Biopharmaceutical Development Program

Standard Operating Procedure

Title: Desalting and Buffer Exchange of Products Using illustra NAP-5 Columns

SOP Number: 22141 **Revision Number: 01**

Supersedes: Revision 00 **Effective Date:** OC- 9 2015

Originator/Date: [redacted]

Approval/Date: [redacted]

Approval/Date: [redacted]

Table of Contents

1.0 Purpose

This proced ure describes the use of GE Healthcare Life Sciences ilustra NAP-5 Columns for rapid and convenient desalting and buffer exchange of nucleic acids, proteins and oligonucleotides (2:1 0-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.

2.0 Scope

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

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BOA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 GE Healthcare Life Sciences illustra NAP-5 Column, BDP PN 21408, (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.

4.2 1.5 mL microcentrifuge tubes, BDP PN 20595 or equivalent.

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

4.4 15 mL of Exchange Buffer (exact buffer formulation will be specific for the product and assay).

5.0 Procedure

5.1 Remove the top cap from the NAP-5 column and pour off the excess liquid.

5.2 Remove the bottom cap from the column.

5.3 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).

5.4 Allow the exchange buffer to completely enter the gel bed.

5.5 Add the sample to the column in a maximum volume of 0.5 mL and allow the sample to completely enter the gel bed.

5.6 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.

5.7 Place a 1.5 mL microcentrifuge tube under the column.

5.8 Elute the sample by adding 1.0 mL of exchange buffer while collecting eluate.

5.9 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***.

5.10 Discard the column and waste solvent in a biomedical waste container.

5.11 A typical elution profile is shown in **Attachment I**.

6.0 Documentation

6.1 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.

7.0 Attachments

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

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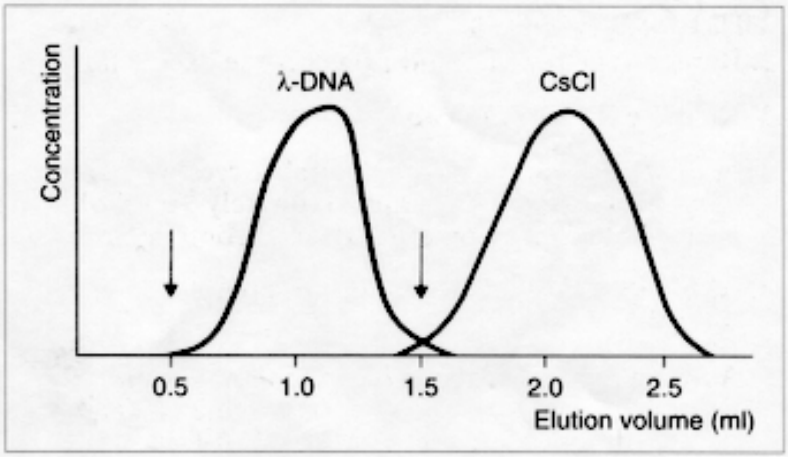
Attachment 1

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



Removal of CsCl from λ-DNA on Pharmacia NAP-5 column.

Equilibration buffer:	10 mM sodium phosphate buffer pH 6.8.
Sample:	25 µg λ-DNA in 0.5 ml 1 M CsCl.
Yield:	97% of DNA was collected in 1.0 ml (between arrows).
Purity:	The purified DNA contained 2% of applied CsCl.

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