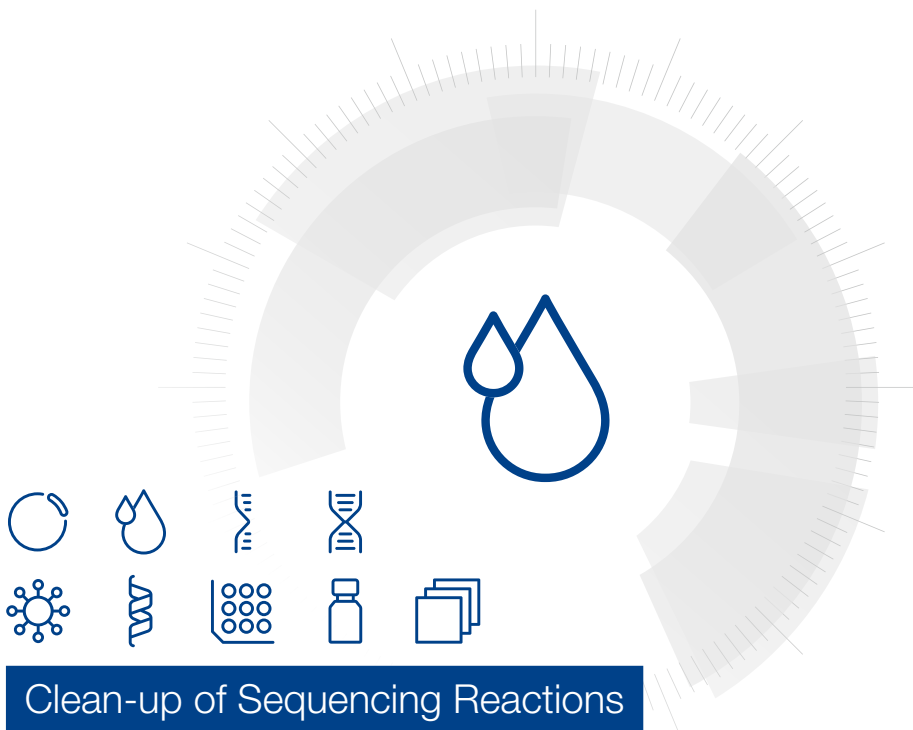


MACHEREY-NAGEL

User manual



Clean-up of Sequencing Reactions

■ NucleoSEQ®

November 2023 / Rev. 06

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1 Components

1.1 Kit contents

NucleoSEQ®			
REF	10 preps 740523.10	50 preps 740523.50	250 preps 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)

1.3 About this user Manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also 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 or updated revisions.

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®
- For research use only

3 Storage conditions

- NucleoSEQ® Columns with dry gel matrix can be stored at room temperature and are stable until: see package Label.
- 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

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




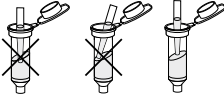

4.1 Disposal

Dispose used materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 Protocol for sequencing reaction clean-up

General procedure

(For details on each step see page 7.)

<p>1 Spin down dried gel resin</p>		<p>750 x g 30 s</p>
<p>2 Hydrate gel resin with 600 µL water, vortex, and incubate at least 30 min for complete hydration</p>		<p>600 µL water RT > 30 min</p>
<p>3 Remove bottom plug and spin down hydrated gel resin</p>		<p>750 x g 2 min</p>
<p>4 Load sample to the center of the column</p>		<p>Load column</p>
<p>5 Spin for 4–6 min at 750 x g to recover purified sample</p>		<p>750 x g 4–6 min</p>

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	<i>Sample added to column improperly</i>
	<ul style="list-style-type: none"> Add sample directly to the center of the settled gel matrix. Dispense sample dropwise. Avoid adding sample at the sides of the gel matrix
Poor signal intensity	<i>Sample volume to high</i>
	<ul style="list-style-type: none"> Add sample in a volume of 20 μL. Higher sample volumes can cause incomplete removal of dye terminators.
Poor signal intensity	<i>Sample volume to small</i>
	<ul style="list-style-type: none"> 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:

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

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

rpm = revolutions per minute

RCF = relative centrifugal force (g force = RCF \times g)

r = radius in mm

6.2 Ordering information

Product	REF	Pack of
NucleoSEQ [®]	740523.10 / .50 / .250	10 / 50 / 250

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

6.3 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-NAGEL's 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 customer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company's website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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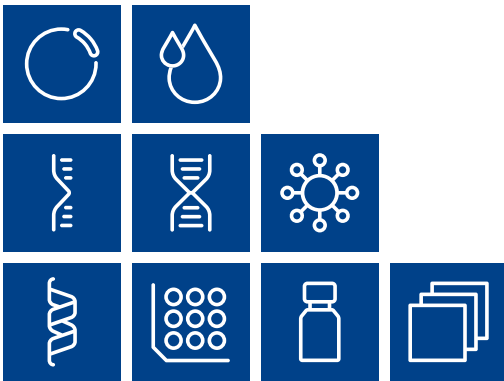
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Trademarks/Disclaimer:

BigDye® is a trademark of a member of Applied Biosystems

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

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