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PCR clean-up

User manual

NucleoMag 96 PCR

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MACHEREY-NAGEL

Table of contents

1 Kit contents

	NucleoMag 96 PCR		
Cat.No.	**1 x 96 preps 744 100.1**	**4 x 96 preps 744 100.4**	**24 x 96 preps 744 100.24**
NucleoMag P-Beads	1.4 ml	5.6 ml	33.6 ml
Binding Buffer MP1	16 ml	64 ml	384 ml
Wash Buffer MP2	36 ml	2 x 72 ml	864 ml
Wash Buffer MP3	15 ml	60 ml	2 x 180 ml
Elution Buffer MP4	7.5 ml	30 ml	180 ml
Elution plate, U-bottom (including one Self-adhering PE foil)*	1	4	24
Protocol	1	1	1

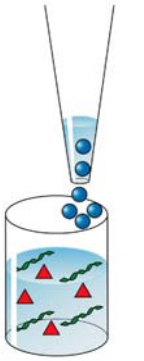
* This plate is intended for the collection and storage of the purified PCR products. If desired, it can alternatively be used as separation plate in combination with the NucleoMag SEP.

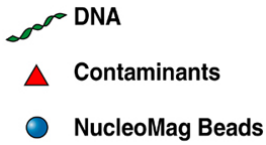
Material to be supplied by user:
Separation plate, e.g. MN Elution Plate, U-Bottom; MN Square-well Block (see ordering information)

2 Product description

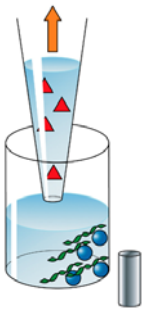
2.1 The basic principle

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.

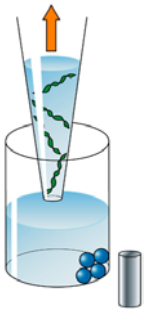




Binding conditions are adjusted and the NucleoMag PCR Beads are added to the sample.



PCR Products are bound to the NucleoMag PCR Beads. Primers, nucleotides, salts, enzymes and other contaminants are removed in subsequent washing steps.



Purified PCR products are eluted in low-salt buffer or water. PCR products are ready-to-use in downstream applications.

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 $A_{260/280} \geq 1.7 – 1.9$.
- 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 PCR Beads** are highly reactive, superparamagnetic beads. The binding capacity is 0.3 µg of DNA per 1 µl of NucleoMag PCR-Bead suspension, 1 µl of suspension contains 150 µg of beads.

2.3 Magnetic separation systems

For use of **NucleoMag 96 PCR** the NucleoMag SEP is recommended. Separation is carried out in a MN Elution Plate, U-Bottom or MN Square-well Block (see ordering information). The kit can also be used with other common separators. See suppliers ordering information for suitable separation plates.

Magnetic separator	**Separation plate**
NucleoMag SEP (Cat. No. 744 900)	Square-well Block (MN, Cat. No. 740 670) Elution Plate, U-Bottom (MN, Cat. No. 740 672)
Promega MagnaBot	96-well microtiter plate (Greiner Cat. No. 650 101; Costar Cat. No. 43119)
Tecan Te-MagS	96-well PCR plate (ABgene Cat. No. AB 0407

Static magnetic pins
Separators with static magnetic pins, e.g. NucleoMag SEP (for manual use and for use on liquid handling workstations): This type of separator is recommended in combination with a plate shaker, e.g. H+P Variomag® Teleshake (H+P Labortechnik AG, Bruckmannring 28, D-85764 Oberschleißheim, Germany, www.hp-lab.de), for

optimal resuspension of the beads during the washing and elution steps. Alternatively, beads can be resuspended in the buffer by pipetting up and down several times. For fully-automated use on liquid handling workstations a gripper tool is required, the plate is transferred to the magnetic separator for separation of the beads and transferred to the shaker module for resuspension of the beads.

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

2.4 Adjusting the shaker settings

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

- Apply 300 µl dyed water (select desired elution buffer volume) to the wells of the separation plate. Position the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off the shaker and check plate surface for small droplets of dyed water.
- Increase speed setting, shake for an additional 30 seconds, and check plate surface for droplets again.
- Continue increasing the speed setting until you observe droplets on top of the separation plate. Reduce speed setting, check again and use this setting for the washing and elution step.

2.5 Handling of beads

Distribution of beads
A homogenous 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 vortex 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 on each system. It is recommended to use the separation plates or tubes specified by the supplier of the magnetic separator.

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

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

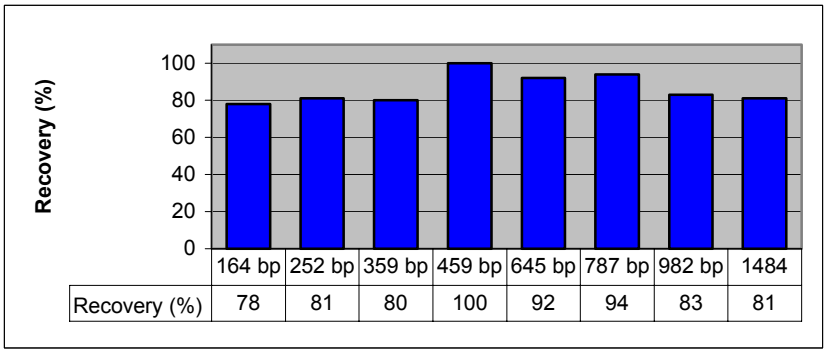
* 8-channel pipetting device

2.6 Elution procedures

Purified PCR products can be eluted directly with the supplied elution buffer 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 dried NucleoMag PCR beads completely with elution buffer during the elution step. The volume of dispensed elution buffer depends on the magnetic separation system (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.

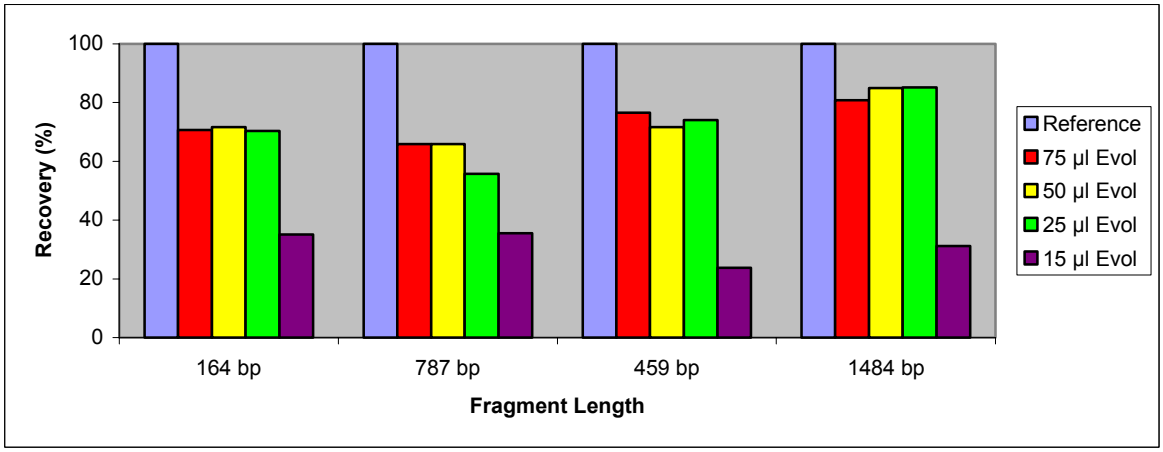
Elution is possible at room temperature. Recovery can be increased by 10 – 15% if elution is performed at 55^{o}C.

The recovery rate depends on the length of the PCR product.



	164 bp	252 bp	359 bp	459 bp	645 bp	787 bp	982 bp	1484
Recovery (%)	78	81	80	100	92	94	83	81

Yield does not depend on the elution volume used (25 to 200 µl).



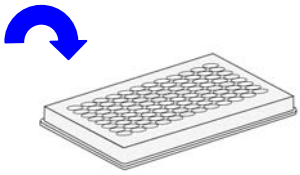
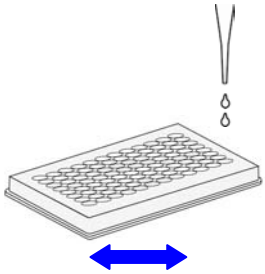
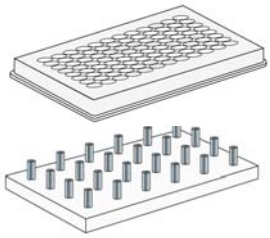
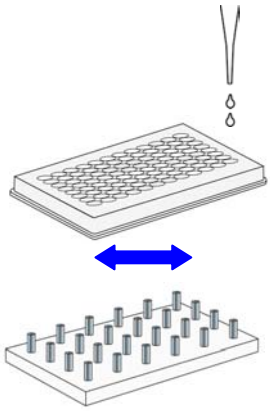
3 Storage conditions and preparation of working solutions

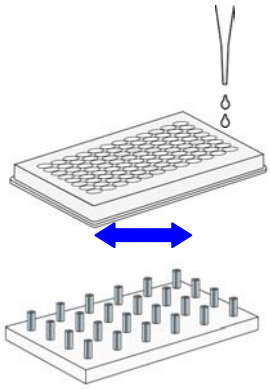
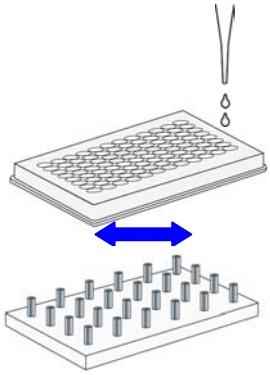
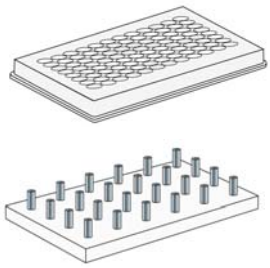
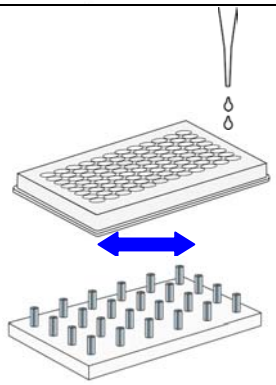
All components of the **NucleoMag 96 PCR** kit should be stored at room temperature (20 – 25°C) and are stable for up to one year.

Before first use of the kit, add the indicated volume of 96% or 100% ethanol to the wash buffer MP3.

	NucleoMag 96 PCR		
	1 x 96 preps	**4 x 96 preps**	**24 x 96 preps**
Cat. No.	**744 100.1**	**744 100.4**	**744 100.24**
Buffer MP3 (concentrate)	15 ml add 60 ml ethanol	60 ml add 240 ml ethanol	2 x 180 ml add 2 x 720 ml ethanol

4 General procedure

1	**Transfer** PCR reaction mixture to appropriate 96-well plate. For PCR reactions < 50 µl: adjust the volume to 50 µl using sterile water.	50 µl PCR reaction	
2	**Add** beads and bind DNA.	12 µl NucleoMag P-Beads 138 µl Binding Buffer MP1 shake 5 min, RT	
3	**Remove** supernatant.	1 min separation	
4	**MP2 wash**.	300 µl MP2	

5	**First MP3 wash**.	300 µl MP3	
6	**Second MP3 wash**.	300 µl MP3	
7	**Dry** the beads.	10 min, RT	
8	**Elute** purified PCR products and transfer to Elution Plate.	25 – 100 µl MP4 shake 5 min, RT 2 min separation transfer Optional: Elution at 55°C	

4.1 Standard protocol for clean-up of 50 µl PCR reactions

This protocol is designed for magnetic separators with static pins (e.g. NucleoMag SEP) and suitable plate shakers (e.g. H+P Variomag® Teleshake). This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments. For the availability of ready-to-run scripts please contact your local distributor or MN directly.

1 Transfer PCR reaction mixture to 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. Elution Plate, U-Bottom; not included in the kit as separation plate) and compatible magnetic separators section 2.3.

2 Add 12 µl NucleoMag P-Beads and 138 µl Binding Buffer MP1 to each well of the separation plate and mix by shaking (5 min) at room temperature.

Alternatively, mix by pipetting up and down 5 times and incubate at room temperature (5 min).

Note: NucleoMag P-Beads and Binding Buffer MP 1 may be premixed. For 96 samples premix 1248 µl of beads with 14.35 ml of buffer MP1 and dispense 150 µl into each well of the separation plate.

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

3 Separate the magnetic beads against the side of the wells by placing the separation plate on the magnetic separator. Wait 60 seconds until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

Remove the separation plate from the magnetic separator.

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

4 Add 300 µl Wash Buffer MP2 to each well and wash the bead/DNA complex by shaking (1 min) until beads are resuspended completely.

Alternatively, pipette up and down 5 times.

Separate all of the magnetic beads against the side of the well by placing the separation plate on the magnetic separator. Aspirate and discard the supernatant.

Remove the plate from the magnetic separator.

5 Add 300 µl Wash Buffer MP3 to each well and wash the bead/DNA complex by shaking (1 min) until beads are resuspended completely.

Alternatively, pipette up and down 5 times.

Separate all of the magnetic beads against the side of the well by placing the separation plate on the magnetic separator. Aspirate and discard the supernatant.

Remove the plate from the magnetic separator.

6 Repeat step 5. Leave separation plate on the magnetic separator after removing the supernatant.

7 Dry the beads by incubating the separation plate 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.

8 Elute DNA from the beads by adding desired volume of Elution Buffer MP4 (25 – 100 µl) to each well and resuspend the bead/DNA complex by shaking (5 min).

Alternatively, pipette up and down 5 times. Incubate at room temperature for 5 min.

Separate all of the magnetic beads against the side of the well by placing the separation plate on the magnetic separator. Transfer the supernatant containing the purified PCR products to the Elution Plate, U-bottom.

Note: The yield can be increased by using prewarmed elution buffer (55°C) or by incubation of the beads/elution buffer suspension for 5 minutes at 55°C.

5 Appendix

5.1 Troubleshooting

Problem	Possible cause and suggestions
Poor DNA yield	*Wash Buffer MP2 did not contain ethanol* • Addition of the indicated volume of 96 – 100% ethanol to the buffer MP2 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 MP2* • Be sure to remove all of the ethanolic wash buffer MP2 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
	Time for magnetic separation too short • Increase separation time to allow the beads to be attracted to the magnetic pins completely.
Carry over of beads	*Aspiration speed too high (elution step)* • High aspiration speeds during the elution step may cause bead carry-over. Reduce aspiration speed for elution step.

5.2 Ordering information

Product	Cat. No.	Pack of
NucleoMag 96 PCR	744 100.1	1 x 96
NucleoMag 96 PCR	744 100.4	4 x 96
NucleoMag 96 PCR	744 100.24	24 x 96
NucleoMag SEP	744 900	1
Elution Plate, U-Bottom (to be used as separation plate)	744 672	20
Square-well Block	740 670	20
Self-adhering PE foil	740 676	50 sheets

5.3 Product Use Restriction / Warranty

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

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

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