5 Wash silica membrane

1st wash

add 500 µl BW to each well of the NucleoSpin® Tissue HC Binding Plate. Centrifuge again at 5,600 – 6,000 × *g* for 2 min.

2nd wash

add 700 µl B5 to each well of the NucleoSpin® Tissue HC Binding Plate. Centrifuge again at 5,600 – 6,000 × *g* for 2 min. Replace MN Square-well Block.

3rd wash

add 700 µl B5 to each well of the NucleoSpin® Tissue HC Binding Plate. Centrifuge again at 5,600 – 6,000 × *g* for 10 min.

During this last step, as much of ethanolic buffer B5 as possible is removed by centrifugation.

6 Elute highly pure DNA

Place NucleoSpin® Tissue HC Binding Plate on the Round-well Block (low). Dispense 100 µl pre-warmed buffer BE (70°C) to each well of the NucleoSpin® Tissue HC Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for 1 min. Centrifuge at 5,600 – 6,000 × *g* for 2 min. Remove the NucleoSpin® Tissue HC Binding Plate from the Round-well Block (low).

Elution buffer volume may be increased to 150 µl or elution step may be repeated.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or poor DNA yield	<p><i>Incomplete lysis</i></p> <ul style="list-style-type: none"> • Sample not completely submerged during heat incubation. Cut samples into small pieces. Mix well. Be sure that the samples are fully submerged in T1/Proteinase K mixture. Incubate until the samples are completely lysed. • Buffer T1 and Proteinase K premixed more than 15 min before addition to the substrate. Proteinase K tends to self digestion under optimal reaction conditions in buffer T1 without substrate.
	<p><i>Reagents not applied properly</i></p> <ul style="list-style-type: none"> • Prepare buffer B5 and Proteinase K solution according to instructions (see section 3). Add BQ1 and ethanol to the lysates before loading them to the wells of the NucleoSpin[®] Tissue HC Binding Plate.
	<p><i>Suboptimal elution of DNA from the column</i></p> <ul style="list-style-type: none"> • Preheat buffer BE to 70°C before elution. Apply buffer BE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically if elution is done with buffers with pH < 7. Use slightly alkaline elution buffer like buffer BE (pH 8.5).
	<p><i>RNA in sample</i></p> <ul style="list-style-type: none"> • If DNA free of RNA is desired, add 20 µl of an RNase A solution (20 mg/ml) before addition of buffer BQ1.
Suboptimal performance of genomic DNA in enzymatic reactions	<p><i>Carryover of ethanol</i></p> <ul style="list-style-type: none"> • Centrifuge ≥ 10 min at 5,600 – 6,000 x g in order to remove ethanolic buffer B5 and evaporate residual ethanol.
	<p><i>Contamination of DNA with inhibitory substances</i></p> <ul style="list-style-type: none"> • Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in buffer BE.

Problem	Possible cause and suggestions
	<p><i>Too much starting material</i></p> <ul style="list-style-type: none">• Repeat the procedure, using two mouse tail sections of maximally 4 – 6 mm length. If processing rat tails, one 0.6 cm-long tail tip section is sufficient.
	<p><i>Hair or bones left in the lysate after step 2</i></p> <ul style="list-style-type: none">• Centrifuge the Round-well Block (low) for 3 min at 5,600 – 6,000 × g. Transfer lysates to a new Round-well Block without disturbing the debris pellet.
Clogged wells	<p><i>Incomplete passage of lysate in step 4</i></p> <ul style="list-style-type: none">• If no more than 300-500 µl of lysate is remaining in the columns, continue with step 5. Through the addition of buffer BW the sample is diluted and thus the sample will pass the column more easily.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® 96 Tissue HC	740 750.4	4 x 96
NucleoSpin® 96 Tissue HC	740 750.24	24 x 96
Lyophilized Samples	740 766	1 set of 4 vials
Hardware Dummy Kit	740 767	1 kit
Lysis buffer T1	740 760.1	1 l
Binding buffer BQ1	740 761.1	1 l
Proteinase K	740 764	100 mg
RNase A	740 765	100 mg

6.3 References

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76**: 615-619.

6.4 Product Use Restriction / Warranty

NucleoSpin® 96 Tissue HC kit components were developed, designed and sold **for research purposes only**. They are suitable **for in vitro uses only**. Furthermore is no claim or representation intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather in the responsibility of the user to verify the use of the **NucleoSpin® 96 Tissue HC** kits for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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

MACHERY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of

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