Bioanalysis

MACHEREY-NAGEL

User manual

PCR clean up Gel extraction

- NucleoTrap® CR
- NucleoTrap®

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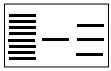
PCR clean up, gel extraction

Protocol at a glance (Rev. 08)

	NucleoTrap®CR PCR clean up		NucleoTrap® Gel extraction
1 NucleoTrap®: **Excise DNA fragment /** **Solubilize gel slice**			
NucleoTrap®CR: **Adjust binding conditions**	4 vol NT2/1 vol sample		300 µL NT1/100 mg gel
2 Bind DNA	10 µL silica matrix / 100 µL sample RT, 10 min 10,000 x *g*, 30 s		4 µL silica matrix/µg DNA 50 °C, 5 – 10 min 10,000 x *g*, 30 s
3 Wash silica matrix	1st 400 µL NT2 2nd 400 µL NT3 3rd 400 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s		1st 500 µL NT2 2nd 500 µL NT3 3rd 500 µL NT3 10,000 x *g*, 30 s 10,000 x *g*, 30 s 10,000 x *g*, 30 s
4 Dry silica matrix	RT or 37 °C, 10 – 15 min		RT or 37 °C, 10 – 15 min
5 Elute DNA	25 – 50 µL NE RT, 10 – 15 min 10,000 x *g*, 30 s		25 – 50 µL NE RT, 10 – 15 min 10,000 x *g*, 30 s

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1 Components

1.1 Kit contents

	NucleoTrap®CR	
REF	**10 preps 740587.10**	**100 preps 740587**
NucleoTrap®CR Suspension	100 µL	1000 µL
Buffer NT2	10 mL	100 mL
Wash Buffer NT3 (Concentrate)*	6 mL	25 mL
Elution Buffer NE**	13 mL	13 mL
User manual	1	1

	NucleoTrap®	
REF	**10 preps 740584.10**	**100 preps 740584**
NucleoTrap® Suspension	100 µL	1000 µL
Buffer NT1	6 mL	60 mL
Buffer NT2	10 mL	100 mL
Wash Buffer NT3 (Concentrate)*	6 mL	25 mL
Elution Buffer NE**	13 mL	13 mL
User manual	1	1

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

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

1.2 Consumables and equipment to be supplied by the user

Consumables:

- 96 – 100 % ethanol
- 1.5 mL microcentrifuge tubes

Equipment:

- Centrifuge for microcentrifuge tubes
- Manual pipettors and disposable tips
- Vortex mixer
- Heating-block
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended to read the detailed protocol sections of this user manual if using the NucleoTrap®CR/NucleoTrap® kits for the first time. However, experienced users may refer to the Protocol at a glance. 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***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

With the NucleoTrap®CR/Trap® method, DNA binds in the presence of chaotropic salts (Buffer NT1 and Buffer NT2) to specially activated silica particles (matrix). Buffer NT1 contains additional components in order to dissolve agarose gel slices. Afterwards, the NucleoTrap®CR/Trap® matrix is added to the binding mixtures. Contaminations like salts and soluble macromolecular components are removed by a simple washing step with ethanolic Wash Buffer NT3. Pure DNA is finally eluted under low ionic strength conditions with slightly alkaline Elution Buffer NE (5 mM Tris-Cl, pH 8.5).

2.2 Kit specifications

- The NucleoTrap®CR kit is designed for direct purification of PCR products.
- The NucleoTrap® kit is designed for the purification of DNA from TAE / TBE agarose gels.
- In contrast to the NucleoTrap® matrix, the NucleoTrap®CR matrix will not bind DNA fragments < 100 bp due to a larger pore size of the silica matrix.
- Standard as well as low melting agarose gels can be used.
- The prepared DNA fragments can be used directly in applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

Table 1: Kit specifications at a glance

Parameter	NucleoTrap®CR	NucleoTrap®
Technology	Silica matrix	Silica matrix
Format	Silica bead suspension	Silica bead suspension
Sample material	< 400 µL PCR reaction mixture	< 200 mg agarose gel
Use	For research use only	For research use only
DNA fragments from agarose gels	-	++
Desalting, removal of enzymes, nucleotides and / or labeling reagents like biotin or radioactive ATP, etc.	++	+
Direct purification of amplified DNA	++	-
Fragment size	100 bp–approx. 50 kbp	20 bp–approx. 50 kbp
Typical recovery	70 – 80 %	50 – 90 %
A_{260}/A_{280}	1.7 – 1.9	1.7 – 1.9
Elution volume		20 – 50 µL
Preparation time		60 min/6 preps
Binding capacity		6 µg/10 µL matrix

-not recommended +possible ++optimal

2.3 Elution procedures

For the elution of DNA one of the following solutions can be used: Buffer NE (supplied) / TE buffer, pH 8.5 / distilled water, pH 8.5.

If water is used, the pH should be checked and adjusted to pH 8 – 8.5 since deionized water usually exhibits a pH below 7. Furthermore, absorption of CO2 Leads to a decrease in pH of unbuffered solutions.

Note: EDTA in TE buffer may cause problems in subsequent reactions. See Table 2 for correlation between fragment size and typical recoveries for purification of 1 – 5 µg of PCR fragments (for gel extraction, recoveries are approximately 10 % lower).

Table 2: DNA recovery with NucleoTrap®CR / NucleoTrap®

Fragment length	NucleoTrap®CR	NucleoTrap®
20 bp	0 %	50 %
40 bp	0 %	68 %
120 bp	68 %	78 %
200 bp	76 %	85 %
520 bp	80 %	87 %
2.5 kbp	81 %	88 %
5.3 kbp	80 %	86 %
8.7 kbp	76 %	80 %
19.4 kbp	74 %	74 %

3 Storage conditions and preparation of working solutions

Attention: Buffers NT1 and NT2 contain chaotropic salts! Wear gloves and goggles!

The NucleoTrap®CR/NucleoTrap® kits should be stored at room temperature and are stable until: see package label.

Before starting any NucleoTrap®CR/NucleoTrap® protocol prepare the following:

Wash Buffer NT3: Add the indicated volume of 96 – 100 % ethanol to Wash Buffer NT3 Concentrate.

	NucleoTrap®CR	
REF	**10 preps** **740587.10**	**100 preps** **740587**
Wash Buffer NT3 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol

	NucleoTrap®	
REF	**10 preps** **740584.10**	**100 preps** **740584**
Wash Buffer NT3 (Concentrate)	6 mL Add 24 mL ethanol	25 mL Add 100 mL ethanol

4 Safety instructions

When working with the **NucleoTrap®CR/NucleoTrap®** 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 http://***www.mn-net.com/msds***).

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 NucleoTrap®CR protocol – direct purification of PCR products

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding conditions

Add **4 volumes** of **Buffer NT2** to **1 volume** of sample (e.g., 400 µL Buffer NT2 and 100 µL PCR reaction mixture).

For sample volumes < 100 µL adjust the volume of the reaction mix to 100 µL using TE buffer (pH 7.5).

Note: If the volume of the PCR reaction mixture is > 100 µL, the volumes of Buffer NT2 and NucleoTrap®CR Suspension must be increased proportionally. Example: a volume of 150 µL reaction mixture needs 600 µL of Buffer NT2, and 15 µL NucleoTrap®CR Suspension to adjust proper binding conditions.

+4 vol NT2 per 1 vol sample

2 Bind DNA

Vortex the NucleoTrap®CR Suspension thoroughly until a homogeneous mixture results. Add **10 µL** of **NucleoTrap®CR Suspension** to each **100 µL** of **reaction mixture**.

Incubate the mixture for **10 min** at **room temperature** and vortex briefly every 2 – 3 min.

Centrifuge the sample at **10,000 x *g*** for **30 s** and discard the supernatant.

10 µL silica matrix RT, 10 min

10,000 x *g*, 30 s

3 Wash silica matrix

1 st wash

Add **400 µL Buffer NT2** to the pelleted silica matrix and vortex briefly for resuspension of the pellet. Centrifuge for **30 s** at **10,000 x *g*** and remove the supernatant completely.

+400 µL NT2

10,000 x *g*, 30 s

2nd wash

Add **400 µL Buffer NT3** to the pelleted silica matrix and vortex briefly. Centrifuge for **30 s** at **10,000 x *g*** and remove the supernatant completely.

+400 µL NT3

10,000 x *g*, 30 s

3rd wash

Add **400 µL Buffer NT3** to the pelleted silica matrix and vortex briefly. Centrifuge for **30 s** at **10,000 x *g***. Remove the supernatant and centrifuge the pellet again briefly. Remove residual Buffer NT3 completely.

+400 µL NT3

10,000 x *g*, 30 s

4 Dry silica matrix

RT or 37 °C, 10 – 15 min

Dry the pelleted silica matrix at **room temperature** or at **37 °C** for **10 – 15 min**.

It is not recommended to dry the sample by vacuum since over-dried pellets lead to lower recoveries.

Residual ethanol from Buffer NT3 would inhibit subsequent reactions and has to be removed in this step.

5 Elute DNA

+25 – 50 µL NE

10,000 x *g*, 1 min

Add **25 – 50 µL Buffer NE** to the silica matrix. Resuspend the pellet by vortexing. Incubate the mixture at **room temperature for 10 – 15 min**. Vortexing the mixture 2 – 3 times during incubation is recommended. Centrifuge the sample at **10,000 x *g*** for **30 s** and transfer the DNA containing supernatant to a clean tube (not provided). Repeating this step will increase the yield by approximately 10 %.

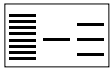
Yield of larger fragments (> 5 – 20 kbp) can be increased by performing the incubation at 55 °C.

6 NucleoTrap® protocol – DNA extraction from agarose gels

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.
- Set heating block to 50 °C.

1 Excise DNA fragment / Solubilize gel slice



Take a clean scalpel to excise the DNA fragment from agarose gel. Excise gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer it to a clean tube (not provided).

For each **100 mg** agarose gel add **300 µL NT1**.

+300 µL NT1

For gels containing > 2 % agarose, double the volume of Buffer NT1.

<u>Note:</u> If the weight of the gel slice is > 100 mg, the volume of Buffer NT1 must be increased proportionally. Example: a 150 mg gel slice (< 2 % agarose) needs 450 µL Buffer NT1.

2 Bind DNA

4 µL silica matrix/µg DNA

Vortex the NucleoTrap® Suspension thoroughly until a homogeneous mixture results. **For each µg of DNA** add **4 µL** of the **NucleoTrap® Suspension**, but at least 10 µL.

50 °C, 5 – 10 min

Incubate sample at 50 °C until the gel slice is dissolved (5 – 10 min). Vortex the sample briefly every 2 – 3 min until the gel slice is dissolved completely.

10,000 x *g*, 30 s

Centrifuge for **30 s** at **10,000 x *g*** and discard supernatant.

3 Wash silica matrix

1st wash

Add 500 µL Buffer NT2 to the pelleted silica matrix and vortex briefly for resuspension of the pellet. Centrifuge for 30 s at 10,000 x *g* and remove the supernatant completely.

+500 µL NT2

10,000 x *g*, 30 s

2nd wash

Add 500 µL Buffer NT3 to the pelleted silica matrix and vortex briefly. Centrifuge for 30 s at 10,000 x *g* and remove the supernatant completely.

+500 µL NT3

10,000 x *g*, 30 s

3rd wash

Add 500 µL Buffer NT3 to the pelleted silica matrix and vortex briefly. Centrifuge for 30 s at 10,000 x *g*. Remove the supernatant and centrifuge the pellet again briefly. Remove residual Buffer NT3 completely.

+500 µL NT3

10,000 x *g*, 30 s

4 Dry silica matrix

Dry the pelleted silica matrix at **room temperature** or at **37 °C** for **10 – 15 min**.

RT or 37 °C, 10 – 15 min

It is not recommended to dry the sample by vacuum since over-dried pellets lead to lower recoveries.

Residual ethanol from Buffer NT3 would inhibit subsequent reactions and has to be removed in this step.

5 Elute DNA

Add **25 – 50 µL Buffer NE** to the silica matrix. Resuspend the pellet by vortexing. Incubate the mixture at room **temperature** for **10 – 15 min**. Vortexing the mixture 2 – 3 times during incubation is recommended. Centrifuge the sample at **10,000 x *g*** for **30 s** and transfer the DNA containing supernatant to a clean tube (not provided). Repeating this step will increase the yield by approximately 10 %.

+20 – 50 µL NE

10,000 x *g*, 30 s

Yield of larger fragments (> 5 – 20 kbp) can be increased by performing the incubation at 55 °C.

7 Protocol for concentration, desalting, removal of enzymes, etc.

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding conditions

Add **4 volumes Buffer NT2** to **1 volume** of DNA containing **sample** (e.g., 400 µL Buffer NT2 and 100 µL reaction mixture).

+4 vol NT2 per 1 vol sample

2 **Bind DNA**

Vortex the NucleoTrap®CR / NucleoTrap® Suspension thoroughly until a homogeneous mixture results. For **each µg of DNA** add **4 µL** of **silica matrix, but at least 10** µL.

4 µL silica matrix/µg DNA

Incubate the mixture for **10 min** at **room temperature** and vortex briefly every 2 – 3 min.

50 °C, 5 – 10 min

Centrifuge for **30 s** at 10,000 x *g* and discard supernatant.

10,000 x *g*, 30 s

Important note: Be aware of the NucleoTrap® Suspension binding fragments down to 20 bp (see Table 2, section 2.3).

Continue with **section 5, step 3**.

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of agarose slices	*High concentration of agarose* • Use doubled volumes of Buffer NT1 for highly concentrated agarose gels. *Wrong buffer* • Buffer NT2 cannot be used for gel dissolution. *Time and temperature* • Check incubation temperature. Depending on the weight of gel slice, incubation (section 6, step 2) can be prolonged up to 20 min. Vortex every 2 min and check integrity of the gel slice. Heavy weight gel slices may be quenched or crushed before addition of Buffer NT1.
No DNA yield	*Reagents not applied properly* • Add indicated volume of 96 – 100 % ethanol to Wash Buffer NT3 Concentrate and mix well before use. *Insufficient drying of the NucleoTrap®CR/NucleoTrap® silica matrix* • Ethanolic Wash Buffer NT3 has to be removed quantitatively before elution. Prolong the drying time up to 30 min. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). *Isolation of large DNA fragments* • Add room-temperature Elution Buffer NE and incubate at 55 °C for 10 – 15 min.

Problem	Possible cause and suggestions
Suboptimal performance of DNA in sequencing reactions	*Carry-over of ethanol/ethanolic Buffer NT3* • Make sure to dry the silica matrix in order to achieve complete removal of ethanolic Buffer NT3 after the washing step. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). • Buffers other than Buffer NE, for example TE buffer (Tris/EDTA), were used for elution of DNA. Note: EDTA may inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Buffer NE or water. *Not enough DNA used for sequencing reaction* • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions. *NucleoTrap®CR or NucleoTrap® particles were not removed quantitatively* • Centrifuge the eluate again and transfer the supernatant to a new tube.

8.2 Ordering information

Product	REF	Pack of
NucleoTrap®CR	740587.10 740587	10 preps 100 preps
NucleoTrap®	740584 740584	10 preps 100 preps
NucleoTrap® Suspension	740568	100 preps
Buffer NT1	740596.100	2 × 50 mL
Buffer NT2	740597	2 × 50 mL
Buffer NT3 Concentrate (for 100 mL Buffer NT3)	740598	20 mL
Collection Tubes (2 mL)	740600	1000

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

8.3 References

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615 – 619.

8.4 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 NAGELS 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.

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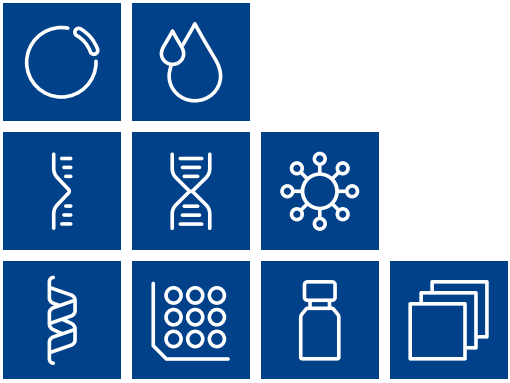

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