PCR clean-up

User manual

NucleoMag® 96 PCR

May 2014 / Rev. 03

MACHEREY-NAGEL
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1 Components

1.1 Kit contents

	NucleoMag® 96 PCR		
	1x 96 preps	**4 x 96 preps**	**24 x 96 preps**
REF	**744100.1**	**744100.4**	**744100.24**
NucleoMag® P-Beads	1.4 mL	5.6 mL	33.6 mL
Binding Buffer MP1	20 mL	80 mL	500 mL
Wash Buffer MP2	40 mL	160 mL	1000 mL
Wash Buffer MP3 (Concentrate)*	25 mL	100 mL	2 x 200 mL
Elution Buffer MP4	15 mL	30 mL	250 mL
Elution Plate U-bottom (including Self-adhering PE Foil)	1	4	24
User manual	1	1	1

1.2 Equipment and consumables to be supplied by user

Product	REF	Pack of
• **Magnetic separation system** e.g., NucleoMag® SEP (see section 2.3)	744900	1
• **Separation plate for magnetic beads separation,** e.g., Square-well Block (96-well block with 2.1 mL square-wells)	740481 740481.24	4 24

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

2 Product description

2.1 The basic principle

The **NucleoMag® 96 PCR** procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Adjusting the PCR reaction to binding conditions and addition of paramagnetic beads can be carried out simultaneously. After magnetic separation and removal of supernatant, the beads are washed to remove contaminants and salt. A short drying step is necessary to remove ethanol from previous washing steps. Finally, highly purified DNA is eluted with low salt elution buffer or water and can be used directly for downstream applications. The **NucleoMag® 96 PCR** kit can be used either manually or automated on standard liquid handling instruments.

2.2 Kit specifications

- **NucleoMag® 96 PCR** is designed for rapid manual and automated clean-up of PCR fragments using the NucleoMag® SEP Magnetic Separator (see ordering information) other magnetic separation systems (see section 2.3). Manual processing time for 96 samples is about 45 min.
- **NucleoMag® 96 PCR** is easily adapted to common liquid handling instruments. The actual processing time and sample volume to be processed depends on the configuration of your instrument and the used magnetic separation system. Typically, 96 samples can be purified in about 30–45 min.
- The kit provides reagents for the purification of 3–5 µg DNA from 50 µL PCR samples.
- Typical concentration of the purified PCR samples of 75–200 ng/µL can be achieved.
- The purity of recovered PCR products is $A_{260}/A_{280} \geq 1.7–1.9$.
- The kit is designed for use with or without detergent containing PCR buffers.
- Purified PCR products are ready-to-use for downstream applications, like automated fluorescent sequencing, labeling, microarray analysis, cloning, or restriction digestion.
- **NucleoMag® 96 PCR** can be processed completely at room temperature. Elution at 55 °C will increase the recovery by about 10–15 %.
- NucleoMag® P-Beads are highly reactive, superparamagnetic beads. The binding capacity is 0.3 µg of DNA per 1 µL of NucleoMag® P-Bead suspension, 1 µL of suspension contains 150 µg of beads.

2.3 Magnetic separation systems

For use of **NucleoMag® 96 PCR,** the use of the magnetic separator NucleoMag® SEP is recommended. Separation is carried out in a Square-well Block (see ordering information). The kit can also be used with other common separators.

Magnetic separator	Separation plate or tube
NucleoMag® SEP (MN REF 744900)	Square-well Block (MN REF 740481)
Tecan Te-MagS™	1.5 mL tubes without lid (Sarstedt)

Static magnetic pins

Separators with static magnetic pins, for example, NucleoMag® SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations, a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

Movable magnetic systems

Separators with moving magnetic pins: Magnetic pins/rods are moved from one side of the well to the other and vice versa. Beads follow this movement and are thus pulled through the buffer during the wash and elution steps. Separation takes place when the system stops.

Automated separators

Separators with moving magnets: Magnetic beads are transferred into suitable plates or tubes. Beads are resuspended from the rod-covered magnets. Following binding, washing or elution beads are collected again with the rod-covered magnets and transferred to the next plate or tube.

2.4 Adjusting the shaker settings

When using a plate shaker for the washing and elution steps, the speed settings have to be 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 wash steps:

- Load 300 µL dyed water (select to the wells of the separation plate. Place the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check the plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, 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.

2.5 Handling of beads

Distribution of beads

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

Magnetic separation time

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

Washing the beads

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

Resuspension by pipetting up and down, however, is more efficient than mixing by a shaker or magnetic mix.

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

+: acceptable, ++: good, +++: excellent, * 8-channel pipetting device

2.6 Elution procedures

Purified DNA can be eluted directly with the supplied Elution Buffer MP4 or water (pH 7.5–8.5). Elution can be carried out in a volume of ≥ 25 µL (per 12 µL bead suspension). It is essential to cover the NucleoMag® P-Beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (e.g., the position of the pellet inside the separation plate). For efficient elution, the magnetic bead pellet should be resuspended completely in the elution buffer. For some separators, high elution volumes might be necessary to cover the whole pellet.

Elution is possible at room temperature. Yield can be increased by 10–15 % if elution is performed at 55 °C.

* 8-channel pipetting device

3 Storage conditions and preparation of working solutions

Attention: *Buffers MP1 and MP2 contain chaotropic salt! Wear gloves and goggles!*

CAUTION: Buffers MP1 and MP2 contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Storage conditions:

- All components of the **NucleoMag® 96 PCR** kit should be stored at room temperature (18–25 °C) and are stable for up to one year.

Before starting any **NucleoMag® 96 PCR** protocol, prepare the following:

- **Wash Buffer MP3:** Add the indicated volume of ethanol (96–100 %) to Buffer MP3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer MP3 is stable at room temperature (18–25 °C) for at least one year.

	NucleoMag® 96 PCR		
	1 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
REF	**744100.1**	**744100.4**	**744100.24**
Buffer MP3 (Concentrate)	25 mL Add 100 mL ethanol	100 mL Add 400 mL ethanol	2 x 200 mL Add 800 mL ethanol to each vial

4 Safety instructions

The following components of the **NucleoMag® 96 PCR** kits contain hazardous contents.

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

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
MP1	Guanidine hydrochloride 36–50 % *Guanidinhydrochlorid 36–50 %*	 Warning *Achtung*	302, 319	280, 301+312, 305+351+338, 330, 337+313
MP2	Guanidine hydrochloride 24–36 % *Guanidinhydrochlorid 24–36 %*	 Warning *Achtung*	302	301+312, 330

Hazard phrases

H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*

Precaution phrases

P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/…/if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 305+351+313	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter ausspülen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 337+313	Get medical advice / attention. *Bei anhaltender Augenreizung: Ärztliche Rat einholen / ärztliche Hilfe hinzuziehen.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com)***.

Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)***.

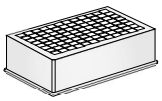
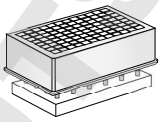
5 Protocol for clean-up of 50 µL PCR reactions

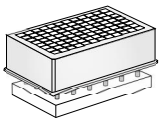
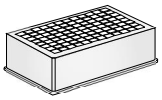
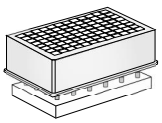
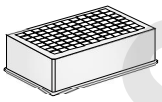
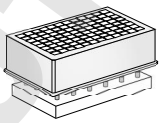
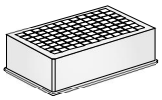
Protocol-at-a-glance

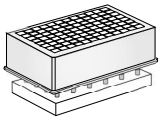
- For additional equipment and hardware requirements, refer to section 1.2 and 2.3, respectively.
- For detailed information on each step, see page 17.

Before starting the preparation:

- Check if Buffer MP3 was prepared according to section 3.

1	**Transfer** PCR reaction mixture to appropriate 96-well plate	**50 µL PCR reaction** For PCR reactions < 50 µL, adjust the volume to 50 µL using sterile water	
2	**Bind** DNA to NucleoMag® P-Beads	**12 µL NucleoMag® P-Beads** **138 µL MP1**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
3	**Wash** with MP2	Remove Square-well Block from NucleoMag® SEP **300 µL MP2**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	

		Remove supernatant after 2 min separation	
4	**1st wash** with MP3	Remove Square-well Block from NucleoMag® SEP **300 µL MP3**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
5	**2nd wash** with MP3	Remove Square-well Block from NucleoMag® SEP **300 µL MP3**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
6	**Dry** the beads	**10 min at RT**	
7	**Elute** DNA	Remove Square-well Block from NucleoMag® SEP **25–100 µL MP4** *(Optional: Elute at 55 °C)*	

Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
Separate 2 min and transfer DNA into Elution Plate U-bottom	

Detailed protocol

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Before starting the preparation:

- Check if Buffer MP3 was prepared according to section 3.

1 Transfer PCR reaction mixture

Transfer PCR reaction mixture to an appropriate 96-well plate.

For PCR reaction volumes < 50 µL, adjust the volume to 50 µL using sterile water.

Note: See recommendations for suitable plates (e.g., Square-well Block; not included in the kit) and compatible magnetic separators section 2.3.

2 Bind DNA to NucleoMag® P-Beads

Add **12 µL NucleoMag® P-Beads** and **138 µL Binding Buffer MP1** to each well of the separation plate. Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature.** Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Note: NucleoMag® P-Beads and Binding Buffer MP1 may be premixed. For 96 samples, premix 1248 µL of NucleoMag® P-Beads with 14.35 mL of Buffer MP1, mix by vortexing. Use 150 µL of the suspension per well. Be sure to resuspend the NucleoMag® P-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.

Separate the magnetic beads against the side of the wells by placing the 96-well plate on the NucleoMag® SEP magnetic separator. Wait at least **2 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. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.

3 Wash with MP2

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **300 µL Buffer MP2** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

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.

4 1st wash with MP3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **300 µL Buffer MP3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

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.

5 2nd wash with MP3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **300 µL Buffer MP3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

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.

6 Dry the beads

Dry the beads by incubating the Square-well Block **10 min** at **room temperature** with the particles held against the magnet in order to allow the remaining traces of alcohol to evaporate.

Note: Allow the pellet to dry sufficiently so that there is no visible droplets of buffer in the bottom of the tube. Allowing the pellet to dry completely, indicated by visible cracking. Do not overdry beads (e.g., by prolonged drying at 55 °C). This will reduce yield.

7 Elute DNA

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **Elution Buffer MP4 (25–100 µL)** to each well and resuspend the by shaking **5–10 min** at **56 °C.** Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5–10 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 PCR products to the Elution Plate U-bottom.

Note: Yield can be increased by 15–20 % by using pre-warmed elution buffer (55 °C) or by incubating the bead / elution buffer suspension at 55 °C for 5 min.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Wash Buffer MP3 did not contain ethanol* • Addition of the the indicated volume of 96–100 % ethanol to Buffer MP3 Concentrate is required before use. *Elution buffer volume insufficient* • Bead pellet must be covered completely with elution buffer. *Insufficient performance of elution buffer during elution step* • Remove residual wash buffers during the separation steps completely. Remaining buffers decrease efficiency of following wash steps and elution step. *Beads overdried* • Do not dry beads longer than 20 min at room temperature. Overdrying of beads may result in lower elution efficiencies.
Suboptimal performance of DNA in downstream applications	*Carry-over of ethanol from Wash Buffer MP3* • Be sure to remove all of the ethanolic Wash Buffer MP3 after the final wash step. Dry beads 10–15 min at room temperature. *Elution of DNA with TE buffer* • Use supplied elution buffer or sterile water. Do not use TE buffer. EDTA may inhibit sequencing reactions. Repurify or precipitate DNA by ethanol and elute / redissolve in Elution Buffer MP 4 buffer or water. *Eluted DNA contains residual primers / primer dimers* • Minimize amount of primers in PCR reaction mixture. Do not use higher volumes of binding buffer than specified.

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® 96 PCR	744100.1 744100.4 744100.24	1 x 96 preps 4 x 96 preps 24 x 96 preps
NucleoMag® SEP	744900	1
Square-well Blocks	740481 740481.24	4 24
Elution Plate U-bottom	740486.24	24
Self-adhering PE Foil	740676	50 sheets

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

6.3 Product use restriction/warranty

NucleoMag® 96 PCR kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

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Last updated: 07/2010, Rev. 03

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