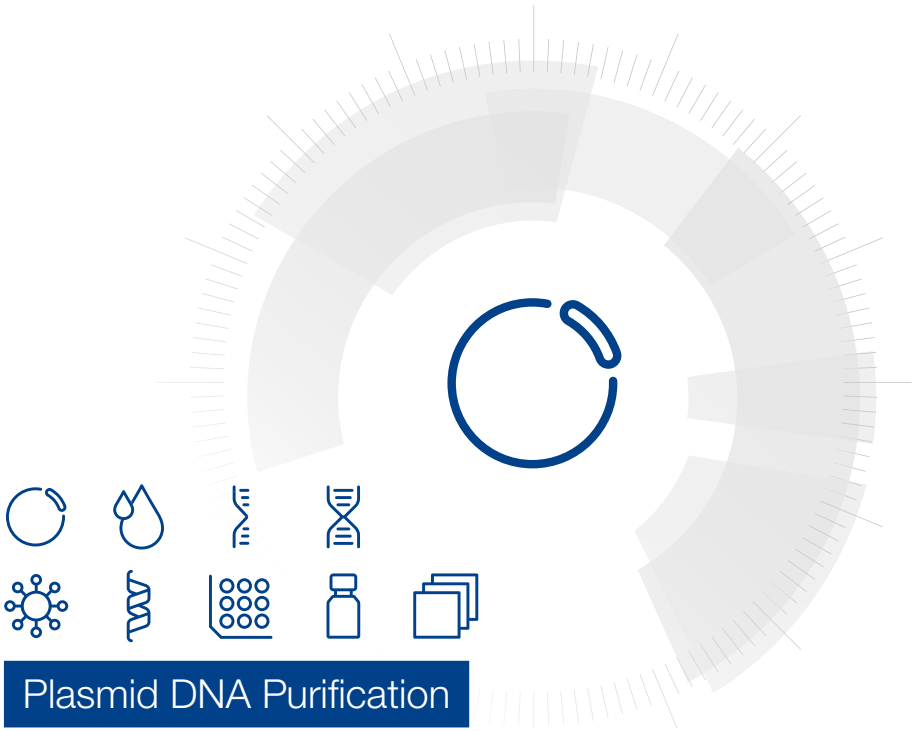
Bioanalysis

MACHEREY-NAGEL

User manual



Plasmid DNA Purification

- NucleoMag® Plasmid

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1 Components

1.1 Kit contents

	NucleoMag® Plasmid	
REF	**1 × 96 preps 744750.1**	**4 × 96 preps 744750.4**
Resuspension Buffer A1	15 mL	75 mL
Lysis Buffer A2	15 mL	100 mL
Neutralization Buffer S3	25 mL	100 mL
Binding Buffer PAB	2 × 35 mL	300 mL
NucleoMag® M-Beads	2 × 1 mL	8 × 1 mL
Detoxification Buffer ERB	2 × 125 mL	2 × 400 mL
Wash Buffer AQ (Concentrate)*	2 × 25 mL	2 × 100 mL
Elution Buffer AE**	30 mL	60 mL
RNase A (lyophilized)*	12 mg	60 mg
Leaflet	1	1

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (non-denatured)
- Lysate clarification via magnetic beads: NucleoMag® Clearing Beads (REF: 744751.1)
- Endotoxin-free water H_2O-EF (REF 740798.1) or ddH_2O

Consumables

Lysate clarification via centrifugation

- 2 mL microcentrifuge tubes for sample lysis

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

** Composition of Elution Buffer AE: 5mM Tris/HCl, pH 8.5

or

- 96 deep-well plate suitable for centrifugation (e.g. Square well Block REF: 740481)
- 96 deep-well plate suitable for the respective magnetic rod instrument
- Rod sleeves/Tip combs suitable for the respective magnetic rod instrument

KingFisher™ Flex

- 96-well Accessory Kit A for KingFisher™ (REF: 744950)

IsoPure™ Mini / MagnetaPure32 Plus

- 96-Deep-Well plates for magnetic rod systems (REF: 744955)
- 8-well Tip Combs for magnetic rod systems (REF: 744960)

For use on compatible liquid handling instruments

- Magnet for magnetic beads separation e.g. NucleoMag® SEP (REF: 744900)
- Separation plate for magnetic beads separation e.g. Square-well Bock (REF 740481 / .24)

Equipment

- 8-channel pipette or dispenser pipette for setup of reagent plates

Lysate clarification via centrifugation

- Centrifuge for microcentrifuge tubes

or

- Centrifuge with swing-bucket rotor capable of at least 2,000 × *g* (optimal 4,000 × *g*) in combination with the compatible rotor and rotor buckets for 96-well deep-well plates

1.3 About this user Manual

It is strongly recommended that first time users of the **NucleoMag® Plasmid** 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 Basic principle

The **NucleoMag® Plasmid** procedure utilizes a modified alkaline lysis protocol in combination with the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Pelleted bacteria are resuspended in Buffer A1. Plasmid DNA is liberated from the cells by Lysis Buffer A2 followed by a subsequent neutralization and precipitation of the lysate using Buffer S3. The crude lysate can be cleared either by centrifugation or using NucleoMag® Clearing Beads, specialized paramagnetic beads for lysate clearing. For binding of nucleic acids to the paramagnetic beads, Binding Buffer PAB and the NucleoMag® M-Beads are added to the cleared lysate. After magnetic separation, endotoxins and proteins are removed by the patented Detoxification Buffer ERB. Further contaminations such as salts or residual ethanol are removed with Wash Buffer AQ and air drying. Pure plasmid DNA is eluted with low-salt elution buffer or water and is ready for any common downstream application including transfection (research use only). The **NucleoMag® Plasmid** kit has been designed for the use on automated magnetic rod-systems.

2.2 Kit specifications

NucleoMag® Plasmid kit is designed for the rapid automated small-scale purification of transfection-grade plasmid DNA from *E. coli* using magnetic-rod systems. The kit allows for easy automation on common magnetic rod instruments such as KingFisher™ systems, MagnetaPure32 Plus, IsoPure Mini or others.

Table 1: Kit specifications at a glance

Parameter	NucleoMag® Plasmid
Technology	Magnetic bead technology
Format	Magnetic beads
Sample material	≤ 5 mL E. coli culture
Typical yield	1 – 50 µg, depending on copy number, plasmid size, culture media, culture condition and bacterial host strain
Typical purity	A_{260}/A_{280}: ≥ 1.8
	A_{260}/A_{230}: ≥ 2.0
Typical Endotoxin level	Lysate clearance via centrifugation – 3 wash steps: ≤ 50 EU/µg
	Lysate clearance via centrifugation – 4 wash steps: ≤ 10 EU/µg
	Lysate clearance via magnetic beads: ≤ 50 EU/µg

Table 1: Kit specifications at a glance	
Vector size	< 25 kbp
Elution volume	50 – 200 µL
Preparation time	Depending on instrument type, script and configuration
Processing	Automated
Use	For research use only

2.3 Automated processing on robotic platforms

The NucleoMag® Plasmid kit has been designed for the use on magnetic rod-based instruments, such as the KingFisher™ systems, Auto-Pure, MagnetaPure32 Plus or IsoPure™ instruments.

The use of the NucleoSpin® 8 / 96 Plasmid (Transfection-grade) kits is recommended in combination with liquid handling platforms equipped with vacuum manifolds or a positive pressure unit, due to the lower tip consumption, full automation and processing time.

The NucleoMag® Plasmid kit can also be used on liquid handling platforms equipped with a gripper, a shaker module and a static magnetic separator. The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads.

For the availability of scripts and general considerations about adaptations to a certain instrument, please contact MN.

Visit MN online 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.

2.4 Growth of bacterial cultures

Plasmid yield and quality highly depend on the bacterial culture which is influenced by many factors. Besides culture medium, selective antibiotic, bacterial host strain and type of plasmid, the oxygen availability is of crucial importance for bacterial growth. Anaerobic metabolism of nutrients will result in suboptimal energy uptake and accumulation of organic acids as metabolic end products which inhibit further cell growth.

When incubating bacterial cultures in small volumes with limited surface (as in a 96-deep-well plates or culture plates (see ordering information section 8.2), take care to shake the plates vigorously (200 – 400 rpm) to maintain a proper aeration of the culture. To avoid cross contamination due to spillage during incubation, cover the 96-deep-well plate with a gas-permeable foil (supplied with culture plate). Do not exceed a total culture volume of 1.5 mL (recommended volume approx. 1 mL) when working with the culture plates. If an increased total culture volume is desired, it is possible to grow bacteria in several culture plates with identical layout or in 24 deep-well plates. Either way take care not to exceed the total resuspension volume of 150 µL per sample. The total volume of 150 µL per sample

may either be split into the corresponding number of plates and pooled after resuspension or the total amount may directly be dispensed into a first plate and completely transferred to succeeding plates after resuspension.

The **NucleoMag® Plasmid** kit is optimized for the purification of plasmid DNA from volumes between 1 – 1.5 mL of bacterial cultures but can be used with up to 5 mL with an OD_{600} of 4 or up to 3 mL of a culture with an OD_{600} of 8. Cultures with a higher OD_{600} can be used by reducing the sample volume to 0.5 mL. Using significantly more bacteria without adapting the protocol will overload the lysis capacity, complicate bead resuspension and result in reduced yield and purity.

2.5 Lysate clearance

The NucleoMag® Plasmid kit offers two options for lysate clearance. The most common process of lysate clearance displays the centrifugation of neutralized lysate and the subsequent transfer of the cleared lysate to a fresh reaction vessel or plate.

Alternatively, lysate clearance can be performed by using NucleoMag® Clearing Beads during the neutralization and clarification step. Cellular debris and other contaminants are aggregated by the NucleoMag® Clearing Beads and removed from the supernatant via magnetic separation.

Both options possess advantages and disadvantages in respect to their versatility, hands-on-time, and purity of plasmid DNA.

Method	Culture volume	OD_{600} (Maximum)	Purity	Hands-on-time
Centrifugation	0.5 – 5 mL	4 – 8	+++	+
Magnetic beads	0.5 – 1.5 mL	3	+	++

2.6 Handling of beads

Distribution of beads

A homogeneous distribution of the magnetic beads (NucleoMag® Clearing Beads, NucleoMag® M-Beads) into the individual wells of the separation plate is essential for a high well-to-well consistency. Therefore, before dispensing the beads, make sure that the beads are completely resuspended. Vortex the storage tubes vigorously and check if the beads are completely resuspended.

Magnetic separation time

Attraction of the magnetic beads to the magnetic rods depends on the magnetic strength of the rods, the geometry of the rod sleeve and the processing plate. The individual times for complete attraction of the beads to the magnetic rods should be checked and adjusted on each system. A slower magnetic separation and a longer separation time is recommended to reduce bead carry over.

Attraction of the magnetic beads to the magnetic pins of a static magnetic separator depends on the magnetic strength of the pins, the selected separation plate, distance of

the separation plate to 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.

2.7 Adjusting the shaker settings

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

Adjusting shaker speed for binding and washing 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 s. Turn off the shaker and check the plate surface for small droplets of dyed water.

Increase speed setting, shake for an additional 30 s, and check the 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.

Adjusting shaker speed for the elution step:

Load 100 µL dyed water to the wells of the collection plate and proceed as described above.

2.8 Elution procedures

Purified plasmid DNA can be eluted directly with the supplied Elution Buffer AE (5 mM Tris/HCl, pH 8.5). Elution can be carried out in a volume of ≥ 50 µL. It is essential to cover the NucleoMag® M-Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic rod system (e.g., the geometry of the rod sleeve and plate used). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, higher elution volumes might be necessary to cover the whole pellet and reduce the dead volume of the rod sleeve. A second magnetic separation or a slower magnetic separation is recommended to reduce bead carry over.

3 Storage conditions and preparation of working solutions

Attention: *Buffer PAB contains guanidinium thiocyanate!* ***Wear gloves and goggles!***

CAUTION: Buffer PAB contains guanidinium thiocyanate, which can form highly reactive compounds when combined with bleach (sodium hypochloride). DO NOT add bleach or acidic solutions directly to the sample preparation waste.

- Store NucleoMag® M-Beads upon arrival at 2 – 8 °C.
- All other components can be stored at 15 – 25 °C and are stable until: see package label.
- Always keep buffer bottles tightly closed.
- Sodium dodecyl sulfate (SDS) in **Buffer A2** may precipitate if stored at temperatures below 20 °C. Precipitated SDS might form a firm layer at the bottom of the bottle, which is difficult to see from the side or above. Invert the bottle carefully several times (avoid extensive foaming) and check the bottom and solution for white flocculates. If a precipitate is observed in Buffer A2, incubate bottle at 30 – 40 °C for several minutes and mix well.
- Buffer ERB may form crystals. The crystals must be redissolved by heating to 50 – 60 °C whilst shaking. The bottle should be always closed during the heat incubation.
  Before starting the extraction process, the ERB buffer must be cooled down to room-temperature.

Before starting the **NucleoMag® Plasmid** protocol, prepare the following:

- **Wash Buffer AQ**: Add the indicated volume of ethanol (96 – 100 %) to Buffer AQ Concentrate before use. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer AQ at 15 – 25 °C for up to one year.
- **RNase A**: Add 1 mL of Buffer A1 to the RNase A vial and vortex. Transfer the resulting solution into the buffer A1 bottle and mix thoroughly. Indicate the date of RNase A addition. Store Buffer A1 containing RNase A at 2 – 8 °C for up to 6 months.

	NucleoMag® Plasmid	
REF	**1 × 96 preps** **744750.1**	**4 × 96 preps** **744750.4**
Wash Buffer AQ (Concentrate)	2 × 25 mL Add 100 mL ethanol to each bottle	2 × 100 mL Add 400 mL ethanol to each bottle

4 Safety instructions

When working with the NucleoMag® Plasmid 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 ***www.mn-net.com/msds***).

Caution: Guanidinium thiocyanate in buffer PAB can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the NucleoMag® Plasmid kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according to 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 Protocol for isolation of plasmid DNA

5.1 General overview

The NucleoMag® Plasmid kit is specially designed for the use on magnetic rod systems, such as the KingFisher™, Auto-Pure, MagnetaPure32 Plus, IsoPure Mini or other magnetic rod-based instruments.

The use of the NucleoSpin® 8/96 Plasmid (Transfection-grade) kits is recommended in combination with liquid handling platforms equipped with vacuum manifolds or a positive pressure unit, due to the lower tip consumption, degree of automation and lower processing time.

The NucleoMag® Plasmid kit can also be used on liquid handling platforms equipped with a gripper, a shaker module and a static magnetic separator. The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads .

For manual preparations the use of the NucleoSpin® Plasmid (Transfection-grade) kit is recommended.

Please contact our application support team (automation-bio@mn-net.com) for method files or more detailed information on specific automation platforms.

5.2 Overview

This overview highlights the different protocol options for magnetic rod devices.

		Lysate clarification via Centrifugation		Lysate clarification via NucleoMag® Clearing Beads
Typical EU-level		≤ 50 EU/µg	≤ 10 EU/µg	≤ 50 EU/µg
1	**Cultivate and harvest bacterial cells**	0.5 mL–5 mL LB or up to 2.5 mL 2 x YT or TB		0.5 mL–1.5 mL LB or 2 x YT or TB
		5 min, 11,000 × *g* (2 mL tubes)		5 min, 11,000 × g (2 mL tubes)
		5 min, 4,000 × *g* (96 deep-well plate)		5 min, 4,000 × g (96 deep-well plate)
2	**Resuspend bacterial cells**	150 µL A1		90 µL A1
		Mix or shake		Resuspension on instrument

3	**Lyse bacterial cells**	150 µL A2 RT, 2 – 5 min Mix, invert 5 times or shake gently		120 µL A2 Lysis on instrument
4	**Neutralize**	210 µL S3 Mix, invert 10 times or shake gently		120 µL S3 Neutralization on instrument
5	**Clarify lysate**	Centrifugation 10 min, 11,000 × *g* (2 mL tubes) 10 min, 4,000 × *g* (96 deep-well plate)		Magnetic Beads 35 µL NucleoMag® Clearing Beads, magnetic separation
6	**Transfer cleared lysate**	Transfer up to 450 µL of cleared lysate		Removal of cellular debris via NucleoMag® Clearing Beads
7	**Bind** DNA to NucleoMag® M-Beads	20 µL NucleoMag® M-Beads and 530 µL PAB *Mix and resuspend for at least 5 min*		20 µL NucleoMag® M-Beads and 390 µL PAB *Mix and resuspend for at least 5 min*
		Remove supernatant after 1 min separation		Remove supernatant after 1 min separation
8	**Wash** with ERB	900 µL ERB *Mix and resuspend for 2 – 5 min*		900 µL ERB *Mix and resuspend for 2 – 5 min*
		Remove supernatant after 1 min separation		Remove supernatant after 1 min separation
9	**2nd Wash** with ERB	–	900 µL ERB *Mix and resuspend for 2 – 5 min*	900 µL ERB *Mix and resuspend for 2 – 5 min*
		–	Remove supernatant after 1 min separation	Remove supernatant after 1 min separation

10	**1st Wash** with AQ	900 µL AQ *Mix and resuspend for 2 – 5 min*	900 µL AQ *Mix and resuspend for 2 – 5 min*
		Remove supernatant after 1 min separation	Remove supernatant after 1 min separation
11	**2nd Wash** with AQ	900 µL AQ *Mix and resuspend for 2 – 5 min*	900 µL AQ *Mix and resuspend for 2 – 5 min*
		Remove supernatant after 1 min separation	Remove supernatant after 1 min separation
12	Dry the beads	15 min, room temperature	15 min, room temperature
13	**Elute** Plasmid DNA	100 µL AE *Mix and resuspend for at least 5 min*	100 µL AE *Mix and resuspend for at least 5 min*
		Transfer the eluate after 2 – 5 min separation to a new reaction vessel	Transfer the eluate after 2 – 5 min separation to a new reaction vessel

6 Protocols for magnetic rod systems

6.1 General information

The NucleoMag® Plasmid kit is specially designed for the use on magnetic rod systems, such as the KingFisher™, Auto-Pure, MagnetaPure32 Plus, IsoPure Mini or other magnetic rod-based instruments.

The use of the NucleoSpin® 8/96 Plasmid (Transfection-grade) kits is recommended in combination with liquid handling platforms equipped with vacuum manifolds or a positive pressure unit, due to the lower tip consumption, degree of automation and lower processing time.

Please contact our application support team (automation-bio@mn-net.com) for method files or more detailed information on specific automation platforms.

6.2 General setup for magnetic rod systems

- This overview serves as a guideline for the general setup of plates, columns, or reaction vessels of the respective instrument for the NucleoMag® Plasmid kit. Depending on the instrument, lysis of sample material can either be performed on the instrument or externally. Positions can represent different formats that are defined by the protocol and instrument (e.g., complete plates (96-well format devices, e.g. KingFisher™ Flex), single rows (12-well format), single columns (8-well format, e.g. MagnetaPure32 Plus, IsoPure™ Mini) or individual wells; cartridge-based systems). Depending on the degree of automation, the setup can differ substantially.

Position	Step	Buffer	Volume
1	Lysis/Binding	Cleared lysate, PAB, M-Beads	1000 µL
2	1st Wash	ERB	900 µL
	Optional Wash	*ERB*	*900 µL*
3	2nd Wash	AQ	900 µL
4	3rd Wash	AQ	900 µL
5	Elution	AE	100 µL

6.3 Detailed protocols for IsoPure™ Mini and MagnetaPure32 Plus

Note: The required method files for processing the NucleoMag® Plasmid kit on the IsoPure™ Mini or MagnetaPure32 Plus instrument are available at the qr.mn-net.com/qr/(241)744750.1 or can be requested at support@mn-net.com.

Two protocol options utilizing different options for lysate clearance (centrifugation or magnetic beads) are available for processing the **NucleoMag® Plasmid** kit on the IsoPure™ Mini or MagnetaPure32 Plus instruments (see section 2.5). Please note, that reagent volumes, plate setup and script differ substantially between these two options. Please make sure to choose the correct script.

6.3.1 Protocol for lysate clearance via centrifugation

For hardware requirements and additional consumables, refer to sections 1.2.

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if 96-Deep-Well plates for magnetic rod systems (REF: 744955) and 8-well Tip Combs for magnetic rod systems (REF: 744960) are available.
- Check if the correct script is installed on your instrument.
  · IsoPure™ Mini: NMPlasmid
  · MagnetaPure32 Plus: NMPlasmid

1 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **5 min** at **11,000 x *g*** (2 mL tubes) or 5 min at 4,000 × *g* (96 deep-well plate)

Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible in order to ensure low endotoxin concentrations.

2 Resuspend bacterial cells

Add **150 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

3 Lyse bacterial cells

Add **150 µL Buffer A2**. Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex to avoid shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 min.

4 Neutralize

Add **210 µL Buffer S3**. Mix by pipetting up and down, inverting several times or shaking.

Note: Do not vortex to avoid shearing of genomic DNA.

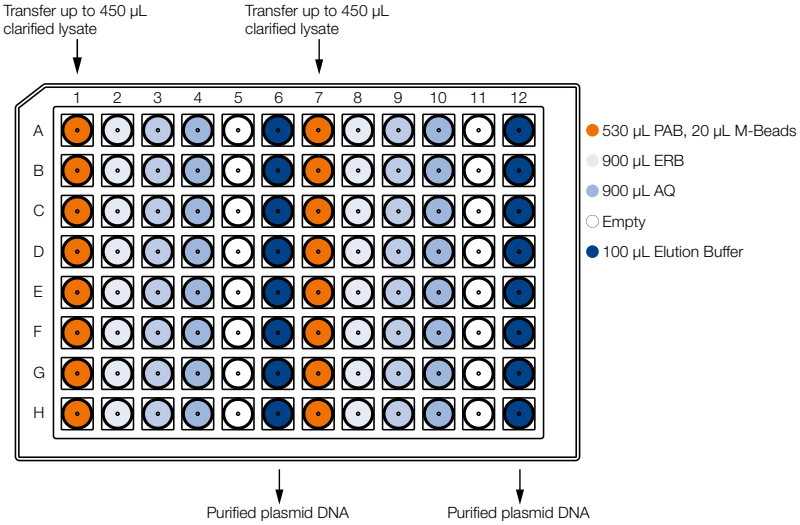
5 Clarify lysate

Centrifuge for **10 min at 11,000** × *g* (2 mL tubes)**/ 10 min at 4,000 × *g*** (96 deep-well plate) at room temperature. Proceed with step 6 in the meantime.

Note: Centrifugation speeds depend on the used reaction vessel. E.g. a 96-deep-well plate: < 5,000 × g; 2 mL tubes: 11,000 × g. Please check the compatibility of the used centrifuge beforehand.

6 Prepare reagent plate(s)

Prepare the reagent plate(s) for IsoPure™ or MagnetaPure32 Plus instruments according to the following loading scheme:



Column 1 and 7: Fill **530 µL Buffer PAB** and **20 µL M-Beads** to each well

Column 2 and 8: Fill **900 µL Detoxification Buffer ERB** to each well

Column 3 and 9: Fill **900 µL Wash Buffer AQ** to each well

Column 4 and 10: Fill **900 µL Wash Buffer AQ** to each well

Column 6 and 12: Fill **100 µL Elution Buffer AE** to each well

Note: Do not moisten the upper rim of the 96 deep-well plate.

7 Transfer cleared lysate

Transfer up to **450 µL** of **cleared lysate** to column 1 and 7 of the prepared reagent plate for IsoPure™ or MagnetaPure32 Plus instruments.

Note: Avoid transferring white flocculants into the reagent plate.

8 Select the respective protocol and start the run

IsoPure™ Mini : NMPlasmid

MagnetaPure32 Plus : NMPlasmid

Load the plate(s) on the instrument.

Insert tip combs on the mounting grooves.

Start the run

9 Transfer eluate

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

6.3.2 Protocol for lysate clearance via magnetic beads

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if 96-Deep-Well plates for magnetic rod systems (REF: 744955) and 8-well Tip Combs for magnetic rod systems (REF: 744960) are available.
- Check if NucleoMag® Clearing Beads (REF: 744751.1) are available.
- Check if the correct script is installed on your instrument.
  · IsoPure™ Mini: NMPlasmidCB

Note: The script includes two manual interventions for the addition of Neutralization buffer S3 and Binding Buffer PAB.

1 Cultivate and harvest bacterial cells

Centrifuge 0.5 – 1.5 mL bacterial cultures for **5 min** at **11,000 x *g* *(2 mL tubes) or 5 min at 4,000 × g*** (96 deep-well plate).

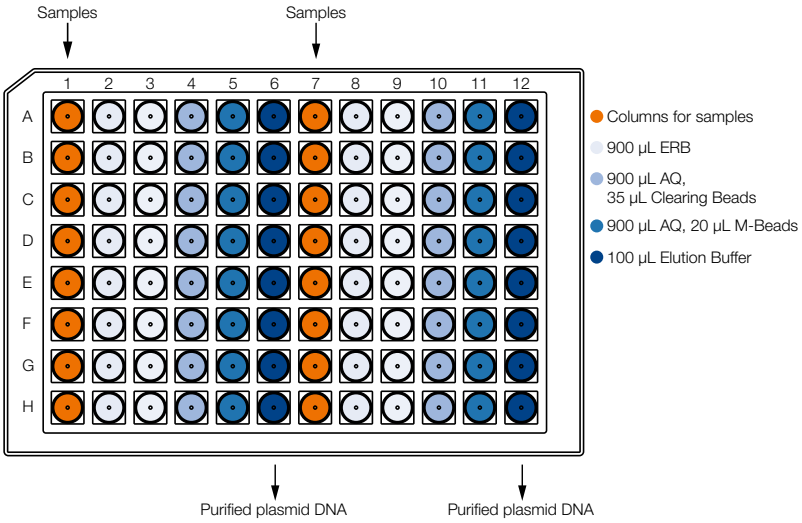
Prepare the reagent plate according to step 2 in the meantime.

Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible in order to ensure low endotoxin concentrations.

2 Prepare reagent plate(s)



Prepare the reagent plate(s) for IsoPure™ instruments according to the following loading scheme:

Column 2 and 8: Fill **900 µL Detoxification Buffer ERB** to each well

Column 3 and 9: Fill **900 µL Detoxification Buffer ERB** to each well

Column 4 and 10: Fill **900 µL Wash Buffer AQ** and **35 µL NucleoMag® Clearing Beads** to each well

Column 5 and 11: Fill **900 µL Wash Buffer AQ** and **20 µL NucleoMag® M-Beads** to each well

Column 6 and 12: Fill **100 µL Elution Buffer AE** to each well

Note: Do not moisten the upper rim of the 96 deep-well plate.

3 Resuspend bacterial cells

Add **90 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

4 Transfer samples to reagent plate

Transfer the resuspended bacterial cells into the respective well of column 1 or column 7.

Note: Do not moisten the rim of the reagent plate.

5 Lyse bacterial cells

Select the protocol from the instrument menu:

IsoPure™ Mini: NMPlasmidCB

Add **120 µL Buffer A2** to the resuspended bacterial cells in column 1 and 7. Immediately place the plate onto the instrument, insert tip combs on the mounting grooves and start the run.

Note: Do not allow the lysis reaction to proceed for more than 2 min before placing the plates onto the instrument

Note: Please equip all tip combs to protect the magnetic rods in used and unused wells.

6 Neutralize and lysate clearance

Dispense **120 µL Buffer S3 into the wells of column 1 and 7** when prompted. Continue the run.

Note: Do not allow the lysis reaction to proceed for more than 5 min in total.

7 Add Binding Buffer PAB

Dispense **390 µL Buffer PAB into the wells of column 1 and 7** when prompted. Continue the run.

8 Transfer eluate

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

6.4 Detailed protocols for KingFisher™ Flex

Note: The required method files for processing the **NucleoMag® Plasmid** *kit on the KingFisher™ Flex instrument are available at the qr.mn-net.com/qr/(241)744750.1 or can be requested at support@mn-net.com*

Two protocol options utilizing different options for lysate clearance (centrifugation or magnetic beads) are available for processing the **NucleoMag® Plasmid** kit on the KingFisher™ Flex instruments (see section 2.5). Please note, that reagent volumes, plate setup and script differ substantially between these two options. Please make sure to choose the correct script.

6.4.1 Protocol for lysate clearance via centrifugation

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if 96-well Accessory Kit A for KingFisher™ is available (REF: 744950)
- Check if the correct script is installed on your instrument.
- KingFisher™ Flex: NucleoMag®_Plasmid_Flex

1 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **5 min** at **4,000 x *g*** (96 deep-well plate).

Centrifugation at higher *g*-forces might produce tight pellets, which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible in order to ensure low endotoxin concentrations.

2 Resuspend bacterial cells

Add **150 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

3 Lyse bacterial cells

Add **150 µL Buffer A2**. Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex to avoid shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 min.

4 Neutralize

Add **210 µL Buffer S3**. Mix by pipetting up and down, inverting several times or shaking.

Note: Do not vortex to avoid shearing of genomic DNA.

5 Clarify lysate

Centrifuge for **10 min** at **4,000 × *g*** (96 deep-well plate) at room temperature. Proceed with step 6 in the meantime.

Note: Centrifugation speeds depend on the used reaction vessel. E.g. a 96-deep-well plate < 5,000 × g. Please check the compatibility of the used centrifuge beforehand.

6 Prepare reagent plate(s)

Prepare the reagent plate(s) for KingFisher™ Flex instruments according to the following loading scheme:

Plate 1: Fill **530 µL Buffer PAB** and **20 µL M-Beads** to each well of a deep-well plate for KingFisher™

Plate 2: Fill **900 µL Detoxification Buffer ERB** to each well of a deep-well plate for KingFisher™

Plate 3: Fill **900 µL Wash Buffer AQ** to each well of a deep-well plate for KingFisher™

Plate 4: Fill **900 µL Wash Buffer AQ** to each well of a deep-well plate for KingFisher™

Plate 5: Fill **100 µL Elution Buffer AE** to each well of an elution plate for KingFisher™

Note: Do not moisten the upper rim of the 96 deep-well plate.

7 Transfer cleared lysate

Transfer up to 450 µL of cleared lysate to plate 1 of the prepared reagent plate for KingFisher™ Flex instruments.

Note: Avoid transferring white flocculants into the reagent plate.

8 Select the respective protocol and start the run

KingFisher™ Flex: NucleoMag®_Plasmid_Flex

Load the plates as indicated on the KingFisher™ Flex instrument display.

Start the run.

9 Transfer eluate

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

6.4.2 Protocol for lysate clearance via magnetic beads

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if 96 Deep-well plates for magnetic rod systems (REF 744955) and Deep-well Tip Combs for KingFisher (REF 744956) are available.
- Check if NucleoMag® Clearing Beads (REF: 744751.1) are available.
- Check if the correct script is installed on your instrument.
- KingFisher™ Flex:
  NM_Plasmid_CB_PartA
  NM_Plasmid_CB_PartB

Note: The script includes two manual interventions for the addition of Neutralization buffer S3 and the change of scripts/plates on the instrument

1 Cultivate and harvest bacterial cells

Centrifuge 0.5 – 1.5 mL bacterial cultures for **5 min** at **4,000 x *g* *(96 deep-well plate)***.

Prepare the reagent plates according to step 2 in the meantime.

Centrifugation at higher *g*-forces might produce tight pellets, which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible in order to ensure low endotoxin concentrations.

2 Prepare reagent plates Part A

Prepare the reagent plates for the KingFisher™ Flex instrument according to the following loading scheme:

Plate 2: Fill **900 µL Wash Buffer AQ** and **35 µL NucleoMag® Clearing Beads** to each well of a deep-well plate for KingFisher™.

Note: Do not moisten the upper rim of the 96 deep-well plate

3 Resuspend bacterial cells

Add **90 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

4 Transfer samples to reagent plate

Transfer the resuspended bacterial cells into the respective well of an empty deep-well plate for KingFisher™ (plate 1).

Note: Do not moisten the rim of the reagent plate.

5 Lyse bacterial cells

Select the protocol from the instrument menu:

KingFisher™ Flex: NM_Plasmid_CB_PartA

Add **120 µL Buffer A2** to the resuspended bacterial cells in plate 1 using an 8-channel pipette or multi-dispensing pipette. Immediately place the plate onto the instrument, insert tip comb and start the run.

Note: Do not allow the lysis reaction to proceed for more than 2 min before placing the plates onto the instrument

6 Neutralize and lysate clearance

Dispense **120 µL Buffer S3 into the wells of plate 1** when prompted. Continue the run.

Note: It is recommended to precool the buffer S3 to 2 – 8 °C or on ice prior use.

Note: Do not allow the lysis reaction to proceed for more than 5 min in total.

7 Prepare reagent plates Part B

Prepare the reagent plates for the KingFisher™ Flex instrument according to the following loading scheme:

Plate 3: Fill **900 µL Detoxification Buffer ERB** to each well of a deep-well plate for KingFisher™

Plate 4: Fill **900 µL Detoxification Buffer ERB** to each well of a deep-well plate for KingFisher™.

Plate 5: Fill **900 µL Wash Buffer AQ** and **20 µL NucleoMag® M-Beads** to each well of a deep-well plate for KingFisher™.

Plate 6: Fill **100 µL Elution Buffer AE** to each well of an elution plate for KingFisher™

Note: Do not moisten the upper rim of the 96 deep-well plate.

8 Rearrange plates

Remove the plates from the instrument when finished the run of Part A. Keep both plates (plate 2 with buffer AQ) and the binding plate (plate 1). Discard the used tip comb.

9 Add Binding Buffer PAB

Dispense **390 µL Buffer PAB into the wells of plate 1**. Select the protocol from the run menu: KingFisher™ Flex: NM_Plasmid_CB_PartB

Load the plates as indicated by the instrument and start the run.

Note: It is recommended to use a new tip comb.

10 Transfer eluate

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plates from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

6.5 Support protocol for transfection-grade plasmid DNA isolation

The following support protocol utilizing only centrifugation for lysate clearance was designed to achieve plasmid DNA purifications with a typical endotoxin level ≤ 10 EU / µg Plasmid DNA. If endotoxin levels ≤ 50 EU/µg are sufficient, it is recommended to follow the standard procedure.

6.5.1 Support protocol for IsoPure™ Mini and MagnetaPure32 Plus (TG)

For hardware requirements and additional consumables, refer to sections 1.2.

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if 96-Deep-Well plates for magnetic rod systems (REF: 744955) and 8-well Tip Combs for magnetic rod systems (REF: 744960) are available.
- Check if the correct script is installed on your instrument.

IsoPure™ Mini: NMPlasmidTG

MagnetaPure32 Plus: NMPlasmidTG

1 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **5 min** at **11,000 × *g*** (2 mL tubes) or 5 min at 4,000 × g (96 deep-well plate)

Centrifugation at higher g-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible to ensure low endotoxin concentrations. Repeat the centrifugation step to ensure the complete removal of residual media.

2 Resuspend bacterial cells

Add **150 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

3 Lyse bacterial cells

Add **150 µL Buffer A2**. Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex to avoid shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 min.

4 Neutralize

Add **210 µL Buffer S3**. Mix by pipetting up and down, inverting several times or shaking.

Note: Do not vortex to avoid shearing of genomic DNA.

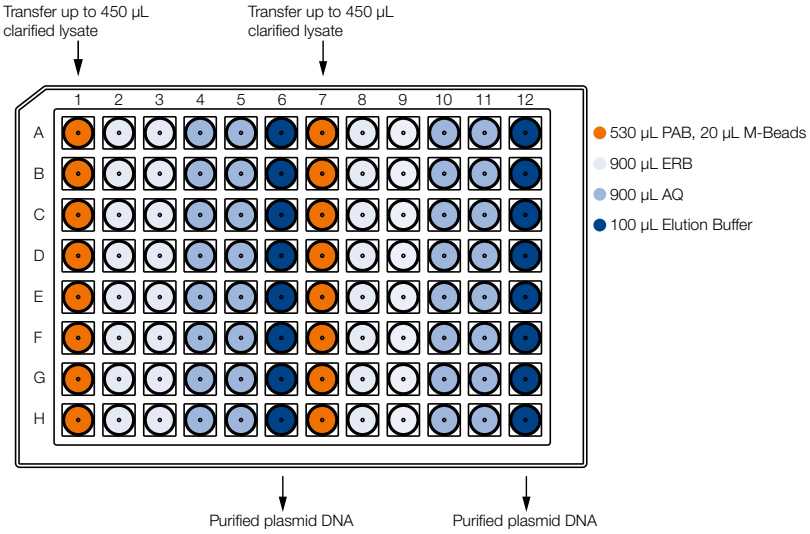
5 Clarify lysate

Centrifuge for **10 min** at **11,000 × *g*** (2 mL tubes) / **10 min at 4,000 × *g*** (96 deep-well plate) at room temperature. Proceed with step 6 in the meantime.

Note: Centrifugation speeds depend on the used reaction vessel. E.g. a 96-deep-well plate: < 5,000 × g; 2 mL tubes: 11,000 × g. Please check the compatibility of the used centrifuge beforehand.

6 Prepare reagent plate(s)

Prepare the reagent plate(s) for IsoPure™ or MagnetaPure32 Plus instruments according to the following loading scheme:



Column 1 and 7: Fill **530 µL Buffer PAB** and **20 µL M-Beads** to each well

Column 2 and 8: Fill **900 µL Detoxification Buffer ERB** to each well

Column 3 and 9: Fill **900 µL Detoxification Buffer ERB** to each well

Column 4 and 10: Fill **900 µL Wash Buffer AQ** to each well

Column 5 and 11: Fill **900 µL Wash Buffer AQ** to each well

Column 6 and 12: Fill **100 µL Elution Buffer AE** to each well

Note: Do not moisten the upper rim of the 96 deep-well plate.

7 Transfer cleared lysate

Transfer up to **450 µL** of **cleared lysate** to column 1 and 7 of the prepared reagent plate for IsoPure™ or MagnetaPure32 Plus instruments.

Note: Avoid transferring white flocculants into the reagent plate.

8 **Select the respective protocol and start the run**

IsoPure™ Mini : NMPlasmidTG

MagnetaPure32 Plus : NMPlasmidTG

Load the plate(s) on the instrument.

Insert tip combs on the mounting grooves.

Start the run

9 Transfer eluate

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

6.5.2 Support protocol for KingFisher™ Flex (TG)

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if 96-well Accessory Kit A for KingFisher™ is available (REF: 744950)
- Check if the correct script is installed on your instrument.

KingFisher™ Flex: NucleoMag®_Plasmid_TG_Flex

1 **Cultivate and harvest bacterial cells**

Centrifuge the bacterial cultures for **5 min** at **4,000 × *g*** (96 deep-well plate)

Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible to ensure low endotoxin concentrations. Repeat the centrifugation step to ensure the complete removal of residual media.

2 Resuspend bacterial cells

Add **150 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

3 Lyse bacterial cells

Add **150 µL Buffer A2**. Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex to avoid shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 min.

4 Neutralize

Add **210 µL Buffer S3**. Mix by pipetting up and down, inverting several times or shaking.

Note: Do not vortex to avoid shearing of genomic DNA.

5 Clarify lysate

Centrifuge for **10 min** at **11,000 × *g*** (2 mL tubes) / **10 min at 4,000 × *g*** (96 deep-well plate) at room temperature. Proceed with step 6 in the meantime.

Note: Centrifugation speeds depend on the used reaction vessel. E.g. a 96-deep-well plate: < 5,000 × g; 2 mL tubes: 11,000 × g. Please check the compatibility of the used centrifuge beforehand.

6 Prepare reagent plate(s)

Prepare the reagent plate(s) for KingFisher™ Flex instruments according to the following loading scheme:

Plate 1: Fill **530 µL Buffer PAB** and **20 µL M-Beads** to each well of a deep-well plate for KingFisher™

Plate 2: Fill **900 µL Detoxification Buffer ERB** to each well of a deep-well plate for KingFisher™

Plate 3: Fill **900 µL Detoxification Buffer ERB** to each well of a deep-well plate for KingFisher™

Plate 4: Fill **900 µL Wash Buffer AQ** to each well of a deep-well plate for KingFisher™

Plate 5: Fill **900 µL Wash Buffer AQ** to each well of a deep-well plate for KingFisher™

Plate 6: Fill **100 µL Elution Buffer AE** to each well of an elution plate for KingFisher™

Note: Do not moisten the upper rim of the 96 deep-well plate

7 Transfer cleared lysate

Transfer up to 450 µL of cleared lysate to plate 1 of the prepared reagent plate for KingFisher™ Flex instruments.

Note: Avoid transferring white flocculants into the reagent plate.

8 Select the respective protocol and start the run

KingFisher™ Flex: NucleoMag®_Plasmid_TG_Flex

Load the plates as indicated on the KingFisher™ Flex instrument display.

Start the run

9 Transfer eluate

The instrument stops after the final elution step. Remove and discard tip combs. Unload the plate(s) from the instrument and transfer the eluate for further processing into a suitable plate or vessel.

7 Support protocols for liquid handling systems

7.1 General information

The NucleoMag® Plasmid kit can also be used on liquid handling platforms equipped with a gripper, a shaker module and a static magnetic separator, if no magnetic rod system, positive pressure unit or vacuum manifold is available.

The gripper needs to transfer the plate to the magnetic separator for the separation of the beads and then to the shaker module for resuspension of the beads.

The adapted NucleoMag® Plasmid kit for liquid handling platforms is only available with four wash steps, either using centrifugation or magnetic beads for lysate clarification, achieving endotoxin levels below 50 EU/µg.

Please contact our application support team (automation-bio@mn-net.com) for method files or more detailed information on specific automation platforms.

7.2 Protocol for lysate clearance via centrifugation

For hardware requirements, additional consumables and shaker settings, refer to sections 1.2 and 2.7.

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if a suitable 96-Deep-Well plate (e.g. Square-well Block) and magnetic separator (e.g. NucleoMag® SEP) are available.

1 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **5 min** at **11,000 x *g*** (2 mL tubes) or **5 min** at **4,000 × *g*** (96 deep-well plate)

Centrifugation at higher g-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible in order to ensure low endotoxin concentrations.

2 Resuspend bacterial cells

Add **150 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

3 Lyse bacterial cells

Add **150 µL Buffer A2**. Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex to avoid shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 min.

4 Neutralize

Add **210 µL Buffer S3**. Mix by pipetting up and down, inverting several times or shaking.

Note: Do not vortex to avoid shearing of genomic DNA.

5 Clarify lysate

Centrifuge for **10 min at 11,000 × *g*** (2 mL tubes) **/ 10 min at 4,000 × *g*** (96 deep-well plate) at room temperature. Proceed with step 6 in the meantime.

Note: Centrifugation speeds depend on the used reaction vessel. E.g. a 96-deep-well plate: < 5,000 × g; 2 mL tubes: 11,000 × g. Please check the compatibility of the used centrifuge beforehand.

6 Transfer cleared lysate

Transfer up to **450 µL** of **cleared lysate** to a suitable processing plate or reaction vessel (e.g. Square-well Block).

Note: Avoid transferring white flocculants into the reagent plate.

7 Bind DNA to NucleoMag® M-Beads

Add **20 µL of NucleoMag® M-Beads** and **200 µL Binding Buffer PAB**. Mix by shaking for 10 min at room temperature preferably with 1200 rpm.

Note: Please check section 2.7 in order to adjust the shaker settings in an appropriate manner.

Note: Be sure to resuspend the NucleoMag® M-Beads before usage. Vortex storage bottle until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 5 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant.

8 Wash with ERB

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer ERB** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

9 Wash with ERB (2nd)

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer ERB** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

10 Wash with AQ

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer AQ** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

11 Wash with AQ (2nd)

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer AQ** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended : 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

12 Air dry magnetic beads

Air dry the magnetic beads for 15 min at room temperature.

Note: Elevated temperatures can be used to increase the efficiency of drying

13 Transfer eluate

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add desired volume of **Elution buffer AE (50 – 100 µL)** to each well of the Square-well Block and resuspend the beads by shaking 5 min at room temperature.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified DNA to suitable elution plate.

Note: Slow aspiration speeds are highly recommended in order to avoid bead carry over.

7.3 Protocol for lysate clearance via magnetic beads

For hardware requirements, additional consumables and shaker settings, refer to sections 1.2 and 2.7.

Before starting the preparation:

- Check if the magnetic beads are resuspended completely according to section 2.6
- Check if RNase A was added to buffer A1 according to section 3.
- Check buffers A2 and ERB for precipitates according to section 3.
- Check if Wash buffer AQ was prepared according to section 3.
- Check if NucleoMag® Clearing Beads (REF: 744751.1) are available.
- Check if a suitable 96-Deep-Well plate (e.g. Square-well Block) and magnetic separator (e.g. NucleoMag® SEP) is available are available.

1 Cultivate and harvest bacterial cells

Centrifuge the bacterial cultures for **5 min** at **11,000 x *g*** (2 mL tubes) or **5 min** at **4,000 × *g*** (96 deep-well plate)

Centrifugation at higher *g*-forces might produce tight pellets which are more difficult to resuspend.

Discard supernatant. Remove residual medium by tapping tube or plate upside down on a clear paper sheet or soft tissue.

Note: It is important to remove as much residual media as possible in order to ensure low endotoxin concentrations.

2 Resuspend bacterial cells

Add **150 µL Buffer A1 with RNase A**. Resuspend the cell pellet completely by vortexing, shaking or mixing by pipetting up and down. Resuspend bacterial cells completely.

Note: No clumps should be visible.

3 Lyse bacterial cells

Add **150 µL Buffer A2**. Incubate at **room temperature** for a maximum of **5 min** with moderate shaking (300 rpm).

Note: Do not vortex to avoid shearing of genomic DNA. Do not allow the lysis reaction to proceed for more than 5 min.

4 Neutralize

Add **210 µL Buffer S3**. Mix by pipetting up and down, inverting several times or shaking at 600 rpm for 1 min.

Note: Do not vortex to avoid shearing of genomic DNA.

5 Clarify lysate

Add **35 µL NucleoMag® Clearing Beads** to each neutralized sample. Shake for 2 min at 800 rpm at room temperature.

Note: Be sure to resuspend the NucleoMag® Clearing Beads before usage. Vortex storage bottle until a homogenous suspension has formed.

Note: Please check section 2.7 in order to adjust the shaker settings in an appropriate manner. Do not exceed 500 rpm in this case.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 5 min until all the beads including the cellular debris have been attracted to the magnets.

6 Transfer cleared lysate

Transfer up to **350 µL** of **cleared lysate** to a suitable processing plate or reaction vessel (e.g. Square-well Block).

Note: Avoid transferring white flocculants into the reagent plate.

Note: Use slow aspiration speeds and start aspiration from top.

7 Bind DNA to NucleoMag® M-Beads

Add **20 µL of NucleoMag® M-Beads** and **155 µL Binding Buffer PAB**. Mix by

shaking for 10 min at room temperature preferably with 1200 rpm.

Note: Please check section 2.7 in order to adjust the shaker settings in an appropriate manner.

Note: Be sure to resuspend the NucleoMag® M-Beads before usage. Vortex storage bottle until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 5 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant.

8 Wash with ERB

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer ERB** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

9 Wash with ERB (2nd)

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer ERB** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

10 Wash with AQ

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer AQ** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

11 Wash with AQ (2nd)

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add **900 µL Buffer AQ** to each well and resuspend the beads by shaking until the beads are resuspended completely for at least 5 min (recommended: 7 min at 1200 rpm).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

12 Air dry magnetic beads

Air dry the magnetic beads for 15 min at room temperature

Note: Elevated temperatures can be used to increase the efficiency of drying

13 Transfer eluate

Remove the Square-well Block from the NucleoMag® SEP magnetic separator. Add desired volume of **Elution buffer AE (50 – 100 µL)** to each well of the Square-well Block and resuspend the beads by shaking 5 min at room temperature.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified DNA to suitable elution plate.

Note: Slow aspiration speeds are highly recommended in order to avoid bead carry over.

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	*Cell pellet not properly resuspended* • It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Buffer A2. Use the centrifugation speed and times given in the manual to avoid tight pellets. *SDS in Buffer A2 precipitated* • SDS in Buffer A2 may precipitate upon storage. If this happens a white precipitate is visible at the bottom of the bottle. Incubate Buffer A2 at 30 – 40 °C for several minutes and mix well before use. *Too many bacterial cells used* • Usage of LB as the growth medium is recommended. When using rich media like 2 x YT or TB, cultures may reach very high cell densities. Reduce culture volume.

Problem	Possible cause and suggestions
Poor plasmid yield	*Incomplete lysis of bacterial cells* • See “Possible *cause* and suggestions” above *No plasmid contained in bacteria* • Cells carrying the plasmid of interest may become overgrown by non-transformed cells due to insufficient amounts of selective antibiotics. • Do not incubate cultures for more than 16 h as this may result in many dead and starving cells with degraded DNA. *Use of low-copy plasmid* • Getting acceptable plasmid yields for transfection requires high-copy plasmids in a miniprep scale or a switch to large scale kits (NucleoBond® Xtra Midi / Maxi). *Suboptimal elution conditions* • Elution efficiency will decrease with larger constructs. When working with large constructs, the elution buffer volume should be increased or the elution process should be prolonged. *Suboptimal protocol conditions* • Make sure to use verified scripts on the magnetic rod systems. Contact our support if script support is needed. *Buffer AQ not prepared correctly* • Add the indicated amount of 96 – 100 % ethanol to each bottle of Buffer AQ. Keep bottles closed tightly to prevent evaporation. • Suggestions specific to liquid handling systems *Insufficient Elution buffer volume* • Bead 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 efficiency of following wash steps and elution step.

Problem	Possible cause and suggestions
Poor plasmid yield	*Aspiration of attracted bead pellet* • Do not disturb the attracted beads while aspirating the supernatant, especially when the magnetic pellet is not visible in the lysate. *Aspiration and loss of beads* • Time for magnetic separation was too short or aspiration speed was too high.
Genomic DNA contamination	*Excessive mixing steps* • Cell lysate was vortexed or mixed too vigorously after addition of Buffer A2 or Buffer S3. Genomic DNA was sheared and thus liberated. • Reduce number of mixing cycles, reduce shaker speed after addition of Lysis Buffer A2 and Neutralization Buffer S3. Excessive mixing can cause shearing of chromosomal DNA, leading to a copurification during the preparation of plasmid DNA. *Lysis was too long* • Lysis was too long and must not exceed 5 min.
RNA contamination	*RNA was not degraded completely* • Ensure that RNase A was added to Buffer A1 and mixed well before use.
Endotoxin levels are too high	*Carry over of residual cultivation media* • Precisely remove the residual cultivation media after harvesting the cells. Perform a second centrifugation step and remove residual media. Suboptimal cultivation conditions • Optimize cultivation conditions in order the lower the amount of dead cells.
Suboptimal performance 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. *RNA contamination* • RNA might influence the photometric measurements resulting in an overestimation of plasmid DNA. Make sure RNase A is added to Buffer A1.

8.2 Ordering information

Product	REF	Pack of
NucleoMag® Plasmid	744750.1 744750.4	96 preps 384 preps
NucleoMag® Clearing Beads	744751.1	2 × 1.75 mL (96 preps)
Buffer A1 (without RNase A)	740911.1	1 L
Buffer A2 without LyseControl	740912.1	1 L
Buffer A2 with LyseControl	740328.100	100 mL
Buffer S3	740518.1	500 mL
Buffer AQ (Concentrate) (for 125 mL Buffer AQ)	740995	25 mL
H_2O-EF	740798.1	1 L
RNase A (lyophilized)	740505 740505.50	100 mg 50 mg
96-well Accessory Kit A for KingFisher™	744950	1 set
96 Deep-well plates for magnetic rod systems	744955	25
8-well Tip Combs for magnetic rod system	744960	50
Deep-well Tip Combs for KingFisher™	744956	4
Culture Plate (with Gas-permeable Foil)	740488 740488.24	4 sets 24 sets
NucleoMag® SEP	744900	1
Square-well Blocks	740481	4
	740481.24	24
Gas-permeable Foil	740675	50
Self adhering Foil	740676	50

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

8.3 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.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

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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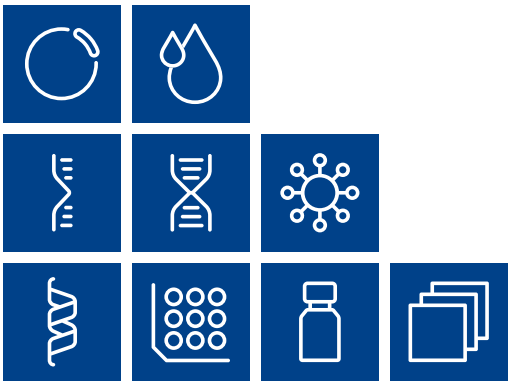
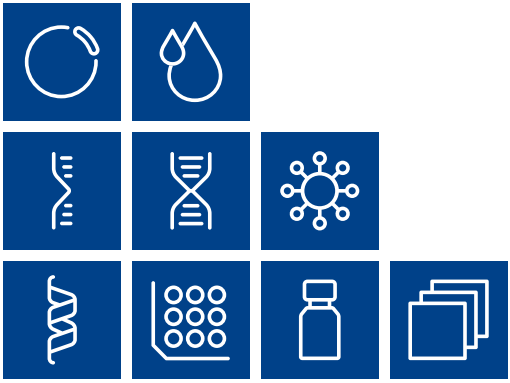


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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