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PCR clean-up
Gel extraction

User Manual

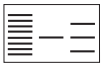
NucleoTrap®

NucleoTrap®CR

July 2010/Rev. 04

PCR clean-up, Gel extraction

Protocol-at-a-glance (Rev. 04)

		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	
	10 preps	**100 preps**
Cat. No.	**740587.10**	**740587**
NucleoTraP®CR Suspension	100 µl	1000 µl
Buffer NT2	10 ml	2 x 50 ml
Wash Buffer NT3 (Concentrate)*	4 ml	20 ml
Elution Buffer NE**	5 ml	15 ml
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	NucleoTrap®	
	10 preps	**100 preps**
Cat. No.	**740584.10**	**740584**
NucleoTrap® Suspension	100 µl	1000 µl
Buffer NT1	6 ml	2 x 30 ml
Buffer NT2	10 ml	2 x 50 ml
Wash Buffer NT3 (Concentrate)*	4 ml	20 ml
Elution Buffer NE**	5 ml	15 ml
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* 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 that first-time users of the **NucleoTrap®CR/NucleoTrap®** kit read the detailed protocol sections of this User Manual. 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*.

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®
DNA fragments from agarose gels	-	++
Desalination, removal of enzymes, nucleotides and/or labeling reagents like biotin or radioactive ATP etc.	++	+
Direct purification of amplified DNA	++	-
Elution volume	20 – 50 µl	20 – 50 µl
Binding capacity	6 µg/10 µl matrix	6 µg/10 µl matrix
Time/prep	45 min for 6 preps	60 min for 6 preps

- 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 CO_2 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 for up to one year.

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	
	10 preps	**100 preps**
Cat. No.	**740587.10**	**740587**
Wash Buffer NT3 (Concentrate)	4 ml Add 16 ml ethanol	20 ml Add 80 ml ethanol

	NucleoTrap®	
	10 preps	**100 preps**
Cat. No.	**740584.10**	**740584**
Wash Buffer NT3 (Concentrate)	4 ml Add 16 ml ethanol	20 ml Add 80 ml ethanol

4 Safety instructions – risk and safety phrases

The following components of the **NucleoTraP®CR/NucleoTrap®** kits contain hazardous contents.

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

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
NT1	Sodium perchlorate	 O**  Xn*	Explosive when mixed with combustible material - Harmful if swallowed	R 9-22	S 13-27
NT2	Sodium perchlorate	 O**  Xn*	Explosive when mixed with combustible material - Harmful if swallowed	R 9-22	S 13-27

Risk phrases

R 9	Explosive when mixed with combustible material
R 22	Harmful if swallowed

Safety phrases

S 13	Keep away from food, drink, and animal feedstuffs
S 27	Take off immediately all contaminated clothing

* Hazard labeling not necessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

** Hazard labeling not necessary if quantity per bottle below 50 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 Protocol for direct purification of PCR products using NucleoTraP®CR

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

1st 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 — **+ 400 µl NT3**

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.

10,000 x *g*
30 s

3rd wash — **+ 400 µl NT3**

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.

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%.

+ 25 – 50 µl NE

10,000 x *g*
1 min

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

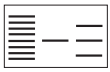
6 Protocol for DNA extraction from agarose gels using NucleoTrap®

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**.

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

Note: 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.

+ 300 µl NT1

2 Bind 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.

4 µl silica matrix / µg DNA

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.

50°C
5 – 10 min

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

10,000 x *g*
30 s

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

+ 500 µl NT3

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.

10,000 x *g*
30 s

3rd wash

+ 500 µl NT3

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.

10,000 x *g*
30 s

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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.

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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.

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7 Support protocol for concentration, desalination, removal of enzymes, etc.

Before starting the preparation:

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

1 Adjust DNA binding conditions

+ 4 vol NT2 per 1 vol sample

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

2 Bind DNA

4 µl silica matrix / µg 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.

RT
10 min

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

10,000 x *g*
30 s

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

<u>Important note</u>: 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	Cat. No.	Pack of
NucleoTraP®CR	740587.10 740587	10 preps 100 preps
NucleoTrap®	740584.10 740584	10 preps 100 preps
NucleoTraP®CR Suspension	740564	100 preps
NucleoTrap® Suspension	740569	100 preps
Buffer NT1	740596.100	2 x 50 ml
Buffer NT2	740597	2 x 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

NucleoTraP®CR/NucleoTrap® 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 **NucleoTraP®CR/NucleoTrap®** 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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