NGS clean-up and size selection

User manual

NucleoMag® NGS Clean-up and Size Select

May 2014 / Rev. 01

MACHEREY-NAGEL
www.mn-net.com

Table of contents

1 Components

1.1 Kit contents

	NucleoMag® NGS Clean-up and Size Select		
	50–100 preps*	**250–500 preps***	**2500–5000 preps***
REF	**744970.5**	**744970.50**	**744970.500**
NucleoMag® NGS Bead Suspension	5 mL	50 mL	500 mL
User manual	1	1	1

** Note: The number of preps is calculated according to a sample volume of 50–100 µL and a ratio (bead suspension to sample) of 1.0.*

1.2 Equipment and consumables to be supplied by user

Reagents:

- 80% ethanol (non-denatured)
- Elution buffer (10 mM Tris-HCl (pH 8) or water)

Consumables:

- Disposable pipette tips

Equipment:

- Well calibrated pipettors
- Vortex mixer
- Magnetic separation system
  e.g., NucleoMag® SEP (REF 744900, see section 2.3)
- Separation plate for magnetic beads separation,
  e.g., 96-well 0.3 mL microtiterplate (Elution Plate U-bottom; REF 740486.24)
- Plate seal,
  e.g., Self-adhering PE Foil (REF 740676)

2 Product description

2.1 The basic principle

The **NucleoMag® NGS Clean-up and Size Select** is designed for rapid clean-up and size selection of DNA fragments in the library construction process for next generation sequencing (NGS). The NucleoMag® NGS Bead Suspension contains paramagnetic beads that are suspended in a special binding buffer. Paramagnetic beads selectively bind DNA fragments based on the volume ratio of bead suspension and sample. After magnetic separation and removal of supernatant, the beads are washed with ethanol. A short drying step is necessary to remove ethanol from previous washing steps. Finally, highly purified DNA fragments are eluted with low salt elution buffer or water that can be used directly for downstream applications. The purified DNA fragment library is free of any contaminants, such as nucleotides, primers, adapters, adapter dimers, enzymes, buffer additives, and salts. The **NucleoMag® NGS Clean-up and Size Select** kit can be used either manually or automated on standard liquid handling instruments.

2.2 Kit specifications

- **NucleoMag® NGS Clean-up and Size Select** is designed for rapid manual and automated clean-up and size selection of DNA fragments from a variety of reaction mixtures that are used in the library construction process for next generation sequencing, such as
  - Fragmentation mixtures
  - End-repair mixtures
  - A-tailing mixtures
  - Adapter ligation mixtures
  - PCR amplicifation mixtures
- The typical sample amount of double stranded DNA fragments is 5 ng to 1 µg.
- By using the tunable size selection method DNA fragment libraries with a size range of 150 bp to 800 bp can be produced.
- To assure accurate and precise pipetting the sample volume should be ≥ 50 µL.
- The **NucleoMag® NGS Clean-up and Size Select** can be processed completely at room temperature.

2.3 Magnetic separation systems

For use of **NucleoMag® NGS Clean-up and Size Select,** the use of the magnetic separator NucleoMag® SEP (see ordering information) is recommended. Separation is carried out in a 96-well microtiterplate with 300 µL u-bottom wells. The kit can also be used with other common separators.

2.4 Handling of beads

Liquid handling

Precise pipetting of the NucleoMag® NGS Bead Suspension and sample is essential for reliable results. Variations in volume will affect size selection performance. Therefore we recommend to use well calibrated pipettes and new tips after each well (single channel) or column (multichannel pipette). A good technique for pipetting the slightly viscous bead suspension is to pipette very slowly. Aspirate slowly and make sure that there are no liquid droplets on the outside of the tip and do not aspirate any air. Dispense slowly to ensure that the bead suspension is transferred completely into the wells.
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.

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 using the separation plates or tubes specified by the supplier of the magnetic separator.

Volume ratio

NucleoMag® NGS paramagnetic beads selectively bind DNA fragments based on the volume ratio of bead suspension and sample. In general, increasing the volume ratio will favor the adsorption of shorter fragments to the paramagnetic beads. This user manual exemplary presents the most commonly used protocols for distinct size range profiles that are optimal for NGS applications using Ilumina sequencing systems. By altering the volume ratio DNA fragment libraries with a size range of 150 bp to 800 bp for any sequencing platform can be produced. The NucleoMag® NGS Bead Suspension is similar to other well known producs in the market. Therefore you can use the same volume ratios that are recommended in your NGS library Kit preparation protocol.

3 Storage conditions and preparation of working solutions

- The **NucleoMag® NGS Clean-up and Size Select** kit is shipped at ambient temperature. The bead suspension should be stored at 4–8 °C upon arrival and is stable for up to six months under proper storage conditions.
- The NucleoMag® NGS Bead Suspension is delivered ready-to-use.

4 Safety instructions

The **NucleoMag® NGS Clean-up and Size Select** kit does not contain hazardous contents.

5 Protocols

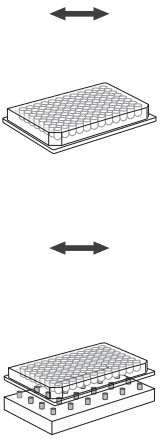
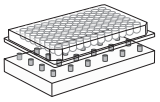
5.1 Protocol for DNA clean-up and single size selection

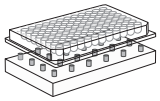
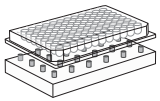
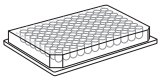
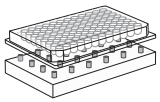
Protocol-at-a-glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 10.

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approxmately 30 min to bring the bead suspension to room temperature.

1	**Bind** target DNA to NucleoMag® NGS Beads	**Mix until suspension is homogeneous** **100 µL NucleoMag® NGS Beads** **100 µL DNA sample** **Mix by pipetting up and down** **Incubate for 5 min** **Remove supernatant after 5 min separation**	
2	**1st wash** with 80 % ethanol	Leave the 96-well plate on NucleoMag® SEP **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	

3	**2nd wash** with 80 % ethanol	Leave the 96-well plate on NucleoMag® SEP **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	
4	**Dry** the beads	**5–15 min at RT**	
5	**Elute** DNA	Remove the 96-well plate from NucleoMag® SEP **10–50 µL elution buffer** **Mix by pipetting up and down** **Incubate for 2–5 min** **Separate 5 min and transfer DNA into a new 96-well plate**	 

Detailed protocol

This protocol can be used to remove contaminants (such as, nucleotides, primers, adapters, enzymes, buffer additives, salts) and shorter DNA fragments from a sample. The method utilizes a single-size selection step (also called left side selection): After adding the appropriate volume of NucleoMag® NGS Bead Suspension to the DNA sample beads will bind larger fragments. The supernatant contains smaller fragments and contaminants that are discarded. For most NGS sequencing applications it is optimal to remove all fragments below 150–200 bp. This can be achieved by using a volume ratio (bead suspension to sample) of 1.0, which is described in the following protocol (e.g., add 100 µL of bead suspension to 100 µL of sample). To assure accurate and precise pipetting the sample volume should be ≥ 50 µL. However, volume ratio may be altered to fit the special application of the library construction process.

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approxmately 30 min to bring the bead suspension to room temperature.

---

1 Bind target DNA to NucleoMag® NGS Beads

Vortex the **NucleoMag® NGS Bead Suspension** well until it appears homogeneous in colour. Add **100 µL** of well dispersed **bead suspension** to each well of the separation plate.

Add **100 µL of DNA sample** (the volume ratio of bead suspension to sample is 1.0). Adjust the pipette to 200 µL and **mix by pipetting** up and down 10 times.

Incubate the separation plate at **room temperature** for **5 min**.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets or until the liquid appears clear.

Remove and discard supernatant by pipetting.

The supernatant contains unwanted low molecular weight contaminants and unwanted smaller DNA fragments.

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

---

2 1st wash with 80 % ethanol

Leave the 96-well plate on the magnetic separator during the following washing step.

Add **200 µL 80 % ethanol** without disturbing the pellet. **Incubate** the separation plate at room temperature for at least **30 s**. Carefully remove and discard supernatant by pipetting.

3 2nd wash with 80 % ethanol

Leave the 96-well plate on the magnetic separator during the following washing step.

Add **200 µL 80 % ethanol** without disturbing the pellet. **Incubate** the separation plate at room temperature for at least **30 s**. Carefully remove and discard supernatant by pipetting.

Note: remove supernatant completely, including residual droplets.

4 Dry the beads

Leave the 96-well plate on the magnetic separator and **incubate** at room temperature for **5–15 min** in order to allow the remaining traces of alcohol to evaporate.

Note: Allow the pellet to dry sufficiently that there are no visible droplets of the supernatant at the bottom of the wells. Do not overdry beads. Yield may decrease since longer DNA fragments will elute slower.

5 Elute DNA fragment library

Remove the 96-well plate from the NucleoMag® SEP magnetic separator.

Add **10–50 µL elution buffer** and **resuspend** the pellet by pipetting up and down 10 times or by shaking (e.g., at 1100 rpm using an eppendorf Thermomixer®).

Incubate the separation plate at room temperature for **2–5 min**.

Note: 10 mM Tris-HCl (pH 8) or water can be used as elution buffer.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets or until the liquid appears clear.

Transfer the supernatant containing the **purified DNA fragment library** to a new **96-well plate**. Proceed to the next step of your library preparation process.

5.2 Protocol for removing adapter dimers

This protocol can be used to remove adapter dimers after an adapter addition reaction.

The method utilizes two successive purification steps according to protocol 5.1.

In the first step a ratio (bead suspension to sample) of 1.0 is used to remove DNA precipitating agents from the ligation reaction buffer that interfere with the size selection process. The following step eliminates adapter dimers by using the same procedure but with a ratio of 0.8.

1 Exchange ligation reaction buffer

Perform purification procedure as described in 5.1 with a ratio of 1.0 and elute in 50 µL.

2 Remove adapter dimers

Perform purification procedure as described in 5.1, but with a ratio of 0.8 (to 50 µL of eluate from step 1, add 40 µL of NucleoMag® NGS Bead Suspension). Elute in 30 µL.

Proceed to the next step of your library construction process.

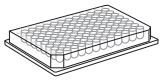
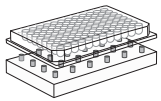
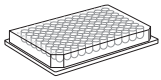
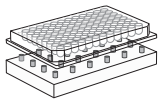
5.3 Protocol for DNA double size selection

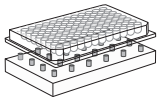
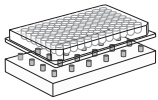
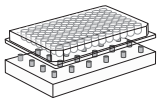
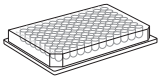
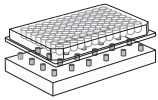
Protocol-at-a-glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 16.

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approxmately 30 min to bring the bead suspension to room temperature.

1	**Remove** unwanted larger DNA fragments	**Mix until suspension is homogeneous**	
		40 µL NucleoMag® NGS Beads **100 µL DNA sample**	
		Mix by pipetting up and down **Incubate for 5 min**	
		Remove and safe supernatant after 5 min separation **Transfer supernatant into a new 96-well plate** **Discard beads**	
2	**Remove** unwanted smaller DNA fragments	**20 µL NucleoMag® NGS Beads to supernatant of step 1**	
		Mix by pipetting up and down **Incubate for 5 min**	
		Remove and discard supernatant after 5 min separation	

3	**1st wash** with 80 % ethanol	Leave the 96-well plate on NucleoMag® SEP **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	
4	**2nd wash** with 80 % ethanol	Leave the 96-well plate on NucleoMag® SEP **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	
5	**Dry** the beads	**5–15 min at RT**	
6	**Elute** DNA	Remove the 96-well plate from NucleoMag® SEP **10–50 µL elution buffer** **Mix by pipetting up and down** **Incubate for 2–5 min** **Separate 5 min and transfer DNA into a new 96-well plate**	  

Detailed protocol

This protocol can be used to generate DNA fragment libraries with a certain size range or to narrow fragment-size distribution. The method is called double size selection, because both smaller and larger fragments can be removed. First, an appropriate volume of NucleoMag® NGS Bead Suspension is added to the DNA sample. This step enables binding of all DNA fragments longer than the desired upper limit of the interval. The beads with the unwanted larger DNA fragments are discarded (right side selection). The supernatant, which contains DNA fragments shorter that the upper length cut-off, is transferred to a new tube to perform the second size selection step (left side selection): More bead suspension is added to the supernatant, so that DNA fragments longer than the lower limit of the interval will be bound. After discarding the supernatant, DNA fragments within the desired size range are eluted.

The following protocol exemplifys size selection of DNA fragment libraries with a size range of 400-500 bp. By altering the volume ratios DNA fragment libraries with other size ranges can be obtained.

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approxmately 30 min to bring the bead suspension to room temperature.

---

1 Remove unwanted larger DNA fragments

Vortex the **NucleoMag® NGS Bead Suspension** well until it appears homogeneous in colour. Add **40 µL** of well dispersed bead **suspension** to each well of the separation plate.

Add **100 µL of DNA sample** (the volume ratio of binding buffer and bead suspension to sample is 0.4). Adjust the pipette to 140 µL and **mix by pipetting** up and down 10 times.

Incubate the separation plate at **room temperature** for **5 min**.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets or until the liquid appears clear.

Transfer the supernatant into the well of a new plate and discard the beads that contain the unwanted large fragments.

---

2 Remove unwanted smaller DNA fragments

Vortex the **NucleoMag® NGS Bead Suspension** well until it appears homogeneous in colour. Add **20 µL** of well dispersed **bead suspension** to each well containing supernatants from step 1 (the **total** volume ratio of binding buffer and bead suspension to the original sample is now 0.6; 40 µL and 20 µL to 100 µL). Adjust the pipette to 160 µL and **mix by pipetting** up and down 10 times.

Incubate the separation plate at **room temperature** for **5 min**.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets or until the liquid appears clear.

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.

3 1st wash with 80 % ethanol

Leave the 96-well plate on the magnetic separator during the following washing step.

Add **200 µL 80 % ethanol** without disturbing the pellet. **Incubate** the separation plate at room temperature for at least **30 s**. Carefully remove and discard supernatant by pipetting.

4 2nd wash with 80 % ethanol

Leave the 96-well plate on the magnetic separator during the following washing step.

Add **200 µL 80 % ethanol** without disturbing the pellet. **Incubate** the separation plate at room temperature for at least **30 s**. Carefully remove and discard supernatant by pipetting.

Note: Remove supernatant completely, including residual droplets.

5 Dry the beads

Leave the 96-well plate on the magnetic separator and **incubate** at room temperature for **5–15 min** in order to allow the remaining traces of alcohol to evaporate.

Note: Allow the pellet to dry sufficiently that there are no visible droplets of the supernatant at the bottom of the wells. Do not overdry beads. Yield may decrease since longer DNA fragments will elute slower.

6 Elute DNA fragment library

Remove the 96-well plate from the NucleoMag® SEP magnetic separator.

Add **10–50 µL elution buffer** and **resuspend** the pellet by pipetting up and down 10 times or by shaking (e.g., at 1100 rpm using a thermomixer).

Incubate the separation plate at room temperature for **2–5 min**.

Note: 10 mM Tris-HCl (pH 8) or water can be used as elution buffer.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets or until the liquid appears clear.

Transfer the supernatant containing the **purified DNA fragment library** to a new **96-well plate**. Proceed to the next step of your library preparation process.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Insufficient ratio* • Use volume ratios outlined in this manual, e.g., 1.0. *Insufficient ethanol concentration used for washing step* • Use freshly prepared 80 % ethanol. Over time ethanol becomes more dilute through evaporation and absorption of atmospheric water. As a consequence parts of the DNA pellet goes into solution and DNA fragments are washed away. *Elution buffer volume insufficient* • Bead pellet must be covered completely with elution buffer. *Incubation time for elution insufficient* • Incubate beads in elution buffer for 5 min for optimal yields. *Beads overdried* • Do not dry beads longer than 15 min at room temperature. Overdrying of beads may result in lower elution efficiencies.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol from washing step* • Be sure to remove all of the ethanol after the final washing step. Dry beads 5-10 min at room temperature.
Carry-over of beads	*Time for magnetic separation too short* • Increase separation time to allow the beads to be attracted to the magnetic pins completely. *Aspiration speed too high (elution step)* • High aspiration speeds during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

6.2 Ordering information

Product	REF	Pack of
NucleoMag® NGS Clean-up and Size Select	744970.5 744970.50 744970.500	1 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoMag® SEP	744900	1
Elution Plate U-bottom	740486.24	24
Self-adhering PE Foil	740676	50 sheets

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction/warranty

NucleoMag® NGS Clean-up and Size Select 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.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-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 MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

There is no warranty for and MACHEREY-NAGEL is not liable for damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; defects in products or components not manufactured by MACHEREY-NAGEL, or damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS.

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications

mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Last updated: 07/2010, Rev. 03

Please contact:
MACHEREY-NAGEL GmbH & Co. KG
Tel.: +49 24 21 969-270
tech-bio@mn-net.com