MACHEREY-NAGEL

User manual

Bioanalysis

NGS clean up and size selection

■ NucleoMag® NGS Clean-up and Size Select

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1 Components

1.1 Kit contents

	NucleoMag® NGS Clean-up and Size Select		
REF	**50 – 100 preps* 744970.5**	**250 – 500 preps* 744970.50**	**2500 – 5000 preps* 744970.500**
NucleoMag® NGS Bead Suspension	5 mL	50 mL	500 mL
Leaflet	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) RNase-free or water)

Consumables:

- Disposable pipette tips (Sterile RNase-free and filtered pipette tips recommended)

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)

1.3 RNase-free work environment

Kit components have been tested to ensure they are RNase-free. However, an RNase-free working environment is also a critical factor for performing successful RNA clean-up and handling. Therefore, general recommendations to avoid RNase contamination should be followed:

- Maintain a separate area, dedicated pipettors and materials when working with RNA.
- Wear gloves when handling RNA and reagents to avoid contact with skin, which is a source of RNases. Change gloves frequently.
- Use sterile RNase-free plastic ware.
- Use RNase-free water for elution.
- Keep all kit components sealed when not in use and all tubes tightly closed when possible.

1.4 About this user manual

It is strongly recommended that first time users of the NucleoMag®NGS Clean-up and Size Select kit read the detailed protocol sections of this user manual. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used as a tool for quick referencing while performing the purification procedure.

All technical literature is available at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The **NucleoMag® NGS Clean-up and Size Select** is designed for rapid clean up of RNA and DNA from enzymatic reactions 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 and RNA 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 or RNA fragments are eluted with low salt elution buffer or water that can be used directly for downstream applications. The purified DNA or RNA 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 and RNA 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
- cDNA synthesis
- in vitro transcription reactions
- RNA amplification

Clean-up of DNA: Typical recovery for DNA ≥ 80 %,

Clean-up of RNA: Typical recovery for RNA between 70 – 100 %

Size Selection: 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.

For research use only.

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, such as ring magnetic separators, suitable for microtiterplates or tubes.

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

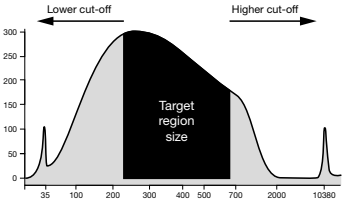
NucleoMag® NGS paramagnetic beads selectively bind DNA fragments based on the volume ratio of bead suspension and sample. In general, increasing the volume ratio will favor the adsorption of shorter fragments to the paramagnetic beads. This user manual exemplary presents the most commonly used protocols for distinct size range profiles that are optimal for NGS applications using Illumina 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 products in the market. Therefore, you can use the same volume ratios that are recommended in your NGS library Kit preparation protocol.

A

Recovery of DNA fragments [%]

Size (bp)	0.5 : 1	0.55 : 1	0.6 : 1	0.65 : 1	0.7 : 1	0.75 : 1	0.8 : 1	0.85 : 1	0.9 : 1	0.95 : 1	1 : 1
100	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	3	5	6	8	13
200	0	0	0	2	3	6	9	20	32	42	59
250	0	1	2	4	8	21	31	54	77	86	91
300	0	0	3	10	21	53	66	82	95	97	95
400	0	5	14	49	75	95	93	94	99	99	95
500	3	20	48	90	109	103	98	99	103	102	98
600	7	45	81	96	96	99	93	96	98	98	94
700	18	70	92	95	95	97	91	93	96	96	92
800	40	81	93	94	94	95	89	91	95	94	91
900	64	84	93	94	95	96	89	91	95	95	90
1000	80	83	91	93	94	95	88	90	94	94	89

B



NucleoMag® NGS Clean-up and Size Select procedure. (A) Recoveries of different fragment sizes. For DNA size selection 100 µL DNA (10 ng/µL) have been added to different volumes of NucleoMag® NGS Clean-up and Size Select beads to achieve the shown ratios (ratio = beads/sample). Input DNA contained fragment size from 100 bp to 1000 bp. The different recoveries of the used ratios (beads: input DNA) are shown in percentage [%]. **(B)** Size selection of fragment mix. For single side size selection (left or right), the sample is mixed with the beads in a certain ratio to exclude larger or smaller fragments until a chosen cut-off. For the double sided size selection two binding steps are performed, to exclude larger fragments above the cut-off and smaller fragments below the lower cut-off.

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 2 – 8 °C upon arrival and is under proper storage conditions stable until: see package label.
- 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.

When working with the **NucleoMag® NGS Clean-up and Size Select** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at http://***www.mn-net.com/msds***).

The waste generated with the **NucleoMag® NGS Clean-up and Size Select** kit has not been tested for residual infectious material. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocols

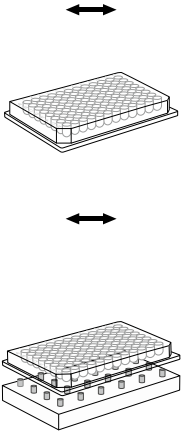
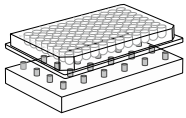
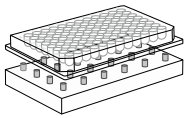
5.1 Protocol for DNA clean up and single size selection

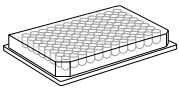
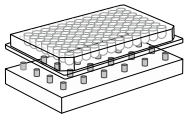
Protocol at a glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 12.

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approximately 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 magnetic separator **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	
3	**2nd wash** with 80 % ethanol	Leave the 96-well plate on magnetic separator **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 magnetic separator	
		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 (see chapter 2.4, page 7).

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approximately 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 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 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), water, or an MN elution buffer (e.g, Buffer BE, see ordering information) can be used as elution buffer.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the 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.

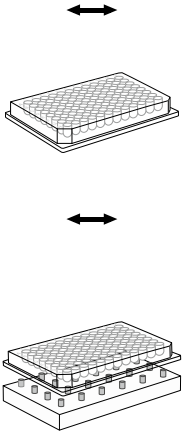
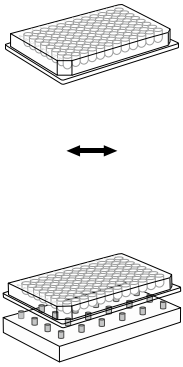
5.3 Protocol for DNA double size selection

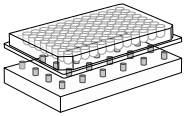
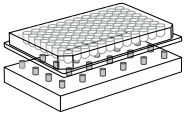
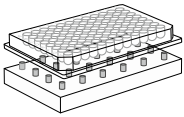
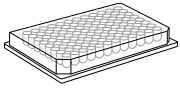
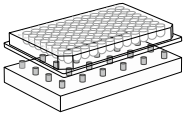
Protocol at a glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 17.

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approximately 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 magnetic separator **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	
4	**2nd wash** with 80 % ethanol	Leave the 96-well plate on magnetic separator **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 magnetic separator **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 exemplifies 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 (see chapter 2.4, page 7).

Before starting the preparation:

- Remove the NucleoMag® NGS Bead Suspension from the refrigerator. Let stand for approximately 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 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 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 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), water, or an MN elution buffer (e.g, Buffer BE, see ordering information) can be used as elution buffer.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the 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.

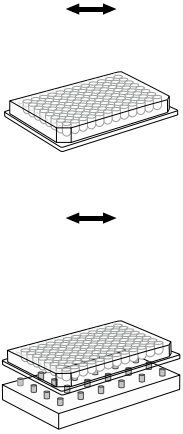
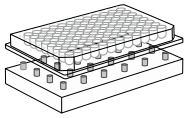
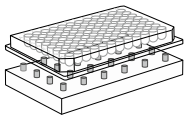
5.4 Protocol for PCR, DNA or RNA clean up

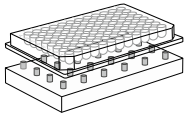
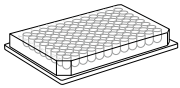
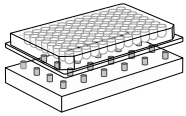
Protocol at a glance

- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 22.

Before starting the preparation:

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

1	**Bind** target DNA or RNA to NucleoMag® NGS Beads	**Mix until suspension is homogeneous** **180 µL NucleoMag® NGS Beads** **100 µL DNA or RNA 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 magnetic bead separator **200 µL 80 % ethanol** **Incubate for 30 s** Remove supernatant carefully	
3	**2nd wash** with 80 % ethanol	Leave the 96-well plate on magnetic bead separator **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 magnetic bead separator	
		10 – 50 µL elution buffer	
		Mix by pipetting up and down	
		Incubate for 2 – 5 min	
		Separate 5 min and transfer DNA or RNA into a new 96-well PCR plate	

Detailed protocol

This protocol can be used to remove contaminants (e.g., nucleotides, primers, adapters, enzymes, buffer additives, salts) from a enzymatic reaction mixtures. To ensure proper binding of nucleic acids, a bead to sample ratio of 1.8 should be used. We recommend that 96-well PCR plates and an appropriate ring-magnet magnetic separator will be used for the clean up.

Table 1: Some common enzymatic reaction volumes and suggested NucleoMag® NGS Bead Suspension volumes

Samples reaction volume	NucleoMag® NGS Bead Suspension volume
10 µL	18 µL
20 µL	36 µL
50 µL	90 µL
100 µL	180 µL

1 Bind target DNA or RNA to NucleoMag® NGS Beads

Vortex the **NucleoMag® NGS Bead Suspension** well until it appears homogeneous in color.

Add 1.8 vol. of NucleoMag® NGS Bead Suspension to each well of the plate. Check Table 1 for some suggested sample and bead volumes.

Note: In case of processing volumes larger than 200 µL, a larger mixing container, e.g. deep-well plate can be used.

Mix by shaking or preferably pipette mixing until the color of the mixture appears homogenous (e.g., 10 times).

Incubate the samples for **5 min** at **room temperature**

Separate the magnetic beads by placing the plate with samples on a suitable magnetic separator (depending on separation plate). Wait at least **2 min** until the beads have been attracted to the magnets.

Remove and discard supernatant by pipetting.

2 1st wash with 80 % ethanol

Leave the separation plate on the magnetic separator.

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

3 2nd wash with 80 % ethanol

Leave the separation plate on the magnetic separator.

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

4 Dry the beads

Leave the separation plate on the magnetic separator and **incubate** at room temperature for **5 – 15 min** in order to allow the remaining traces of ethanol 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 or RNA

Remove the separation plate from the magnetic separator.

Add **10 – 50 µL elution buffer** and **resuspend** the pellet by pipette mixing (e.g. 10 times).

Note: Depending on the expected total yield of DNA or RNA higher elution volumes such as 100 µL or more can be used.

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

Separate the magnetic beads by placing the plate with samples on a suitable magnetic separator (depending on separation plate). Wait at least **2 min** until the beads have been attracted to the magnet.

Transfer the supernatant containing the purified DNA or RNA to a new plate.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA / RNA yield	*Insufficient ratio* • Use volume ratios outlined in this manual, e.g., 1.0. (see chapter 2.4, page 7) *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.
Low RNA recovery / degraded RNA	• RNase contamination • Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, RNase-free consumables is recommended. Keep tubes or plates closed whenever possible during the preparation. Glassware should be oven baked for at least 2 hours at 250 °C before use or treated with RNase decontamination solutions.
Unexpected ratio A_{260}/A_{280}	• RNA type. Ratio A_{260}/A_{280} is base dependent. E.g. Poly-A+ RNA has an A_{260}/A_{280} ratio of 3.3 – 3.7 and an A_{260}/A_{230} ratio of 3.5 – 4.1. Consider RNA base composition for interpretation of absorbance ratios.
Suboptimal performance of DNA / RNA 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.

Problem	Possible cause and suggestions
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	5 mL 50 mL 500 mL
NucleoMag® SEP	744900	1
Elution Plate U-bottom	740486.24	24
Self-adhering PE Foil	740676	50 sheets
RNase free water	740378.1000	1000 mL

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

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL's employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

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Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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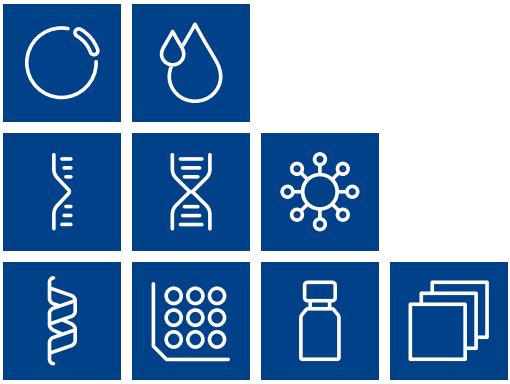
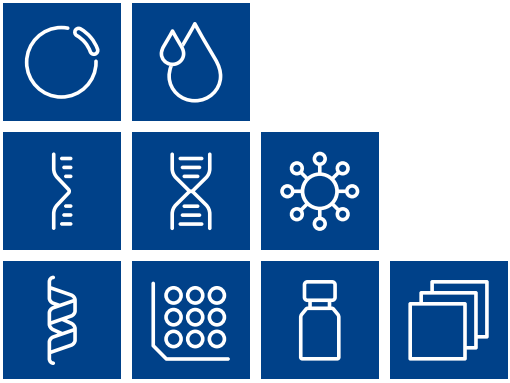


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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