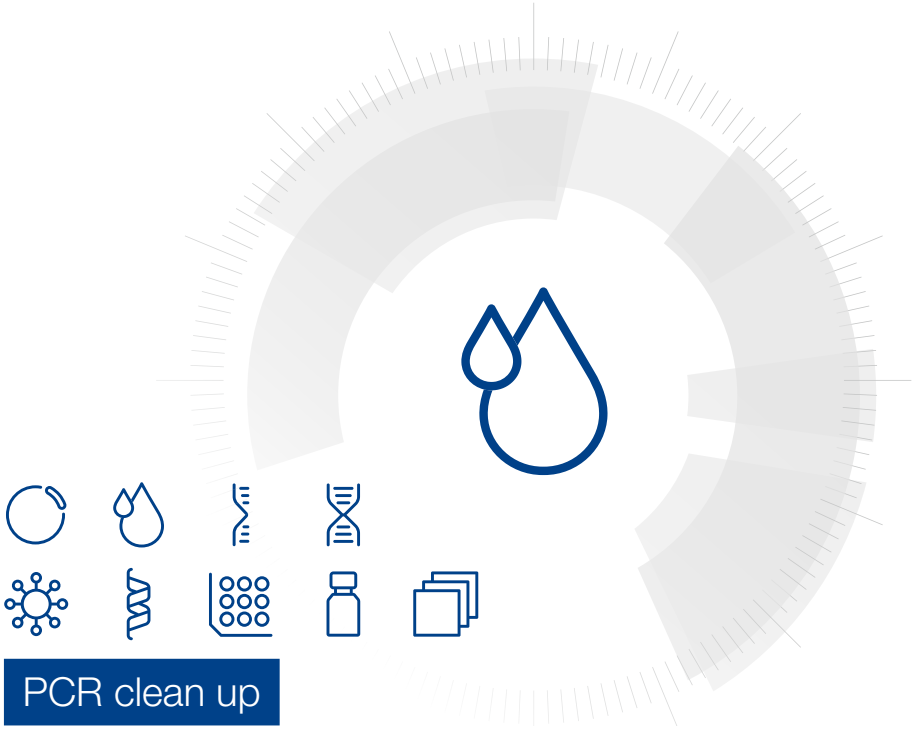
Bioanalysis

MACHEREY-NAGEL

User manual



PCR clean up

■ NucleoFast® 96 PCR

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1 Components

1.1 Kit contents

	NucleoFast® 96 PCR Clean-up Kit		NucleoFast® 96 PCR Plates	
REF	**4 x 96 preps 743500.4**	**24 x 96 preps 743500.24**	**10 x 96 preps 743100.10**	**50 x 96 preps 743100.50**
Recovery Buffer RB*	50 mL	300 mL	-	-
RNase-free H_2O	125 mL	2 x 375 mL	-	-
NucleoFast® 96 PCR Plates	4	24	10	50
Elution Plates (including Self-adhering PE Foil)	4	24	-	-
User manual	1	1	1	1

1.2 Consumables, and equipment to be supplied by user

Consumables

- Disposable tips

Equipment

- Manual pipettors
- suitable vacuum manifold and pump
- Centrifuge
- Equipment for PCR
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the NucleoFast® 96 PCR is used for the first time. Experienced users, however, may refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

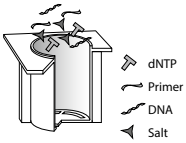
All technical literature is available on the internet at ***www.mn-net.com***.

* Composition of Recovery Buffer RB: 5 mM Tris / HCl, pH 8.5

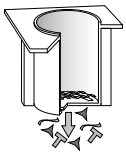
2 Product description

2.1 The basic principle

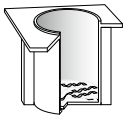
NucleoFast® 96 PCR is based on ultrafiltration and designed for rapid clean up of PCR fragments. During the procedure the PCR samples are applied to the ultrafiltration membrane. Under vacuum or in a centrifuge contaminants (primers, dNTPs, salts) are filtered to waste. The desired PCR products are retained on the membrane and can be recovered from the membrane after the addition of water or low salt buffer and a short incubation. The purified PCR fragments can be used directly for further downstream applications, like sequencing or microarray spotting. The **NucleoFast® procedure** eliminates the use of chaotropic salts for binding of nucleic acids and subsequent ethanolic washing steps. **The NucleoFast® 96 PCR Plates** can be used either manually or automated on standard liquid handling instruments.



PCR products are loaded directly onto the **NucleoFast® 96 PCR** filter membrane.



PCR products are collected on the surface of the ultrafiltration membrane while contaminants are filtered to waste. Optionally, the PCR products can be washed with RNase-free H_2O.



PCR products are recovered from the membrane after addition of water or recovery buffer. PCR products are ready-to-use for downstream applications.

2.2 Kit specifications

- **NucleoFast® 96 PCR** is designed for the rapid manual clean up of PCR fragments using NucleoVac 96 (see ordering information, section 6.2), other suitable vacuum manifolds, or microplate centrifuges (see section 2.5). Manual processing time for 96 samples is about 20 minutes.
- Besides PCR clean up, **NucleoFast® 96 PCR** can also be used for the concentration of purified genomic DNA or RNA > 150 bp / nt. Due to the low recovery volume (> 25 µL for manual use and > 50 µL for automated use, this is an easy and fast method to concentrate pre-purified samples.
- **NucleoFast® 96 PCR** can easily be adapted to common liquid handling instruments (see section 2.5). The actual processing time for the purification of 96 samples depends on the configuration of the instrument, but can be as short as 15 minutes.
- 20–300 µL PCR reaction mix can be processed per well. If a larger volume is to be processed the sample has to be loaded stepwise. Filtration times will increase as the retained PCR products will decrease the permeability of the membrane.
- The recovery volume is ≥ 25 µL for manual use. For automated use a recovery volume ≥ 50 µL is recommended.
- High DNA recovery of 50–95 % for DNA fragments of ≥ 150 bp.
- The purity of recovered PCR products is A_{260}/A_{280} ≥ 1.7 – 1.8.
- Purified PCR products are ready-to-use for downstream applications like automated fluorescent sequencing, labelling, microarray analysis, cloning, or restriction digestion.
- The sturdy membrane allows easy recovery of purified PCR fragments without the risk of damaging the membrane.
- Low dead volume of the NucleoFast® membrane of 3–4 µL only.

2.3 Filtration conditions

Filtration time depends on sample volume, vacuum strengths, and vacuum pump used. For use of the **NucleoFast® 96 PCR Plates** apply a vacuum of up to - 0.6 bar (reduction of atmospheric pressure, 22.5 inches Hg). Use a portable vacuum pump or suitable house vacuum.

Typically, a 100 µL PCR reaction passes the membrane in 10–15 minutes. When all of the solution has passed the membrane, apply vacuum for an additional 30–60 seconds to allow the liquid to drain off the outlets. Before adding Recovery Buffer RB (or RNase-free H_2O) make sure that vacuum is completely released to prevent the buffer from being sucked through the membrane.

For processing of the **NucleoFast® 96 PCR Plates** in a centrifuge a force of 4,500 x *g* is recommended. Lower g-forces will increase filtration times significantly.

When using less than 96 samples sealing of unused wells is not required.

2.4 Recovery of the purified PCR products

Purified PCR products can be recovered directly from the membrane using Recovery Buffer RB or RNase-free H_2O (both not supplied with 743100.10 and 743100.50). For manual use the recovery volume should be at least 25 µL. Use a multichannel pipettor to recover the buffer containing the purified PCR products completely from the wells. The tips may touch the membrane slightly during the manual recovery process. During the automated use a minimum recovery volume of 50 µL is recommended to improve the recovery and the well-to-well consistency (see section 2.5). It is crucial to collect the Recovery Buffer RB completely from the membrane to get an optimal recovery of PCR products.

The sturdy ultrafiltration membrane allows an easy recovery of purified PCR products without the risk of damaging the membrane. Damaging of the membrane would result in the risk of co-recovering small membrane parts (a common problem with other ultrafiltration membranes). These parts might interfere with subsequent applications, especially capillary sequencing and microarray spotting. With the **NucleoFast® 96 PCR** membrane it is possible to touch the membrane with the tips during the recovery process without the risk of damaging it.

Recovery of DNA can be facilitated either by a short incubation, mixing, or by using a plate shaker after the addition of Recovery Buffer RB or RNase-free H_2O (this is especially recommended for PCR products ≥ 500 bp):

- Incubate for 5 minutes at room temperature without shaking after the addition of Recovery Buffer RB or RNase-free H_2O.Add Recovery Buffer RB or RNase-free H_2O to the membrane and mix by pipetting up and down 5–10 times, or
- shake for 2–5 minutes on a suitable microplate shaker with moderate shaking. For use with a shaker the dispensed recovery buffer volume should be ≥ 50 µL.

When using a plate shaker for recovery the speed settings have to be checked carefully to prevent cross-contamination from well to well. Proceed as follows:

- Apply 50–100 µL of Recovery Buffer RB or RNase-free H_2O with some added dye (e.g., bromphenol blue) to the wells of a **NucleoFast® 96 PCR Plate**. Position the plate on the shaker and start shaking with a moderate speed setting for 30 seconds. Turn off 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 **NucleoFast® 96 PCR Plate**. Reduce speed setting, check again, and use this setting for the recovery step.

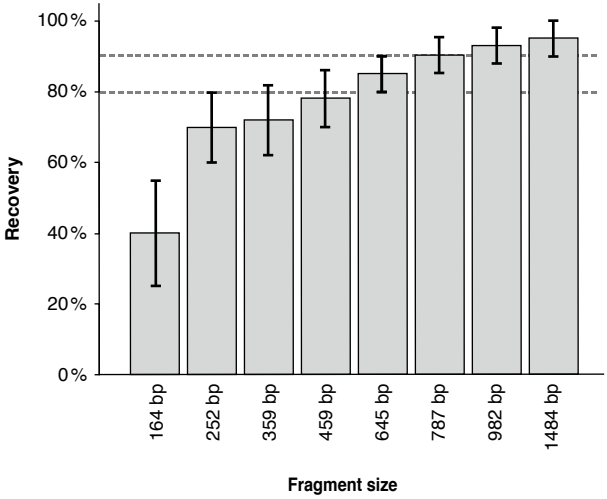


Figure 1 The recovery rate depends on the length of the PCR product: 100 µL of PCR products have been purified using the NucleoFast® 96 PCR Plate under vacuum. Mean values and SD of n = 8.

2.5 Automated processing on robotic platforms

NucleoFast® 96 PCR can easily be automated on common liquid handling instruments. As no reassembly of the vacuum chamber is necessary when processing one plate per run, **NucleoFast® 96 PCR** can be used fully automated even on workstations without integrated gripper tools.

During the automated use a recovery volume of ≥ 50 µL is recommended. Smaller volumes are possible, but may lead to a reduced recovery of PCR products and to a lower well-to-well consistency. Recovery can be improved either by mixing, incubation, or the use of a plate shaker (see section 2.4).

A very crucial step is the effective recovery of PCR products from the membrane. Needles/disposable tips have to be as close to the membrane as possible during the recovery step to recover Buffer RB or RNase-free H_2O completely. Slight touching of the membrane will not result in damage of the membrane, but might block the needles/disposable tips during the recovery process, resulting in a reduced recovery. The height adjustment of the needles/disposable tips has to be optimized for each individual platform with extra care for optimal results.

Make sure that the vacuum is released before recovering the PCR products and adjusting the height of the needles/disposable tips, as the **NucleoFast® 96 PCR Plate** has a lower position inside the manifold under vacuum. This may result in a loss of about 20–30 % of PCR fragments.

If more than one plate is to be processed during the run, the plates stored on the platform and currently not in use can be protected with PE Foil, which is available separately (see ordering information).

NucleoFast® 96 PCR is compatible with common automation workstations.

Please contact MN or your local distributor for technical support regarding hardware, software, setup instructions, and selection of available protocols.

3 Storage conditions

All kit components can be stored at 15–25°C for at least one year.

4 Safety instructions

All kit components are non hazardous. For more information consult the appropriate Material Safety Data Sheets (MSDS available online at http://***www.mn-net.com/msds***).

5 Protocols

5.1 NucleoFast® 96 PCR – vacuum processing

Protocol-at-a-glance

1	**Adjust** the volume of the reaction mixture to 100 µL using RNase-free H_2O	**For PCR samples < 100 µL**
2	**Transfer** PCR samples to NucleoFast® 96 PCR Plate	**100–300 µL**
3	**Remove** contaminants to waste under vacuum or centrifugation	**-0.4 to -0.6 bar*, 10–15 min**
4	**Wash** membrane**	**100 µL RNase-free H2O** **-0.4 to -0.6 bar*, 10–15 min**
5	**Recover** purified PCR samples	**25–100 µL RB or RNase-free H2O**

* Reduction of atmospheric pressure

** Optional for vacuum processing

Detailed protocol

This protocol is designed for PCR reaction volumes of 20–100 µL. For PCR reaction volumes of up to 300 µL, filtration times have to be increased. The protocol is for manual use or for use with common liquid handling systems.

1 Adjust the volume of reaction mixture

Note: Smaller sample volumes should be filled up with RNase-free H_2O to 100 µL to enable a uniform loading of the plate.

2 Transfer PCR samples to NucleoFast® 96 PCR Plate

Note: Slowly dispense samples directly onto the membrane. Avoid dispensing of the samples to the inner wall of the wells.

Unused wells of the NucleoFast® 96 PCR Plate may be left open. Sealing is not required.

3 Remove contaminants by ultrafiltration

Place the NucleoFast® 96 PCR Plate on a suitable vacuum manifold and apply vacuum. Adjust vacuum to -0.4 to -0.6 bar*.

Note: Typically vacuum has to be applied for 10–15 min for a sample volume of 50–100 µL.

After the samples have passed the NucleoFast® 96 PCR Plate completely, apply vacuum for an additional 30–60 s.

4 Optional: Wash membrane

Release vacuum (60– 90 s).

*Dispense **100 µL RNase-free H_2O** into each well of the NucleoFast® 96 PCR Plate and apply vacuum (-0.4 to -0.6 bar*) until water has passed the membrane. Apply vacuum for an additional 30–60 s.*

Note: The optional washing step is recommended if the purity of the PCR samples is considered not sufficient for desired downstream application. If problems after clean up are observed with the downstream application perform the washing step. Typically, the washing step is not required.

5 Recover purified PCR samples

Release the vacuum (60–90 s).

Dispense an appropriate volume (25–100 µL) of Recovery Buffer RB or RNase-free H_2O directly onto the membrane of the NucleoFast® 96 PCR Plate. Recover DNA by incubation, mixing, or shaking. For more information about the recovery process refer to section 2.4.

Note: Make sure that no vacuum is applied to the manifold when dispensing the recovery buffer.

* Reduction of atmospheric pressure

5.2 NucleoFast® 96 PCR – centrifuge processing

Protocol-at-a-glance

1	**Adjust** the volume of the reaction mixture to 100 µL using RNase-free H_2O	**For PCR samples < 100 µL**
2	**Transfer** PCR samples to NucleoFast® 96 PCR Plate	**100–300 µL**
3	**Remove** contaminants to waste under vacuum or centrifugation	**4,500 x *g*, 10–15 min**
4	**Wash** membrane**	**100 µL RNase-free H2O** **4,500 x *g*, 10–15 min**
5	**Recover** purified PCR samples	**25–100 µL RB or RNase-free H2O**

** Optional for vacuum processing

Detailed protocol

This protocol is designed for a PCR reaction volume of 20–100 µL. For PCR reaction volumes of up to 300 µL filtration times have to be increased.

This protocol is for manual processing using a microplate centrifuge. The centrifuge buckets have to be able to hold the NucleoFast® 96 PCR Plate on top of a suitable plate for waste collection (e.g., Square-well Block, Round-well Block; not provided with the kit). Do not use standard microtiter plates for waste collection as they break under the g-forces required to process the NucleoFast® 96 PCR Plate.

If you are not sure that your buckets are able to hold the sandwich of a NucleoFast® 96 PCR Plate and a waste collection plate, place a standard microtiter plate on top of the appropriate waste collection plate and see if this sandwich fits into the bucket. If using a standard Square-well Block for waste collection, the sandwich hight is 58 mm.

1 Adjust the volume of reaction mixture

Note: Smaller sample volumes should be filled up with RNase-free H_2O to 100 µL to enable a uniform loading of the plate.

2 Transfer PCR samples to NucleoFast® 96 PCR Plate

Unused wells of the NucleoFast® 96 PCR Plate may be left open. Sealing is not required.

3 Remove contaminants by ultrafiltration

Place the NucleoFast® 96 PCR Plate onto a suitable waste collection plate (e.g., Square-well Block). Place the sandwich in the centrifuge and spin at 4,500 x *g*.

Note: Typically centrifugation for 5–10 min for a sample volume of 50–100 µL is sufficient.

4 Wash membrane

Dispense **100 µL RNase-free H_2O** into each well of the NucleoFast® 96 PCR Plate. Place the NucleoFast® 96 PCR Plate on top of the waste collection plate and centrifuge for 5–10 min.

Note: The washing step is mandatory if NucleoFast® 96 PCR is used under centrifugation. About 3–5 µL of PCR sample (containing salts, primers, dNTPs) will remain on top of the membrane after the first centrifugation step. To avoid contamination of the purified PCR sample the washing step is mandatory to remove the contaminants.

5 Recover purified PCR samples

Dispense an **appropriate volume (25–100 µL)** of **Recovery Buffer RB** or **RNase-free H_2O** directly onto the membrane of the NucleoFast® 96 PCR Plate. Recover DNA by incubation, mixing, or shaking. For more information about the recovery process refer to section 2.4.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low DNA recovery	*Insufficient mixing or shaking during recovery step* • Increase number of mixing steps, increase incubation time, optimize shaker speed settings. *PCR fragment smaller than 150 bp* • Use the NucleoSpin® 96 PCR Clean-up kit for purification of small PCR products. *Recovery buffer volume too small* • Increase amount of recovery buffer to at least 25 µL for manual use. For automated use a minimum volume of 50 µL is recommended. *DNA fragments dried onto membrane* • Dispense Recovery Buffer RB or RNase-free H_2O and incubate for 15–30 minutes at room temperature to allow DNA to rehydrate before removing DNA.
Samples are contaminated	*Samples not filtered completely* • Allow the samples to pass the filter completely. Wait until the membrane appears dry and shiny. *Samples remain on the well´s inner wall* • Dispense samples directly onto the membrane. Make sure that no sample material sticks to the side of the well, as contaminants might get co-recovered. Avoid tip touch during automated use of NucleoFast® 96 PCR. Perform optional washing step. *No washing step performed while using NucleoFast® 96 PCR under centrifugation* • Perform washing step to remove contaminants.
Low A260/A230 ratios	• Perform an additional equilibration step of the NucleoFast prior loading the sample. Therefore, transfer 300 µL of RNase-free H_2O to the NucleoFast® PCR Plate. Apply vacuum (-0.3 to -0.4 bar) until the water has passed the NucleoFast plate completely (approx. 15–20 min). • Perform the optional washing step (step 4 of the NucleoFast user manual - "wash membrane") by transferring 100 µL of RNase-free H_2O to the NucleoFast® PCR Plate and apply vacuum (-0.4 to -0.6 bar*) until water has passed the membrane. Apply vacuum for an additional 30–60 s.

6.2 Ordering information

Product	REF	Pack of
NucleoFast® 96 PCR Clean-up Kit	743500.4 743500.24	4 x 96 preps 24 x 96 preps
NucleoFast® 96 PCR Plates	743100.10 743100.50	10 plates 50 plates
NucleoVac 96 Vacuum Manifold	740681	1
NucleoVac Vacuum Regulator	740641	1
Buffer RB	740362.50	50 mL
Square-well Block	740481 740481.24	4 24
Round-well Block with Cap Strips	740475 740475.24	4 24
Self-adhering PE Foil	740676	50 sheets

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

6.3 Product use restriction / warranty

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

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

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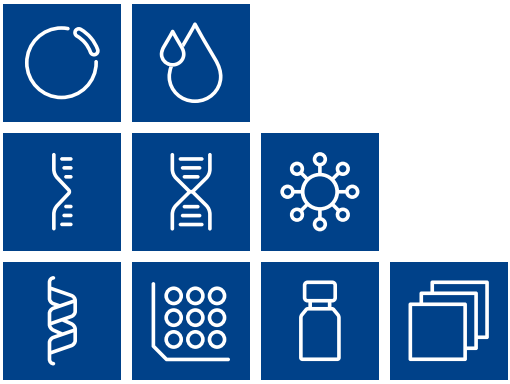
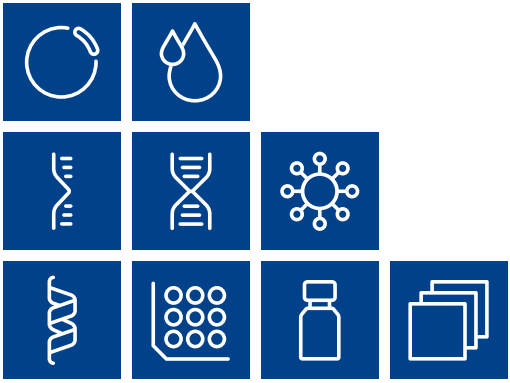


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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