NucleoSpin® Extract II Kits
User Manual

Cat. Nos. 636971
636972
636973
PT3814-1 (PR48598)
Published 10 September 2004

Table of Contents

List of Figures

List of Tables

I. Kit Contents

TABLE I. NUCLEOSPIN® EXTRACT II			
Cat. No.	**10 preps 636971**	**50 preps 636972**	**250 preps 636973**
Buffer NT	6 ml	30 ml	2 x 75 ml
Buffer NT3 (concentrate)	7 ml	2 x 7 ml	80 ml
Buffer NE	5 ml	15 ml	50 ml
NucleoSpin® Extract II columns	10	50	250
NucleoSpin® collecting tubes (2ml)	10	50	250
Protocol	1	1	1

II. Product Description

A. The Basic Principle

With the **NucleoSpin® Extract II** method, DNA binds in the presence of chaotropic salt to a silica membrane. The binding mixture is loaded directly onto **NucleoSpin® Extract II** columns. Contaminations like salts and soluble macromolecular components are removed by a simple washing step with ethanolic buffer NT3. Pure DNA is finally eluted under low ionic strength conditions with slightly alkaline buffer NE (5 mM Tris-Cl, pH 8.5).

B. About This User Manual

Experienced users who are performing the purification of PCR* products or DNA fragments from agarose gels using a **NucleoSpin® Extract II** isolation kit may refer to the Protocol-at-a-glance instead of this user manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this user manual.

C. Kit Specifications

The **NucleoSpin® Extract II** kit is designed for the purification of DNA from TAE/TBE agarose gels and for direct purification of PCR* products (two applications in one kit).

The **NucleoSpin® Extract II** buffer formulation ensures complete removal of primers from PCR* reactions while small DNA fragments are still bound and purified with high yields

With **NucleoSpin® Extract II** even DNA fragments from PCR reaction buffers rich in various detergents can be purified with high recovery.

The adsorption of DNA to the **NucleoSpin® Extract II** membrane is pH dependent. Optimal yields are achieved by using TAE standard gels or reaction mixtures with pH 6-8.

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.

*PCR is patented by Roche Diagnostics

II. Product Description *continued*

TABLE II. KIT SPECIFICATIONS AT A GLANCE	
Parameters	**NucleoSpin® Extract II**
DNA fragments from agarose gels	x
Desalination, removal of enzymes, nucleotides, and/ or labeling reagents like biotin or radioactive ATP etc.	x
Direct purification of amplified DNA	x
Elution volume	15-50 *µl*
Binding capacity	15 *µg*
Time/prep	10 min for 6 preps

D. Elution Procedures

- For elution of DNA one of the following solutions can be used: Buffer NE (supplied) / TE buffer, pH 8.5 / distilled water, pH 8.5.
- Note: EDTA in TE buffer may cause problems in subsequent reactions, and the pH of distilled water should be checked before use to avoid lower recovery yields. See Table 1 for correlation between dispensed elution buffer volumes and typical recovery rates for purification of 1-5 *µg* of PCR* fragments (for gel extraction, recovery rates are approx. 10 % lower).
- With an elution volume of 15 *µl* of buffer NE typical recovery rates of 70-95 % are usually obtained for DNA fragments between 50-10,000 bp resulting in highly concentrated eluates (see Table 1). If larger amounts (5-15 *µg*) of DNA have to be purified (e.g. from PCR* reactions > 100 *µl* or gel slices > 200 mg) elution with at least 50 of buffer NE is recommended. Primers are not bound.
- Yields of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer NE and incubate for 1-2 min before collecting eluate by centrifugation.For fragments > 10 kb the use of our NucleoTraP®CR kit is recommended.

*PCR is patented by Roche Diagnostics

II. Product Description *continued*

TABLE III. RECOVERY RATES NUCLEOSPIN® EXTRACT II

Fragment Length	Elution Volume	NucleoSpin® Extract II
65 bp	15 µl 25 µl 50 µl 100 µl	85 % 90 % 95 % 95 %
400 bp	15 µl 25 µl 50 µl 100 µl	85 % 95 % 100 % 100 %
700 bp	15 µl 25 µl 50 µl 100 µl	85 % 90 % 95 % 95 %

II. Product Description *continued*

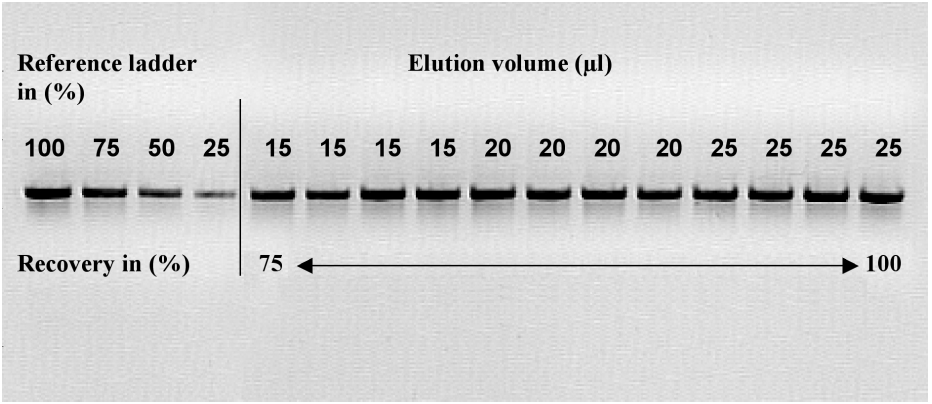

Figure 1: Use of different elution volumes

A PCR sample with a fragment size of 782 bp were purified according to the standard protocol of **NucleoSpin® Extract II** using different elution volumes as shown. All elution volumes were adjusted to 25 *µl* plus 4.5 *µl* loading dye. For analysis the mixture was loaded onto a 1% TAE gel. The recovery rates were estimated by use of a fragment ladder.

III. Storage Conditions and Preparation of Working Solutions

Attention:

Buffer NT contains chaotropic salt. Wear gloves and goggles!

- The **NucleoSpin® Extract II** kit should be stored at room temperature and is stable for up to one year.

Before starting any **NucleoSpin® Extract II** protocol prepare the following:

- Add the indicated volume of 96-100% ethanol to buffer NT3 concentrate.

TABLE IV NUCLEOSPIN® EXTRACT II

Cat. No.	10 preps 636971	50 preps 636972	250 preps 636973
Buffer NT3	7 ml add 28 ml ethanol	2 x 7 ml add 28 ml ethanol	80 ml add 320 ml ethanol

IV. Safety Instructions – Risk and Safety Phrases

The following components of the NucleoSpin® Extract II kits contain hazardous contents.

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

TABLE V SAFETY INSTRUCTIONS--RISK AND SAFETY PHRASES

Buffer	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
NT	Guanidine Thiocyanate	**X** Xn*	Harmful by inhalation, in contact with skin and if swallowed	**R 20/21/22**	**S 13**

Risk Phrases

R 20/21/22 Harmful by inhalation, in contact with skin and if swallowed

Safety Phrases

S 13 Keep away from food, drink and animal feedstuffs

*Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

V. Protocol for DNA Extraction from Agarose Gels

1. Excise DNA fragment

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.

2. Gel lysis

For each **100 mg** agarose gel add **200 *µl* buffer NT.**

For gels containing > 2% agarose, double the volume of buffer NT. The maximum amount of gel slice per NucleoSpin® Extract II column is 400 mg or 200 mg of a high percentage gel > 2%. In this case 2 loading steps are required (step 3).

Incubate sample at **50°C** until the gel slices are dissolved **(5-10 min).** Vortex the sample briefly every 2 3 min until the gel slices are dissolved **completely!**

3. Bind DNA

Place a NucleoSpin® Extract II column into a 2 ml collecting tube and load the sample.

Centrifuge for **1 min at 11,000 x g.** Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

4. Wash silica membrane

Add **600 *µl* buffer NT3.** Centrifuge for **1 min** at **11,000 x g.** Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

V. Protocol for DNA Extraction from Agarose Gels *continued*

5. Dry silica membrane

Centrifuge for **2 min** at **11,000 x g** to remove **buffer NT3** quantitatively. Make sure the spin column doesn't come in contact with the flow-through while removing it from the centrifuge and the collecting tube.

Residual ethanol from buffer NT3 would inhibit subsequent reactions and has to be removed in this step. In addition to centrifugation, total removal can be achieved by incubation of NucleoSpin® Extract II columns for 2-5 min at 70°C prior to elution.

6. Elute DNA

Place the NucleoSpin® Extract II column into a **clean** 1.5 ml microcentrifuge tube. Add **15-50 *µl* elution buffer NE**, incubate at **room temperature** for **1 min** to increase the yield of eluted DNA. Centrifuge for **1 min** at **11,000 x g.**

Yield of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature for 1 min before collecting eluate by centrifugation.

VI. Protocol for Direct Purification of PCR Products

1. Adjust DNA binding conditions

Mix **2 volumes** of **buffer NT** with **1 volume of sample** (e.g. 200 *µl* and 100 *µl* PCR reaction mix).

For sample volumes < 50 µl adjust the volume of the reaction mix to 50 µl using TE buffer (pH 7.5).

2. Bind DNA

Place a NucleoSpin® Extract II column into a 2 ml collecting tube and load the sample.

Centrifuge for **1 min** at **11,000 x g.** Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

3. Wash silica membrane

Add **600 *µl* buffer NT3.** Centrifuge for **1 min** at **11,000 x g.** Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

4. Dry silica membrane

Centrifuge for **2 min at 11,000 x g** to remove **buffer NT3** quantitatively. Make sure the spin column doesn't come in contact with the flow-through while removing it from the centrifuge and the collecting tube.

Residual ethanol from buffer NT3 would inhibit subsequent reactions and has to be removed in this step. In addition to centrigugation, total removal can be achieved by incubation of NucleoSpin® Extract II columns for 2-5 min at 70°C prior to elution.

5. Elute DNA

Place the NucleoSpin® Extract II column into a **clean** 1.5 ml microcentrifuge tube. Add **15-50 *µl* elution buffer NE,** incubate at **room temperature** for **1 min** to increase he yield of eluted DNA. Centrifuge for **1 min** at **11,000 x g.**

Yield of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature f or 1 min before collecting

VII. Support Protocol for Concentration, Desalination, Removal of Enzymes

1. Adjust DNA binding conditions

Add **2 volumes** of **buffer NT** to **1 volume** of the DNA containing **sample** (e.g. 200 *µl* NT and 100 *µl* PCR reaction mix).

If your sample contains large amounts of detergents or other critical substances, double the volume of NT.

2. Bind DNA

Continue with **step 2** of the protocol for purification of PCR products (section 5).

Appendix A: Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of agarose slices	*High amount of agarose* • Use doubled volumes of buffer NT for highly concentrated and/or LMP (low melting point) agarose gels. *Time and temperature* • Check incubation temperature. Depending on the weight of gel slice, incubation (section 4, 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 NT. *Reagents not applied properly* • Add indicated volume of 96-100% ethanol to buffer NT3 concentrate and mix well before use. *Incompletely dissolved gel slice* • Increase time or add another two volumes of NT and vortex the tube every 2 minutes during incubation at 50°C. Small pieces of gel are hardly visible and contain DNA that will be lost for purification.
Low DNA yield	*Insufficient drying of the* ***NucleoSpin® Extract II*** silica *membrane* • Centrifuge 5 min at 11,000 x g before elution to remove ethanolic buffer NT3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Remove the spin cup carefully from centrifuge and and collecting tube and avoid contact of spin cup with flow-through. *Not enough elution buffer* • Especially when larger amounts of DNA (>5) are bound, increase elution buffer volume up to 100 *µl*. *Isolation of large DNA fragments* • Preheat elution buffer NE to 70°C and incubate on the silica membrane at room temperature for 2 min before centrifugation.

Appendix A: Troubleshooting *continued*

Problem	Possible cause and suggestions
	Carry-over of ethanol/ethanolic buffer NT3 • Be sure to centrifuge 3-5 min at 11,000 x g 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).
Suboptimal performance of DNA in sequencing reactions	*Elution of DNA with Buffers other than buffer NE e.g. TE buffer (Tris/EDTA)* • EDTA may inhibit sequencing reactions. In this case it is recommended to repurify 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.

Appendix B: Ordering Information

For a complete listing of all Clontech products, please visit **www.clontech.com**

Product	Pack of	Cat. No.
NucleoSpin® Extract II	10 preps	636971
NucleoSpin® Extract II	50 preps	636972
NucleoSpin® Extract II	250 preps	636973
NucleoSpin® collection tubes (2 ml)	1000	636029

Appendix C: References

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

Appendix D: Product Use Restriction / Warranty

NucleoSpin® Extract II 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 **NucleoSpin® Extract II** 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 warants 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.

MACHEREY-NAGEL does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product; against defects in products or components not manufactured by MACHEREY-NAGEL, or against damages resulting from such non-MACHEREY-NAGEL components or products.

MACHEREY-NAGEL makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, REPRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO MACHEREY-NAGEL PRODUCTS

Appendix D: Product Use Restriction / Warranty *continued*

In no event shall MACHEREY-NAGEL be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of MACHEREY-NAGEL products to perform in accordance with the stated specifications. This warranty is exclusive and MACHEREY-NAGEL makes no other warranty expressed or implied.

The warranty provided herein and the data, specifications and descriptions of this MACHEREY-NAGEL product appearing in MACHEREY-NAGEL published catalogues and product literature are MACHEREY-NAGEL´s sole representations concerning the product and warranty. No other statements or representations, written or oral, by MACHEREY-NAGEL´s employees, agent or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Applications mentioned in MACHEREY-NAGEL literature are provided for informational purposes only. MACHEREY-NAGEL does not warrant that all applications have been tested in MACHEREY-NAGEL laboratories using MACHEREY-NAGEL products. MACHEREY-NAGEL does not warrant the correctness of any of those applications.

Please contact:

MACHEREY-NAGEL Germany
Tel.: +49 (0) 24 21 969 270
e-mail: TECH-BIO@mn-net.com

Notes

Notice to Purchaser

Clontech products are to be used for research purposes only. They may not to be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

NucleoSpin® is a registered trademark of MACHEREY-NAGEL GmbH & Co. KG.