illustra™
NAP-25 Columns
For the purification of oligonucleotides and small DNA fragments.
Desalting and buffer exchange.

Product booklet

Codes:  17-0852-01 (20 columns)
        17-0852-02 (50 columns)
1. Legal

Product use restriction

NAP™-25 columns and components have been designed, developed and sold for research purposes only. They are suitable for in vitro use 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 the responsibility of the user to verify the use of NAP-25 columns for a specific application range as the performance characteristics of this product has not been verified to a specific organism.

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GE Healthcare reserves the right, subject to any regulatory and contractual approval, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation.

Contact your GE Representative for the most current information and a copy of the terms and conditions.

http://www.gehealthcare.com/nap

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK
2. Handling

2.1. Safety warnings and precautions

⚠️ Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage conditions

Store at ambient temperature (4ºC to 30ºC). Do not freeze.

2.3. Expiry

For expiry date please refer to outer packaging label.
3. Components

3.1. Kit contents

<table>
<thead>
<tr>
<th>Pack Size</th>
<th>20</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No.</td>
<td>17-0852-01</td>
<td>17-0852-02</td>
</tr>
</tbody>
</table>

NAP-25 Columns containing Sephadex G-25 DNA Grade

Columns are supplied with Sephadex G-25 DNA Grade in distilled water containing 0.15% Kathon™ CG/ICP Biocide as preservative.

3.2. Materials to be supplied by user

Equilibration buffer, (N.B. any nuclease free buffer is suitable, including water or Tris/EDTA, (TE)).

Collection tubes for sample collection.

3.3. Equipment to be supplied by user

A column support.

A suitable receptacle to catch buffer flowthrough.
4. Description

4.1. The basic principle
NAP columns are disposable columns prepacked with Sephadex G-25 DNA Grade resin and require only gravity to run. They allow DNA purification by the process of gel filtration. Molecules larger than the largest pores in the matrix are excluded from the matrix and elute first. Intermediate size molecules penetrate the matrix to varying extents, depending on their size. Penetration of the matrix retards progress through the column; very small molecules elute last. The volume required to elute these small molecules is dependent on the volume available both inside and outside the pores (i.e. the bed volume).

4.2. Introduction
NAP-25 columns are designed for the rapid purification of DNA, by desalting, buffer exchange, and removal of unincorporated nucleotides from end-labeled oligonucleotides. They can be used for any DNA greater than 10 bases in length and are therefore ideal for the purification of oligonucleotides or very small DNA fragments following synthesis or a labeling reaction. They will not remove or denature enzyme.
4.3. Choosing the appropriate NAP column and buffer volumes

NAP columns are available in three sizes depending on sample volume: up to 0.5 ml (NAP-5), up to 1 ml (NAP-10) or up to 2.5 ml (NAP-25). The table below provides a selection guide:

**Note:** the composition of the equilibration buffer is not critical. Any nuclease free buffer is suitable, including water or Tris/EDTA (TE).

<table>
<thead>
<tr>
<th>Column Type</th>
<th>Sample Volume (ml)</th>
<th>Equilibration Buffer (ml)</th>
<th>Elution Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-5</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.25</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>0.5 max vol</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>NAP-10</td>
<td>0.75</td>
<td>0.25</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>1.0 max vol</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>NAP-25</td>
<td>1.5</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>2.5 max vol</td>
<td>0</td>
<td>3.5</td>
</tr>
</tbody>
</table>
### 4.4 Product Specifications

<table>
<thead>
<tr>
<th>Format</th>
<th>Gravity flow column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle</td>
<td>Gel filtration</td>
</tr>
<tr>
<td>Column matrix</td>
<td>Sephadex G-25 DNA grade</td>
</tr>
<tr>
<td>Column buffer</td>
<td>Distilled water containing 0.15% Kathon</td>
</tr>
<tr>
<td>Yield / Recovery of DNA</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Purity of recovered DNA</td>
<td>Typically &lt;3% salt contamination</td>
</tr>
<tr>
<td>Length of labeled DNA recovered</td>
<td>&gt;10 bp (N.B. there is no maximum length of oligonucleotide that can be purified)</td>
</tr>
<tr>
<td>Major subsequent application</td>
<td>PCR, sequencing, labeling</td>
</tr>
</tbody>
</table>
| Column capacity (maximum amount of DNA that can be loaded onto column) | <1 mg/ml  
  Do not load a DNA solution of a concentration greater than 1 mg/ml. Higher concentrations reduce column resolution and give lower yield due to increased viscosity. Samples at a concentration greater than 1 mg/ml should be diluted with equilibration buffer prior to loading. |
5. Protocol

Prior to commencing, ensure that the NAP-25 columns have equilibrated to ambient temperature.

1. Support the column over a suitable receptacle to catch buffer flow through. Remove the top and bottom caps from the NAP-25 column and allow the excess liquid to flow through the column.

   **Note:** not allowing the excess liquid to flow through the column prior to use will result in slow flow rates.

2. Equilibrate the gel with approximately 25 ml of equilibration buffer.

   **Note:** this volume corresponds to 3 complete refills of the column.

   **Note:** the composition of the equilibration buffer is not critical. Any nuclease free buffer is suitable, including water or Tris/EDTA (TE). It is important to use the same buffer in steps 2, 3, 5 and 7 and so this buffer will be the one that the sample is to be eluted into.

3. Allow the equilibration buffer to completely enter the gel bed by gravity flow. Do not apply positive pressure.

4. Add the sample to the column in a maximum volume of 2.5 ml. If the sample volume is less than 2.5 ml, do not adjust it at this time. Allow the sample to enter the gel bed completely.
Note: if the sample volume is less than 2.5 ml and you wish to elute your sample using the minimum volume of equilibration buffer (see Table 1. ‘Buffer volume guide’ below) it is important not to adjust the sample volume to 2.5 ml with equilibration buffer at this point.

For example: if 1.5 ml sample is loaded onto the column and allowed to enter the gel bed completely (as in step 4), and then 1.0 ml of equilibration buffer is added to the column and allowed to enter the gel bed completely (as in step 5), the sample can be eluted using just 2.5 ml equilibration buffer (as shown in Table 1 below). This is because the sample volume loaded onto the column is small and will elute earlier.

Alternatively, if the sample is adjusted to 2.5 ml using the equilibration buffer and then added to the column in step 4 it would be necessary to elute with 3.5 ml equilibration buffer in order to obtain the maximum yield of the sample.

Table 1. Buffer volume guide

<table>
<thead>
<tr>
<th>Column Type</th>
<th>Sample Volume (ml)</th>
<th>Equilibration Buffer (ml)</th>
<th>Elution Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-25</td>
<td>1.5</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>2.5 max vol</td>
<td>0</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Note: concentration of the DNA sample should be below 1 mg/ml, as higher concentrations tend to reduce resolution and give lower yields due to increased viscosity.
5. For sample volumes less than 2.5 ml, add equilibration buffer so that the combined volume of sample added in step 4 and buffer added in step 5 equal 2.5 ml. Allow the equilibration buffer to enter the gel bed completely.

6. Place an appropriate size collection tube for sample collection under the column.

7. Elute the purified sample with an appropriate volume of equilibration buffer (please refer to Table 1. ‘Buffer volume guide’ which can be found in step 4).

Note: If you require a concentrated sample, collect 0.1 ml fractions as the sample elutes off the column. The concentration of the different fractions can be quantitated using a spectrophotometer or by loading 5 μl of each fraction onto an analytical gel (agarose or acrylamide).
### 6. Appendices

#### 6.1. Related products available from GE Healthcare

<table>
<thead>
<tr>
<th>Application</th>
<th>Product</th>
<th>Product Code</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radioactive labeling</strong></td>
<td>Rediprime™ II DNA labeling system</td>
<td>RPN1633</td>
<td>30 rxns</td>
</tr>
<tr>
<td></td>
<td>Nick Translation kit</td>
<td>NS000</td>
<td>20 rxns</td>
</tr>
<tr>
<td></td>
<td>5’-end labeling kit</td>
<td>RPN1509</td>
<td>20 rxns</td>
</tr>
<tr>
<td><strong>Purification of oligonucleotides</strong></td>
<td>MicroSpin™ G-25 columns</td>
<td>27-5325-01</td>
<td>50 columns</td>
</tr>
<tr>
<td>following synthesis (buffer exchange and desalt). Spin column format</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCR and enzymatic DNA reaction purification</strong></td>
<td>GFX™ PCR and gel band extraction kit</td>
<td>28903470</td>
<td>100 columns</td>
</tr>
<tr>
<td>(enzyme removal, buffer exchange, desalt, primer removal), 100 bp-10 kb size range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraction of DNA from agarose gels</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Rapid and efficient purification of PCR products** for automated sequencing One-tube, one-step method

**Dye terminator removal**
from automated sequencing reactions

**Unincorporated labeled nucleotide removal**
from a DNA labeling reaction
Spin column format

**Unincorporated labeled nucleotide removal**
from a DNA labeling reaction
Gravity flow

A full range of reagents can be found in the GE Healthcare catalogue. If you need any further information, GE Healthcare technical services are always happy to assist.

http://www.gehealthcare.com/nap
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imagination at work
17-0812-01PL Rev A 2006
The next two pages are a protocol card. If required please add to the back page as a tear off addition.

If not then delete these three pages.
illustri™
NAP-25 Columns
17-0852-01 (20 columns)
17-0852-02 (50 columns)
For the purification of oligonucleotides and small DNA fragments.
Desalting and buffer exchange.

**Experienced user protocol**

1. Remove top and bottom caps from the column and allow the excess liquid to flow through the column.

2. Equilibrate the gel with 25 ml equilibration buffer.

3. Add the sample to the column.

4. Elute the purified sample.
NAP-25 Columns  
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Buffer volume guide

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<th>Elution Buffer (ml)</th>
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<tbody>
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<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>2.5 max vol</td>
<td>0</td>
<td>3.5</td>
</tr>
</tbody>
</table>