



BIOPHARMACEUTICAL DEVELOPMENT PROGRAM

SOP Title: Desalting and Buffer Exchange of Products Using illustra NAP-5 Columns
SOP Number: 22141
Revision: 02

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1. PURPOSE

This procedure describes the use of GE Healthcare Life Sciences illustra NAP-5 Columns for rapid and convenient desalting and buffer exchange of nucleic acids, proteins and oligonucleotides (≥ 10 -mers), and will be used primarily as a sample preparation technique for assays in which a specific product formulation may be necessary for a particular analytical technique.

2. SCOPE

Scope text This procedure applies to Process Analytics/Quality Control (PA/QC) personnel.

3. RESPONSIBILITIES

3.1 Director / Process Analytics/Quality Control (PA/QC)

- Defines procedure

3.2 Personnel / PA/QC

- Training laboratory personnel
- Perform procedure
- Reviewing data and documentation
- Document training to Biopharmaceutical Quality Assurance (BQA)

3.3 BQA / QA

- Provides oversight

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4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
21408	GE Healthcare Life Sciences illustra NAP-5 Column, (available as a 20 or 50 pack). NAP-5 columns are prepacked, disposable columns containing Sephadex® G-25 medium of DNA grade in distilled water containing 0.15% Kathon® CG/ICP Biocide as a preservative.	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
20595	1.5 mL microcentrifuge tubes	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
	15 mL of Exchange Buffer (exact buffer formulation will be specific for the product and assay).	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO

5. EQUIPMENT

- (Optional) Automatic Environmental SpeedVac (AES) system, Savant model number AES1010 or equivalent.

6. PROCEDURE

- 6.1 Remove the top cap from the NAP-5 column and pour off the excess liquid.
- 6.2 Remove the bottom cap from the column.
- 6.3 Support the column over a waste receptacle, such as a small beaker, and equilibrate the gel with approximately 10 mL of the exchange buffer (10 mL of buffer corresponds to 3 complete refills of the column).
- 6.4 Allow the exchange buffer to completely enter the gel bed.
- 6.5 Add the sample to the column in a maximum volume of 0.5 mL and allow the sample to completely enter the gel bed.
- 6.6 For sample volumes less than 0.5 mL, add exchange buffer so that the total volume of sample and buffer equals 0.5 mL. Allow the buffer to completely enter the gel bed.
- 6.7 Place a 1.5 mL microcentrifuge tube under the column.

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- 6.8 Elute the sample by adding 1.0 mL of exchange buffer while collecting eluate.
- 6.9 Since the elution volume will be approximately 1.0 mL, the sample will be more dilute than the original. If necessary, the purified sample can be concentrated as per SOP 22916 - Operation of the Savant Automatic Environmental SpeedVac.
- 6.10 Discard the column and waste solvent in a biomedical waste container.
- 6.11 A typical elution profile is shown in Attachment 1.

7. DOCUMENTATION AND RECORDS

Document the column lot number, and expiration date, the sample information, and the exchange buffer formulation, lot number and expiration date, in an issued laboratory notebook or other controlled document.

8. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
22916	Operation of the Savant Automatic Environmental SpeedVac

9. ATTACHMENTS

- Attachment 1 Example Elution Profile (provided in the Amersham Biosciences NAP-5 instruction sheet)

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Attachment 1

Example Elution Profile

(provided in the Amersham Biosciences NAP-5 instruction sheet):

