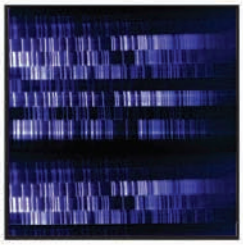


# PCR Clean-up

## User Manual

NucleoFast 96 PCR

March 2010 / Rev. 04

MACHEREY-NAGEL

# Table of contents

# 1 Kit contents

| | NucleoFast 96 PCR | | | |
|---|---|---|---|---|
| | **4 x 96 preps** | **24 x 96 preps** | **10 x 96 preps*** | **50 x 96 preps*** |
| **Cat. No.** | **743500.4** | **743500.24** | **743100.10** | **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 |

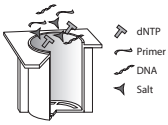
* Cat. No. 743100.10 and 743100.50 do not contain Buffer RB, RNase-free $H_2O$, or Elution Plates.

** 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 (see section 2.3), or microplate centrifuges (see section 5.2). Manual processing time for 96 samples is about 20 minutes.
- **NucleoFast 96 PCR** can easily be adapted to common liquid handling instruments (see section 2.6). 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.
- No detergents leak out of the membrane.
- Low dead volume of the NucleoFast membrane of 3 – 4 µl only.

## 2.3 Suitable vacuum manifolds

**NucleoFast 96 PCR Plates** can be used with common vacuum manifolds:

**Suitable vacuum manifolds**

| Vacuum manifold | Suitability |
|---|---|
| NucleoVac 96 Vacuum Manifold | Yes |
| Millipore/MultiScreen® | Yes |
| Qiagen/QIAvac 96 | Yes |
| Promega/Vac-Man® 96 vacuum manifold | Yes |
| Bio-Rad/Aurum™ vacuum manifold | Yes |
| Eppendorf/Perfect VAC Manifold | No |

## 2.4 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.

## 2.5 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 2.6). 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.

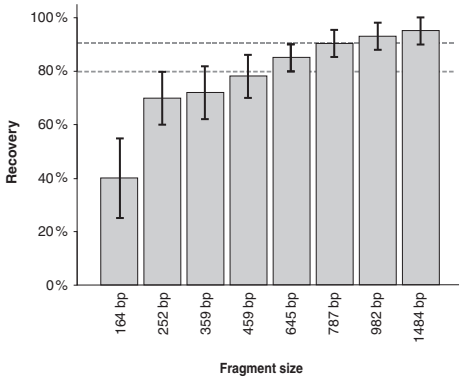



**Figure 1: The recovery rate depends on the length of the PCR product:** 100 µl of PCR products have been purified using the NucleoFast 96 PCR Plate under vacuum. Mean values and SD of n = 8.

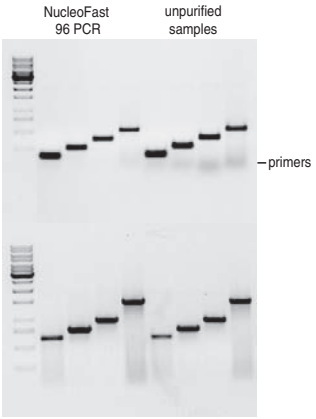



**Figure 2: NucleoFast 96 PCR shows a high recovery even for small fragments:** PCR samples (164, 252, 359, 490 bp, upper panel; 645, 787, 982, 1484 bp, lower panel, 25 µl each) have been purified manually according to the standard protocol. Control: unpurified samples. Note the high recovery even for small fragments and the efficient removal of primers.

## 2.6 Automation of NucleoFast 96 PCR

**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 2.5).

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 cover lids, which are 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.

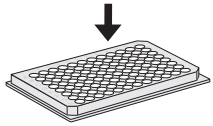
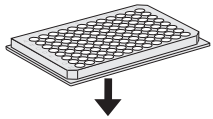
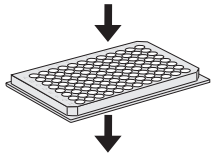
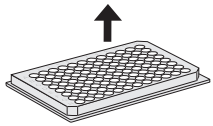
# 3 Storage conditions

All kit components can be stored at room temperature (18 – 25°C) for up to one year.

# 4 Safety instructions

All kit components are non-hazardous.

# 5 General procedure

| | | | |
|---|---|---|---|
| 1 | **Transfer** PCR samples to **NucleoFast 96 PCR Plate** | 20 – 300 µl |  |
| 2 | **Filter** contaminants to waste under vacuum or centrifugation | -0.4 to -0.6 bar* 10 – 15 min<br>or<br>4,500 x *g* 10 – 15 min |  |
| 3 | **Wash** membrane** | *100 µl $H_2O$ (RNase-free)*<br>-0.4 to -0.6 bar* 10 – 15 min<br>or<br>*4,500 x* g *5 – 10 min* |  |
| 4 | **Recover** purified PCR samples | 25 – 100 µl RB or $H_2O$ (RNase-free) |  |

* Reduction of atmospheric pressure

** Optional for vacuum processing

## 5.1 Standard protocol for the purification of PCR products – vacuum processing

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** **Transfer** PCR samples to **NucleoFast 96 PCR Plate.**

*Note: Smaller sample volumes should be filled up with $H_2O$ to 100 µl to enable a uniform loading of the plate.*

*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.

**2** **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.

**3** ***Optional washing step***

*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.*

* Reduction of atmospheric pressure

**4 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 2.5.

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

## 5.2 Standard protocol for the purification of PCR products – centrifuge processing

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.

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**1** **Transfer** the PCR samples (20 – 100 µl) to the **NucleoFast 96 PCR Plate**.

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

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**2** **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.*

---

**3** **Washing step**

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.*

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**4** **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 2.5.

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# 6 Appendix

## 6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Low DNA recovery | *Insufficient mixing or shaking during recovery step*<br>• Increase number of mixing steps, increase incubation time, optimize shaker speed settings.<br><br>*PCR fragment smaller than 150 bp*<br>• Use the NucleoSpin® 96 Extract II kit for purification of small PCR products.<br><br>*Recovery buffer volume too small*<br>• Increase amount of recovery buffer to at least 25 µl for manual use. For automated use a minimum volume of 50 µl is recommended.<br><br>*DNA fragments dried onto membrane*<br>• 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*<br>• Allow the samples to pass the filter completely. Wait until the membrane appears dry and shiny.<br><br>*Samples remain on the well´s inner wall*<br>• 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.<br><br>*No washing step performed while using NucleoFast 96 PCR under centrifugation*<br>• Perform washing step to remove contaminants. |

## 6.2 Ordering information

| Product | Cat. No. | Pack of |
|---|---|---|
| NucleoFast 96 PCR Clean-up Kit | 743500.4<br>7403500.24 | 4 x 96 preps<br>24 x 96 preps |
| NucleoFast 96 PCR Plates | 743100.10<br>743100.50 | 10 plates<br>50 plates |
| Cover Lids for NucleoFast 96 PCR Plates | 743101.50 | 50 lids |
| Self-adhering PE Foil | 740676 | 50 sheets |
| NucleoVac 96 Vacuum Manifold | 740681 | 1 |
| NucleoVac Vacuum Regulator | 740641 | 1 |
| Buffer RB | 740362.50 | 50 ml |
| Square-well Block | 740670 | 20 |
| Round-well Block | 740671 | 20 |

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

## 6.3 Product use restriction / warranty

**NucleoFast 96 PCR Clean-up** kit components were developed, designed, distributed, 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 **NucleoFast 96 PCR Clean-up** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish an extra copy.

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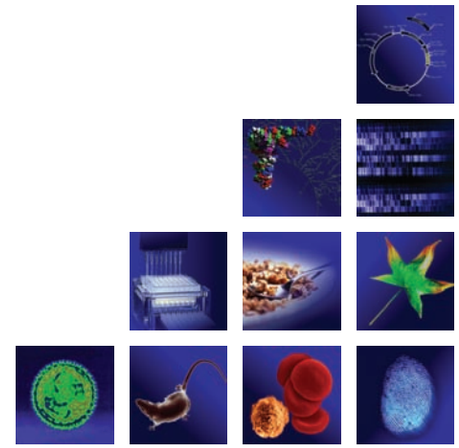

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