Genomic DNA from Forensic Samples

User manual

NucleoMag 96 Trace

August 2006/Rev. 01

MACHEREY-NAGEL

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1 Kit contents

	NucleoMag 96 Trace		
	1 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
Cat. No.	**744 600.1**	**744 600.4**	**744 600.24**
NucleoMag B-Beads	1.7 ml	6.8 ml	40.8 ml
Lysis Buffer FLB	50 ml	200 ml	2 x 600 ml
Binding Buffer MB2	45 ml	2 x 90 ml	9 x 120 ml
Wash Buffer MB3	75 ml	300 ml	2 x 900 ml
Wash Buffer MB4	75 ml	300 ml	2 x 900 ml
Wash Buffer MB5	125 ml	500 ml	3 x 1000 ml
Elution Buffer MB6	25 ml	100 ml	2 x 300 ml
Proteinase K (lyophilized)	50 mg	4 x 50 mg	24 x 50 mg
Proteinase Buffer PB	3,6 ml	15 ml	3 x 35 ml
Protocol	1	1	1

Material to be supplied by user:

Product	**Cat. No.**	**Pack of**
Separation Plate for magnetic beads separation, e.g. Square-well Block, 96-well block with 2.1 ml square-wells	740670	20
Lysis Tubes for incubation of samples and lysis, e.g. MN Tubes Strips, 8-well microtube strips, 1.2 ml wells, rack with 12 strips each	740637	5 racks
Cap Strips for Lysis Tubes, 8-well strips of caps	740638	30 strips
Elution Plate for collecting purified DNA,		
e.g. 96-well 0.3 ml Microtiterplate, u-bottom	740672	20
e.g. 96-well 0.3 ml Microtiterplate, flat-bottom	740673	20

Material to be supplied by user (continued):

Product	Cat. No.	Pack of
For use of kit on KingFisher 96 instrument:		
KingFisher 96 Accessory Kit A Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag 96 Trace preps using King Fisher 96 platform	744950	1 set

2 Product description

2.1 The basic principle

The **NucleoMag 96 Trace** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Lysis is achieved by incubation of samples in a solution containing chaotropic ions in the presence of proteinase K at room temperature or 56°C. For the adjustment of binding conditions under which nucleic acids bind to the paramagnetic beads buffer MB2 and the NucleoMag B-Beads are added to the lysate. After magnetic separation the paramagnetic beads are washed twice to remove contaminants and salts using wash buffers MB3 and MB4. Residual ethanol from previous wash steps is removed by Wash Buffer MB5. Finally, highly purified DNA is eluted with low-salt Elution Buffer (MB6) and can directly be used for downstream applications. The **NucleoMag 96 Trace** kit can be used either manually or automated on standard liquid handling instruments or automated magnetic separators.

2.2 Kit specifications

NucleoMag 96 Trace is designed for rapid manual and automated small-scale preparation of highly pure genomic DNA from buccal swabs or other samples e.g. dried blood spots or cigarette filters. The kit is designed for use with NucleoMag 96 SEP magnetic separator plate (see ordering information) or other magnetic separation systems (see section 2.3). Manual time for the preparation of 96 samples is about 120 minutes. The purified DNA can be used directly as template for PCR, or any kind of enzymatic reactions.

NucleoMag 96 Trace allows easy automation on common liquid handling instruments or automated magnetic separators e.g. Thermo Electron´s KingFisher instruments. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 120 minutes using the NucleoMag SEP on the automation platform.

The kit provides reagents for the purification of up to 7 µg of pure genomic DNA from suitable samples (typical yields for DNA isolation from buccal swabs: 1-3 µg DNA) Depending on the elution volume used concentrations of 10–30 ng/µl can be obtained.

Following lysis of samples with proteinase K at 56°C (recommended, optional: proteinase K treatment can be performed at RT) **NucleoMag 96 Trace** can be processed completely at room temperature, however, elution at 56°C will increase the yield by about 15–20%.

NucleoMag B-Beads are highly reactive, superparamagnetic beads. The binding capacity is 0.4 µg of gDNA per 1 µl of NucleoMag-B-Bead Suspension, 1 µl of suspension contains 130 µg of beads.

2.3 Magnetic separation systems

For use of **NucleoMag 96 Trace** the use of the magnetic separator NucleoMag SEP is recommended. Separation is carried out in a Square-well Block (see ordering

information). The kit can also be used with other common separators. See suppliers ordering information for suitable separation plates.

Magnetic separator	Separation plate or tube
NucleoMag SEP (Cat. No. 744 900)	Square-well Block (MN, Cat. No. 740 670)
Tecan Te-MagS	1.5 ml tubes without lid (Sarstedt)

Static magnetic pins
Separators with static magnetic pins, e.g. NucleoMag SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a plate shaker, e.g. H+P Variomag® Teleshake (H+P Labortechnik AG, Bruckmannring 28, D-85764 Oberschleißheim, Germany, www.hp-lab.de), for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems
Separators with moving magnetic pins, e.g. Te-MagS (for automated use only): Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators
Separators with moving magnets, e.g. ThermoElectron´s King Fisher instruments: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps the speed settings have to be checked carefully for each specific separation plate and shaker to prevent cross-contamination from well to well. Proceed as follows:

Checking shaker speed for wash steps:

- load 600 µl dyed water to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again and use this setting for the washing step.

Checking shaker speed for elution step:

- apply 100-200 µl dyed water to the wells of the collection plate and proceed as described above.

2.5 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads to the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before distributing the beads make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. Premixing magnetic beads with the binding buffer allows easier homogenous distribution of the beads to the individual wells of the separation plate. During automation, a premix step before aspirating the beads/binding buffer mixture from the reservoir is recommended to keep the beads resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic pins depends on the magnetic strength of the magnetic pins, the selected separation plate, distance of the separation plate from the magnetic pins, and the volume to be processed. The individual times for complete attraction of the beads to the magnetic pins should be checked and adjusted on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

Washing the beads

Washing the beads can be achieved by shaking or mixing. In contrast to mixing by pipetting up and down mixing by shaker or magnetic mixing allows simultaneous mixing of all samples. This reduces the time and number of tips needed for the preparation. Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

Method	Resuspension efficiency	Speed	Small elution volume possible	Number of tips needed
Magnetic mix	+	++	+	Low
Shaker	++	++	+++	Low
Pipetting	+++	+*	++	High

* 8-channel pipetting device

2.6 Elution procedures

Purified genomic DNA can be eluted directly with the supplied Elution Buffer MB6. Elution can be carried out in a volume of ≥ 50 µl. It is essential to cover the NucleoMag Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g. the position of the pellet inside the separation plate). For efficient elution the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators high elution volumes might be necessary to cover the whole pellet.

Elution is possible at room temperature. However, the DNA yield can be increased by 15 – 20% if elution is performed at 56°C.

3 Storage conditions and preparation of working solutions

Attention:

Buffers MB2, MB3 and MB4 contain chaotropic salt! Wear gloves and goggles!

All components of the **NucleoMag 96 Trace** kit should be stored at room temperature (20-25°C) and are stable for up to one year.

All buffers are delivered ready-to-use.

Before starting **NucleoMag 96 Trace** protocol prepare the following:

- 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.

	NucleoMag 96 Trace		
	1 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
Cat. No.	**744600.1**	**744600.4**	**744600.24**
Proteinase K (lyophilized)	1 x 50 mg add 2.5 ml Proteinase Buffer	4 x 50 mg add 2.5 ml Proteinase Buffer to each vial	24 x 50 mg add 2.5 ml Proteinase Buffer to each vial

4 Safety instructions – risk and safety phrases

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
MB2, MB3, MB4	Sodium perchlorate + ethanol < 50%	 Xn*	Harmful if swallowed. Flammable	R 10-22	S 7-13-16-27
FLB	guanidine hydrochloride <10%	Substance does not have to be specially labeled as hazardous			
Proteinase K	Proteinase K, lyophilized	 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 10	Flammable.
R 22	Harmful if swallowed
R 36/37/38	Irritating to eyes, respiratory system and skin
R 42	May cause sensitization by inhalation

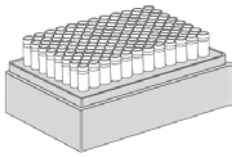
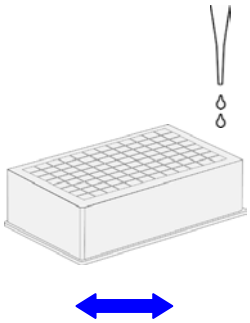
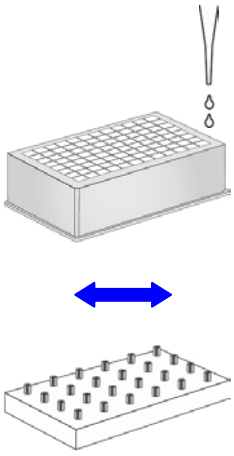
Safety Phrases

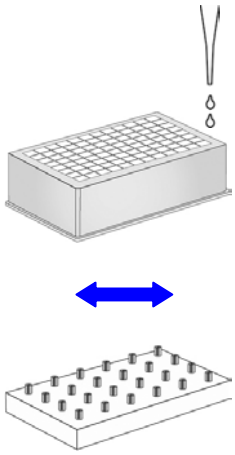
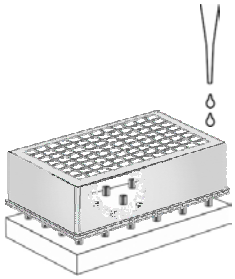
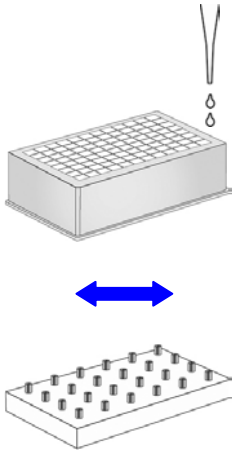
S7	Keep container tightly closed
S13	Keep away from food, drink and animal feeding stuffs
S16	Keep away from sources of ignition – No Smoking!
S 22	Do not breathe dust
S 24	Avoid contact with the skin
S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S 27	Take off immediately all contaminated clothing
S 36/37	Wear suitable protective clothing and gloves

* Label not necessary, if quantity below 50 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

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

5 General procedure

1	Lyse samples (e.g. buccal swabs).	**Add 25 µl proteinase K solution and 200-400 µl buffer FLB mix and incubate at 56°C, 1 h**	
2	Separate lysate from sample material, transfer 225 µl of lysate to a Square- well Block for further processing.		
3	Bind DNA to NucleoMag Beads.	**225 µl lysate 14 µl B-beads 360 µl MB2 shake 5 min at RT (optional: mix by pipetting up and down)**	
4	Remove supernatant.	**after 2 min separation remove supernatant**	
5	MB3 wash step.	**600 µl MB3 shake 5 min, RT (optional: mix by pipetting up and down) after 2 min separation remove supernatant**	

6	MB4 wash step.	**600 µl MB4** **shake 5 min, RT** **(optional: mix by pipetting up and down)** **after 2 min separation remove supernatant**	
7	MB5 wash step.	**900 µl MB5** **60 sec incubation** **aspirate and discard supernatant** ***Note: Do not resuspend beads in MB5 buffer***	
8	Elute genomic DNA and transfer to Elution Plate.	**50 – 200 µl MB6** **shake 5-10 min, RT** **(optional: mix by pipetting up and down)** **after 2 min separation transfer eluted DNA** **Optional: Elution at 56°C**	

5.1 Protocol for the purification of genomic DNA from buccal swabs

This protocol is designed for magnetic separators with static pins (e.g. NucleoMag SEP) and suitable plate shakers (e.g. H+P Variomag® Teleshake). This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Sample collection

Collect the samples with cotton, Dacron, or C.E.P. swabs. Scrape firmly against the inside of each cheek several times and let the swabs air dry.

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

Samples should be processed immediately or stored at 4°C.

1 Lyse samples

Calculate the amount of lysis stock required: for each sample 25 µl of proteinase K solution + 200 µl buffer FLB are required. Prepare lysis stock solution accordingly and vortex.

Never prepare the lysis stock solution more than 15 min before addition to the samples. Proteinase K tends to self digestion when incubated in FLB buffer without substrate.

Transfer 225 µl of the resulting solution to each lysis tube containing the buccal swab head. Close the individual tubes. Mix by vigorous shaking for 10 – 15 s. Spin briefly (15 s; 1,500 × *g*) to collect any sample at the bottom of the tube.

The buccal swab heads should be submerged into the lysis solution. Therefore, depending on type or size of buccal swab used the FLB buffer volume has to be increased to up to 400 µl. Increasing volume of proteinase solution is not required.

Alternatively perform lysis with buffer FLB / proteinase K in a NucleoSpin® Trace Filter Plate (see ordering information, page 18). This plate allows convenient separation of lysate from swab material by centrifugation and reduces loss of lysate.

Incubate the tubes containing the samples at 56°C for 1h or overnight at room temperature. For optimal lysis, mix occasionally during incubation. Make sure that the lysis tubes are securely closed.

Note: Other samples (e.g. dried blood spots, cigarette filters etc.) can be processed accordingly.

2 Separate swab material from lysed sample. Remove buccal swab and squeeze out to obtain 225 µl lysate.

When using increased volumes (>200 µl) of FLB lysis buffer in step 1 of the procedure transfer 225 µl lysed sample to a new Square-well block for further processing.

When using the NucleoSpin® Trace Filter Plate centrifuge the NucleoSpin Trace Filter plate stacked onto a 96 well Square-well Block for 5 min at 5,600 x g to draw the lysate out of the swab material.

3 To each lysate of 225 µl from the previous step add 14 µl of NucleoMag B-Beads and 360 µl of binding buffer MB2. Mix immediately by shaking for 5 min using a microplate shaker.

Alternatively, pipette up and down 10 times and incubate 5 min at room temperature.

Note: NucleoMag B-Beads and Binding Buffer MB2 can be premixed. Per well to be processed mix 14 µl of NucleoMag B-Beads with 360 µl Binding Buffer MB2. Vortex briefly. Depending on the dead volume of the reservoir, additional amounts of bead suspension and binding buffer are required.

Be sure to resuspend the NucleoMag B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

4 Separate the magnetic beads against the side of the wells by placing the separation plate on the magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. Remove supernatant from the opposite side of the well.

Remove the separation plate from the magnetic separator.

5 Add 600 µl Wash Buffer MB3 to each well and wash the bead/DNA complex by shaking (5 min) at room temperature.

Alternatively, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the separation plate on the magnetic separator (at least 2 min). Aspirate and discard the supernatant.

Remove the separation plate from the magnetic separator.

6 Add 600 µl Wash Buffer MB4 to each well and wash the bead/DNA complex by shaking (5 min) at room temperature.

Alternatively, pipette up and down 15 times.

Separate all of the magnetic beads against the side of the well by placing the separation plate on the magnetic separator (at least 2 min). Aspirate and discard the supernatant.

Leave the separation plate on the magnetic separator for step 7!

7 Add 900 µl Wash Buffer MB5 to each well and incubate for 60 s while the beads are still attracted on the magnet. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in Wash Buffer MB 5. This step is to remove traces of ethanol and eliminates a drying step. Resuspension of beads in buffer MB5 will decrease DNA yield.

8 Add desired volume of Elution Buffer MB6 (50 – 200 µl) to each well and resuspend the bead/DNA complex by shaking (5 – 10 min).

Alternatively, pipette up and down 10 times and incubate 5 – 10 min.

Separate the magnetic beads against the side of the wells by placing the separation plate on the magnetic separator. Wait 2 min until all the beads have been attracted to the magnet. Transfer the supernatant containing the purified genomic DNA to the Elution Plate.

Note: The yield can be increased by 15 – 20% by using prewarmed elution buffer (56°C) or by incubating the bead/elution buffer suspension at 56°C for 10 min.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Elution buffer volume insufficient* • Beads pellet must be covered completely with elution buffer *Insufficient performance of elution buffer during elution step* • Remove residual buffers during the separation steps completely. Remaining buffers decrease the efficiency of following wash and elution steps. *Beads dried out* • Do not let the beads dry as this might result in lower elution efficiencies. *Partial elution in Wash Buffer MB5 already* • Keep the beads on the magnet while dispensing Wash Buffer MB5. Do not resuspend beads in this buffer, and do not incubate beads in this buffer for more than 2 min, as this buffer is water-based and might elute the DNA already. *Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic bead pellet is not visible in the lysate. *Incubation after dispensing beads to lysate* • Mix immediately after dispensing NucleoMag Beads/Binding Buffer MB2 to the lysate. *Aspiration and loss of beads* • Time for magnetic separation too short or aspiration speed too high.

Problem	Possible cause and suggestions
Low purity	*Insufficient washing procedure* • Use only the appropriate combinations of separator and plate, e.g. MN Square-well Block in combination with NucleoMag SEP. • Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol from wash buffers* • Be sure to remove all of the ethanolic wash solution, as residual ethanol interferes with downstream applications. *Low purity* • see above
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well. *Aspiration speed too high (elution step)* • High aspiration speed during the elution step may cause bead carry over. Reduce aspiration speed for elution step.
Cross contamination	*Contamination of the rims* • Do not moisten the rims of the Square-well Block when transferring the tissue lysate. If the rim of the wells is contaminated, seal the Square-well Block with self-adhering PE foil (see ordering information) before starting the shaker.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoMag 96 Trace	744 600.1	1 x 96
NucleoMag 96 Trace	744 300.4	4 x 96
NucleoMag 96 Trace	744 300.24	24 x 96
NucleoMag SEP	744 900	1
Square-well Blocks	740 670	20
NucleoSpin Trace Filter Plate	740 677	20
Self-adhering PE foil	740 676	50 sheets
MN Tube Strips	740 637	5 sets
Cap Strips	740 638	30 strips
KingFisher 96 Accessory Kit A Square-well Blocks, Deep-well tip combs, Elution Plates for 4 x 96 NucleoMag 96 Trace preps using King Fisher 96 platform	740950	1 set

6.3 Product use restriction / warranty

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

It is rather the responsibility of the user to verify the use of the **NucleoMag 96 Trace** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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