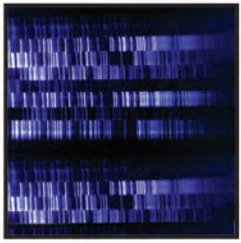
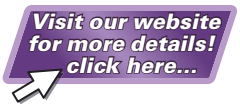


Clean-up of Sequencing Reactions

User Manual

NucleoSEQ®



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MACHEREY-NAGEL

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1 Components

1.1 Kit contents

	NucleoSEQ®		
	10 preps	**50 preps**	**250 preps**
REF	**740523.10**	**740523.50**	**740523.250**
NucleoSEQ® Columns	10	50	250
Collection Tubes	10	50	250
User Manual	1	1	1

1.2 Material to be supplied by user

- Collection tubes (e.g., 1.5 mL microcentrifuge tubes)

2 Product description

2.1 The basic principle

NucleoSEQ® Columns are designed for fast and effective clean-up of nucleic acids. Using gel exclusion in a convenient spin column format allows reliable removal of smaller molecules from nucleic acids. Impurities, for example, salts, excess of labels, nucleotides, traces of organic solvents, primers are retained by the column while nucleic acids of interest are recovered with high yield. The columns are pre-filled with size exclusion matrix.

2.2 Kit specifications

- Maximum sample volume to be loaded onto the column: 20 µL
- Removal of sequencing dye terminators including BigDye®

3 Storage conditions

- NucleoSEQ® Columns with dry gel matrix can be stored at room temperature for 12 months.
- NucleoSEQ® Columns with hydrated gel matrix should be stored at 4 °C. Columns can be stored up to 14 days at 4 °C.

4 Safety instructions – risk and safety phrases

The components of the NucleoSEQ® kits do not contain hazardous contents.

5 Protocol for sequencing reaction clean-up

General procedure

(For details on each step see page 7.)

1 Spin down dried gel resin

750 x *g*
30 s

2 Hydrate gel resin with **600 µL water**, vortex, and incubate at least **30 min** for complete hydration

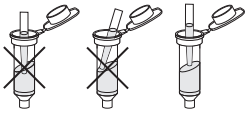
600 µL water

RT
> 30 min

3 Remove bottom plug and spin down hydrated gel resin

750 x *g*
2 min

4 Load sample to the center of the column



Load column

5 Spin for **4 – 6 min** at **750 x *g*** to recover purified sample

750 x *g*
4 – 6 min

Detailed procedure

Perform sequencing reaction according to standard protocols.

We recommend to use not more than 1 – 2 µL of Big Dye® Ready reaction mix in a 20 µL sequencing reaction in order to avoid overloading the column.

1 Centrifuge the NucleoSEQ® Columns for **30 s** at **750 x *g*** to collect the dry gel matrix on the bottom of the cartridge.

2 Add **600 µL dist. water** and vortex to hydrate the gel matrix. Remove air bubbles by vortexing or tapping the column. Incubate at least **30 min** or overnight to hydrate the gel matrix. Incubation can be performed at room temperature or 4 °C. Hydrated columns can be stored at 4 °C for a maximum of 14 days. Resuspend the settled gel matrix by inverting or vortexing the spin column several times. Air bubbles should not be visible now. Remove the bottom plug and place the spin column into a Collection Tube (supplied with the kit).

3 Place the column into an appropriate centrifuge (the hinge of the spin column`s cover lid should be orientated to the outside of the rotor). Centrifuge **2 min** at **750 x *g*** to remove the remaining storage buffer. Discard the collection tube with storage buffer. Place the spin column in an appropriate collection tube (e.g., microcentrifuge tube, not supplied with the kit).

4 Open the lid of the column. Carefully load the sample drop by drop onto the center of the gel resin. Pipetting the sample at the sides of the spin column tube may reduce purification efficiency of the column. Moreover, do not disturb the gel surface. Sample volume should not exceed 20 µL.

5 Place the column in the same orientation as in step 3 into the centrifuge. Elute the sample by centrifuging the column for **4 – 6 min** at **750 x *g***. Discard the spin column.

Dry the sample or use the sample directly.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Dye blobs	*Sample added to column improperly* • Add sample directly to the center of the settled gel matrix. Dispense sample dropwise. Avoid adding sample at the sides of the gel matrix *Sample volume to high* • Add sample in a volume of 20 µL. Higher sample volumes can cause incomplete removal of dye terminators.
Poor signal intensity	*Sample volume to small* • Add sample in a volume of 20 µL. If necessary adjust sample volume to 20 µL using distilled water

Conversion of RCF from different centrifuges:

$$rpm = 100 \times \sqrt{RCF / 1.12r}$$

$$RCF = (rpm / 1000)^2 \times 1.12r$$

rpm = revolutions per minute
RCF = relative centrifugal force (*g* force = RCF x *g*)
r = radius in mm

6.2 Ordering information

Product	REF	Pack of
NucleoSEQ®	740523.10	10 preps
	740523.50	50 preps
	740523.250	250 preps

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

6.3 Product use restriction/warranty

NucleoSEQ® kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

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