MACHEREY - NAGEL

Official Note

From: MACHEREY-NAGEL, Germany	Date: 02. April 2004
To: MN subsidiaries and distributors	

Product: **NucleoSpin® Extract II**

Topic: **Release of Kit**

Summary:

In the past years many different Polymerase buffer systems from several suppliers have been launched which contain varying detergents. During substantial R&D work did we find out, that some of the detergents were not compatible with the NucleoSpin® Extract buffer system resulting in lower recovery rates especially for small fragments and possible failure of down stream application.

In order to meet the wide range of different Polymerase buffer systems MACHEREY-NAGEL has developed an updated version of the NucleoSpin® Extract kit which is called **NucleoSpin® Extract II**.

By the use of a new Solubilisation-Binding **Buffer NT** which substitutes the buffers NT1 and NT2 (no additional buffer is necessary for gel extraction, anymore) we were able to develop an updated NucleoSpin® Extract II kit which shows an excellent performance with all common polymerase buffer systems on the market. Furthermore, the recovery rates for smaller fragments down to 65 bp have been improved.

Also the ratio between sample and Solubilisation-Binding buffer has been changed:

Table 1: Ratios of Sample and Solubilisation-Binding buffer

	NucleoSpin® Extract	NucleoSpin® Extract II
PCR Purification	Sample / NT2 1:4	Sample / NT 1: 2
Gel Extraction	Sample / NT1 1:3	

All other components like silica membrane, Wash Buffer NT3 and Elution Buffer NE have not been changed.

Ordering Information:

Product	Cat. No.	Pack of	Price* 1 – 4 p.	Price* 5 – 24 p.	Price* from 25 p.
NucleoSpin® Extract II	740609.10	10	19 € 1.90 €/prep		
NucleoSpin® Extract II	740609.50	50	70 € 1.40 €/prep	66 € 1.32 €/prep	63 € 1.26 €/prep
NucleoSpin® Extract II	740609.250	250	319 € 1.28 €/prep		
Buffer NT	740614.100	100 ml	24 €		

*prices refer to the MN Bioanalysis 2004 German price list

1. Product description

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).

2. Technical Specifications

Kit specification at a glance	
Parameters	**NucleoSpin® Extract II**
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	15-50 µl
Binding capacity	15 µg
Time/prep	10 min / 6 preps

- not recommended + possible ++ optimal

3. Handling

Detailed information about the contents of the kit, the product specifications, the handling, trouble shooting, and ordering are included in the kit protocol.

4. Safety Instructions – Risk and Safety Phrases

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

Buffer	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
NT	guanidine thiocyanate	 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 the skin and if swallowed

Safety Phrases

S 13 Keep away from food, drink and animal feedstuffs

5. Customers and downstream applications

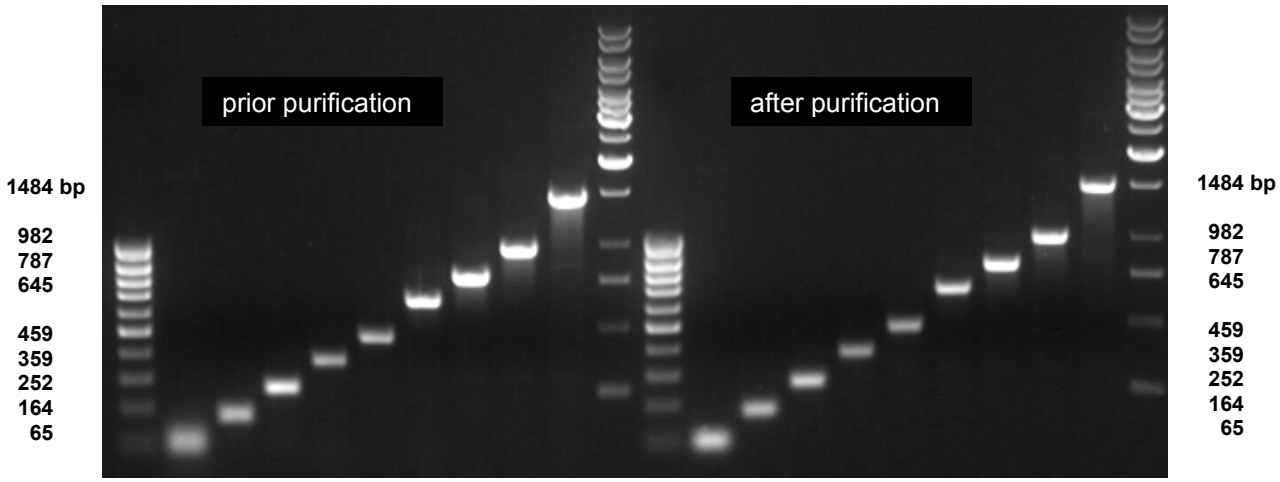
Potential Customers:
Research departments in companies and at universities.

Downstream applications:
The prepared DNA is suitable for all common downstream applications like sequencing, ligation, labeling, cloning and restriction.

* 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)

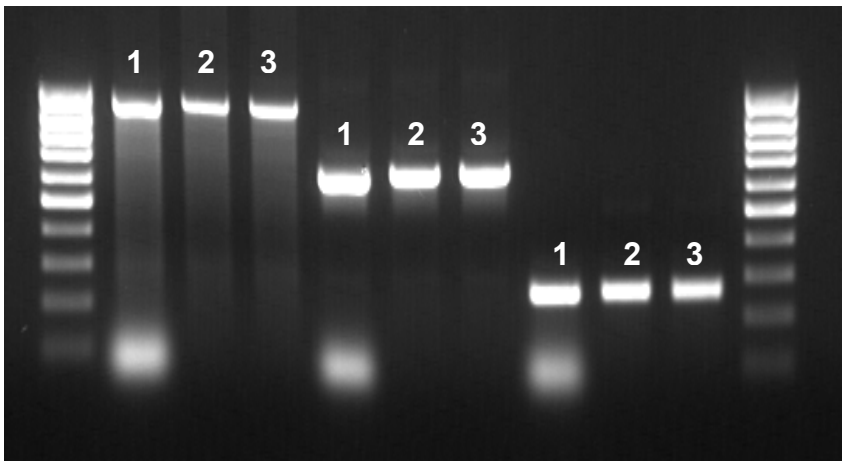
6. Original data

I. Purification of different fragment sizes



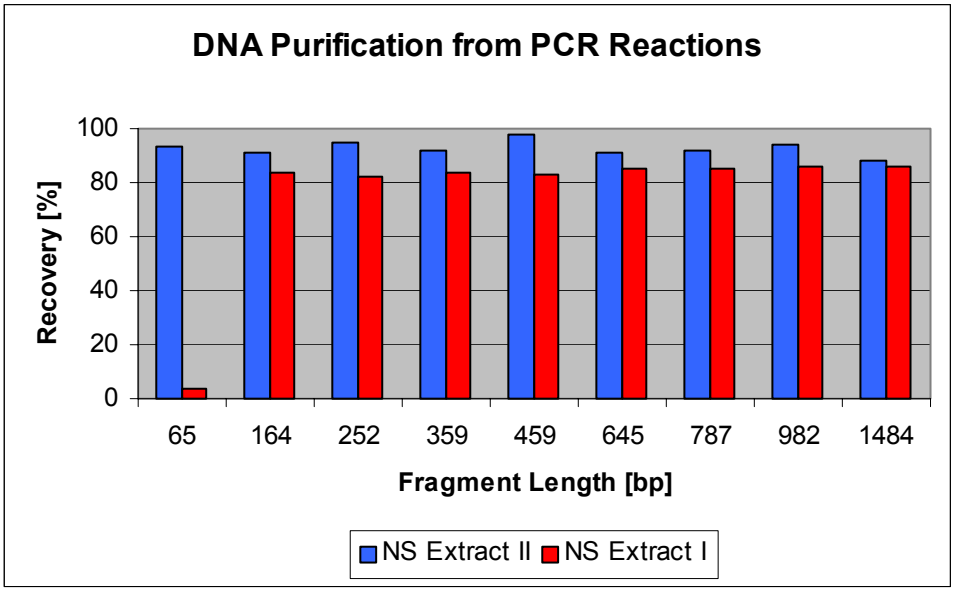
Picture1:
9 fragments different in size (65, 164, 252, 359, 459, 645, 787, 982, 1484 bp) were amplified with Invitrogen Taq and subsequently purified. Elution was performed using 50 µl NE. The same percentage (prior and after purification) was analyzed on a 1% TAE-gel.
(100 bp and 1 kbp DNA ladder marker Fermentas). Primers were used up during amplification and therefore not detectable.

II. Complete removal of primers



Picture 2:
PCR fragments of 982, 645 and 252 bp were amplified using ABgene mastermixes AB 0575 and AB 0938 spiked with additional primer. Elution was performed using 50 µl NE. The same percentage (prior, lane 1 and after purification, lane 2/3) was analyzed on a 1% TAE-gel.

III. NucleoSpin® Extract vs. NucleoSpin® Extract II regarding recovery of different fragment sizes



Picture 3:
PCR Fragments of different sizes were purified using NucleoSpin® Extract and NucleoSpin® Extract II and analyzed on a 1% TAE gel. The recovery was quantified by software ONE Dscan von Scanalytics. The average recovery rates are 84% for NucleoSpin® Extract and 93% for NucleoSpin® Extract II.
Remark: NucleoSpin® Extract II allows recovery rates of > 90% even for very small fragments 65bp.

IV. NucleoSpin® Extract vs. NucleoSpin® Extract II regarding recovery of different fragment sizes and elution volumes

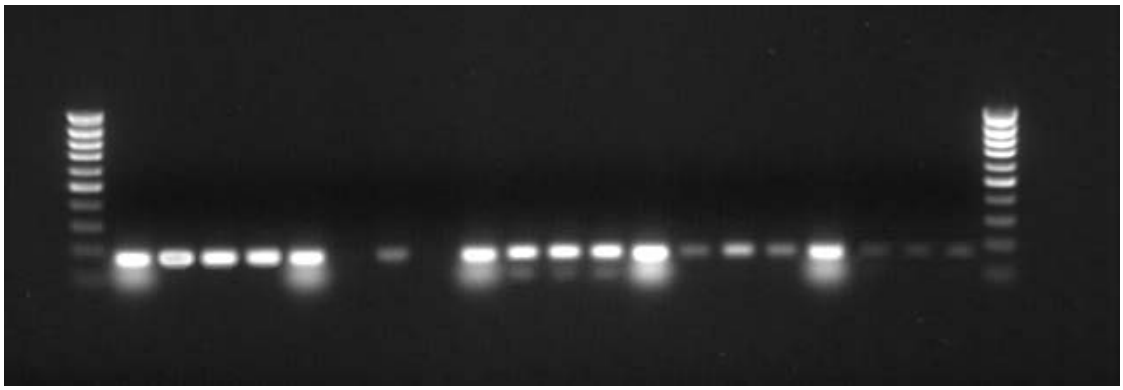
Table 2: Recovery rates NucleoSpin® Extract II versus NucleoSpin® Extract

Fragment length	Elution volume	NucleoSpin® Extract II	NucleoSpin® Extract
65 bp	15 µl	85 %	n.d.
	25 µl	90 %	0 %
	50 µl	95 %	5 %
	100 µl	95 %	5 %
400 bp	15 µl	85 %	n.d.
	25 µl	95 %	80 %
	50 µl	100 %	85 %
	100 µl	100 %	90 %

700 bp	15 µl	85 %	n.d.
	25 µl	90 %	80 %
	50 µl	95 %	90 %
	100 µl	95 %	95 %
1500 bp	15 µl	85 %	n.d.
	25 µl	85 %	75 %
	50 µl	90 %	90 %
	100 µl	95 %	95 %

Table 1:
PCR Fragments of different sizes were purified using NucleoSpin® Extract and NucleoSpin® Extract II and analyzed on a 1% TAE gel. After elution with different volumes the recovery was quantified by software ONE Dscan von Scanalytics.
Remark: NucleoSpin® Extract II allows recovery rates of > 90% even for very small fragments and shows in average 10% higher recovery rates even when small elution volumes (15µl) are used.

V. Comparison data (competitors and use of different DNA polymerases)



	Extract II				Extract				Qiagen				Sigma				Roche		
	a	b	c		a	b	c		a	b	c		a	b	c		a	b	c

Picture 4:
A PCR fragment with a size of 165bp was amplified using different DNA-Polymerases (a-c). Additional primers were added and the mixture was purified using different competitive kits. The elution was performed with 25µl. For analysis the complete eluate was loaded onto a 1% TAE Gel.
In comparison to the NucleoSpin Extract II all other kits show lower recovery rates or inefficient removal of primers. Please note that Qiagen shows a comparable recovery rate but inefficient removal of primers!
REMARK:
We recoverd during substantial R&D work, as mentioned in the summary above that some components of certain polymerase buffer system were not compatible with the NucleoSpin® Extract buffer system resulting in much lower recovery rates for small fragments as shown in picture 4. This also applies to the competitores Sigma and Roche. Please note that this does not apply to all buffer systems available on the market as NucleoSpin® Extract is still one of our best sold products. However, with NucleoSpin® Extract II even “difficult” polymerase buffer systems can be used.

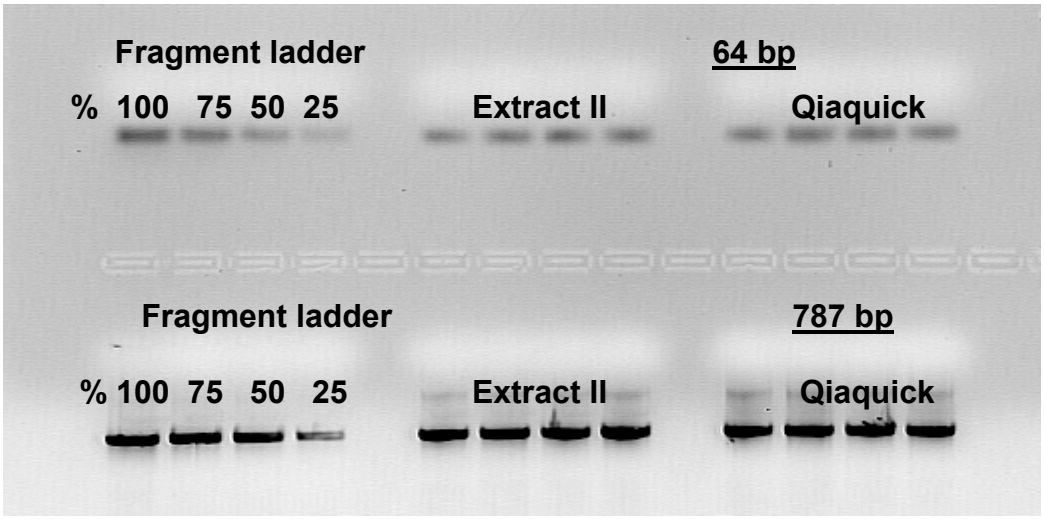
PCR Purification Kits

MN	NS Extract II
MN	NS Extract
Qiagen	QIAquick PCR Purification Kit
Sigma	GenElute PCR Clean-up Kit
Roche	PCR Clean Up Kit

DNA Polymerases (detail)

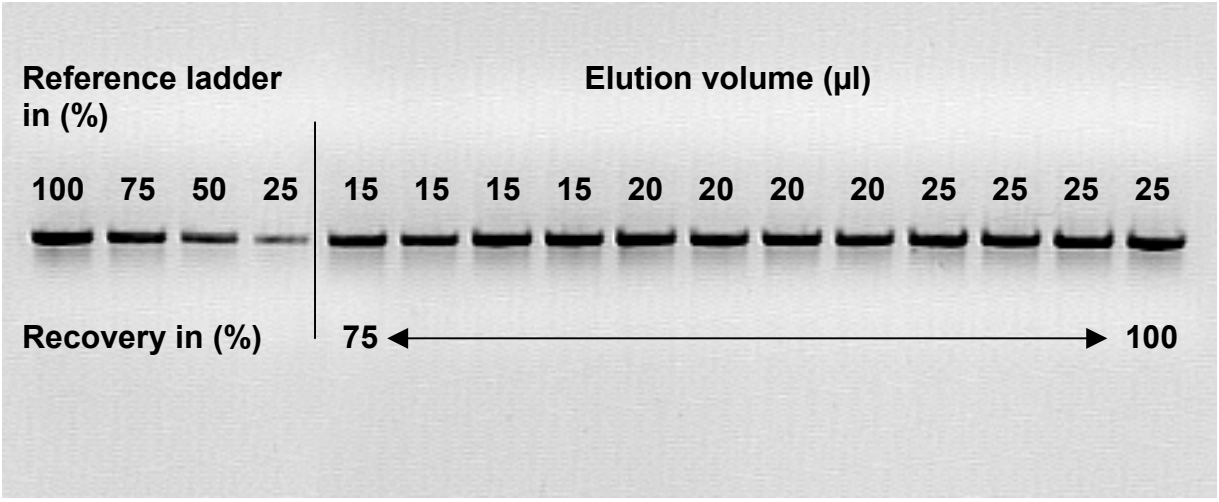
a) Qiagen (with Q Puffer)
b) Stratagene (Extender)
c) ABgene (Buffer 2)

VI. Comparison data (gel extraction)



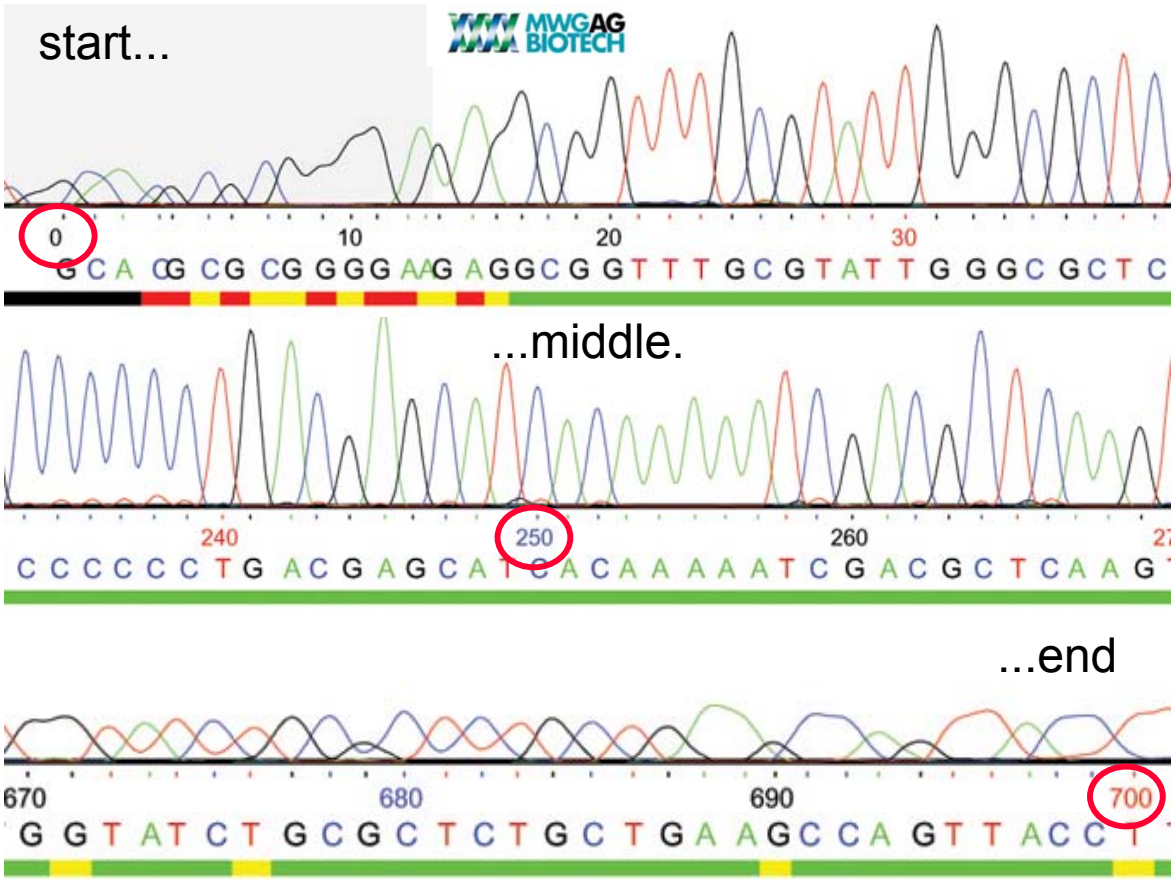
Picture 5:
With each kit (NucleoSpin Extract II and Qiaquick) four samples with a fragment size of 782bp and four samples with a fragment size of 64bp were purified according to the standard protocol. The elution was performed with 20µl for each kit. After the elution 3.5µl loading dye were added. For analysis the mixture was loaded onto a 1% TAE gel. The recovery rates were estimated by use of a fragment ladder.
Both kits show equal performance. The estimated recovery rate is for both kits and for both fragment sizes around 75%.

VII. Use of different elution volumes



Picture 6:
A PCR sample with a fragment size of 782bp were purified according to the standard protocol of NucleoSpin Extract II using different elution volumes as shown. All elution volumes were adjusted to 25µl plus 4.5µl loading dye. For analysis the mixture was loaded onto a 1% TAE gel. The recovery rates were estimated by use of a reference ladder.
Even with an elution volume down to 15µl recovery rates of up to 75-100% can be achieved.

VIII. Sequencing profile



Picture 7:
The PCR fragment was amplified using ABgene mastermix AB 0575 and sequenced by MWG Biotech subsequently. The reading length up to 700 bp was excellent (as indicated by the green bar) up to 800 bp could be analyzed.

IV. Which polymerase buffer systems have been tested?

1. Invitrogen Taq DNA Polymerase (rekomb.) buffer,
2. Stratagene Taq Polymerase buffer,
3. Stratagene Taq Extender buffer,
4. Peqlab Pwo-Polymerase buffer,
5. ABgene Extension High-Fidelity buffer 1,
6. ABgene Extension High-Fidelity buffer 2,
7. Bioline BioTaq NH4 reaction buffer,
8. Qiagen Taq DNA Polymerase with Q-Puffer,
9. Applied Biosystems PCR Gold buffer

7. Sales Arguments for NucleoSpin® Extract II

- 2 applications in one kit: for Gel extraction and for PCR purification (with QIAGEN you need two kits)
- A pH indicator included in the binding buffer is not necessary
- A very fast extraction procedure
- Purification of PCR products which are DIRECTLY used for following cycle sequencing reactions
- Effective removal of primer

NEW!

- One buffer for PCR and Gel extraction
- High recovery rates even for very small fragments (< 100 bp)
- Reduced elution volumes **15** – 50 µl
- Optimized buffer chemistry in regard to different polymerases buffer systems

8. Marketing Strategy

In the long run it is planned to replace the NucleoSpin® Extract by the NucleoSpin® Extract II kit.
In order to make this replacement for the customers as smooth as possible, meaning that every customers worldwide should have the possibility for testing, we decided to sell the updated version NucleoSpin® Extract II in parallel.

We would recommend the following procedure:

1. All customers in your area who observed problems regarding recovery or failed downstream application with the standard kit should be sampled with the updated NucleoSpin® Extract II.
2. To all new customers NucleoSpin® Extract II should be sold.

For questions please contact:
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