Econo-Pac® 10DG Columns

Instruction Manual

Catalog Number

732-2010

BIO-RAD

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Section 1
Introduction

The Econo-Pac 10DG columns allow quick and economical desalting and buffer exchange of a wide range of samples. The columns are packed with Bio-Gel® P-6DG gel. This polyacrylamide gel allows high recovery, without interference from biologically active material leaching from the matrix.

In desalting, a chromatography matrix is chosen for its ability to exclude the solutes of interest from the pores of the gel, and to retain the smaller contaminants. The Econo-Pac 10DG columns are packed with a matrix which excludes solutes greater than 6,000 daltons, allowing them to elute in the void volume. Therefore, desalting occurs between the excluded components larger than 6,000 daltons and the included components smaller than 6,000 daltons.

Desalting is not a capacity dependent separation; the efficiency is not limited by the concentration of the sample. Therefore the only limiting parameter is sample volume, which is limited to the void volume of the

column. The Econo-Pac 10DG columns have a void volume of approximately 3.3 ml.

This manual includes a general procedure for desalting or buffer exchange, as well as procedures for specific applications.

Section 2
Column Features

Figure 2.1 provides a summary of the features of the Econo-Pac 10 columns.

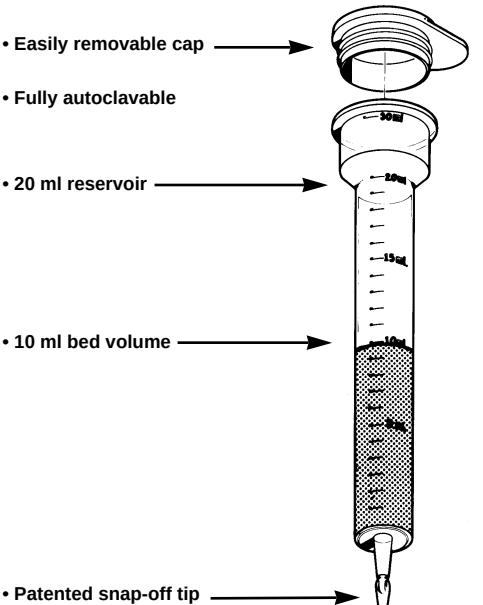

Fig. 2.1. Econo-Pac 10DG column.

Section 3
Column Specifications

Bed volume	10 ml
Total column volume	30 ml
Maximum sample volume	3.3 ml
Recommended sample volume	3.0 ml
Void volume	3.3 ml
Packing buffer	10 mM sodium phosphate, 10 mM NaCl, pH 7.0, with 0.02% sodium azide
Flow rate range	1.1 - 1.5 ml/min
Column material	Polypropylene
Frit material	Polyethylene
Desalting gel	Bio-gel P-6DG gel
pH range	2 - 10
Operating temperature range	2 - 45 °C

Section 4
Buffer Considerations

The Econo-Pac 10DG columns are shipped containing sodium azide as a preservative. Before using the column, remove this buffer and equilibrate the column in a suitable buffer for desalting.

Most buffers are suitable for desalting. The low residual charge of Bio-Gel P-6DG gel makes deionized water a good choice, since salts are not needed to overcome any ionic interactions between the gel and the solute. However, before desalting or exchanging a sample into a buffer, the solubility of the sample in the new buffer should be tested. For example, most antibodies require an ionic strength of at least 10 mM to remain in solution. IgM antibodies require as much as 40 mM to stay soluble.

Section 5
Procedures

Three procedures are included in this section. The first procedure is for general desalting or buffer exchange. The other two procedures are for specific applications.

5.1 Desalting or Buffer Exchange Protocols

The following desalting protocols are recommended for sample volumes ranging from 100 µl to 3.0 ml. For volumes < 100 µl use Bio-Spin chromatography columns. For volumes > 3.0 ml use Bio-Gel P-6DG gel packed into an appropriate column.

General Protocol

1. Remove the upper cap, and pour off the excess buffer above the top frit.
2. Add 20 ml of the appropriate buffer to the column (fill to the 30 ml mark), and snap off the bottom tip to start the column flowing.
3. Allow the buffer to drain to the top frit. The column will not run dry. Flow will stop when the buffer level reaches the top frit.
4. Add a 3.0 ml sample to the column. If sample is less than 3.0 ml add buffer to reach a total volume of 3.0 ml or perform minimal dilution protocol to minimize sample dilution.

5. Allow entire sample to enter the column and discard the first 3.0 ml of effluent.

6a. Add 4.0 ml of buffer to elute the higher molecular weight component(s), while collecting the 4.0 ml fraction from the column. For a more precise collection method refer to step 6b.

6b. Elute the desired components with 8 ml of buffer, while collecting 1 ml fractions from the column. Analyze the fractions with the appropriate detector and plot the results against the fraction number. Pool the desired fractions (See Figure 5.1).

Minimal Dilution Protocol

1. Remove the upper cap, and pour off the excess buffer above the top frit.
2. Add 20 ml of the appropriate buffer to the column (fill to the 30 ml mark), and snap off the bottom tip to start the column flowing.
3. Allow the buffer to drain to the top frit. The column will not run dry. Flow will stop when the buffer level reaches the top frit.
4. Measure sample volume, then add sample to the column.

5. Allow entire sample to enter the column. Add (3.0 ml - sample volume) of buffer. Allow buffer to enter the column and discard the effluent.

6a. Add 1.5 x sample volume or 4ml of buffer (whichever is less) to elute the higher molecular weight component(s). Collect this fraction from the column. For a more precise collection method refer to step 6b.

6b. Elute the desired components with 8 ml of buffer, while collecting 1 ml fractions from the column. Analyze the fractions with the appropriate detector and plot the results against the fraction number. Pool the desired fractions (See Figure 5.1).

Conditions

Column:	Econo-Pac 10 DG desalting column
Sample:	BSA in 250 mM NaCl
Buffer:	H_2O
Flow rate:	1.0 ml/min

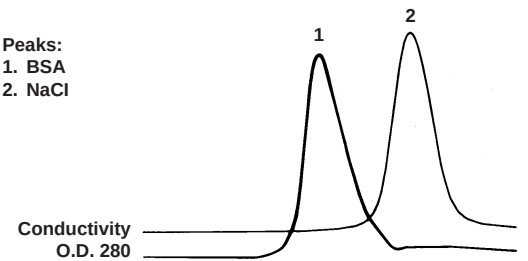

Fig. 5.1. Protein desalting with the Econo-Pac 10 DG desalting column.

5.2 Application 1: Separation of Incorporated Radiolabeled Proteins from Unincorporated Radiolabel

1. Follow steps 1 through 3 in the general desalting procedure, Section 5.1.
2. Apply the radiolabeled protein solution to the column, allowing the sample to run completely into the column. (For best results, the sample volume should be between 1.0 and 3.0 ml.)
3. Elute with 10 ml of buffer, collecting 1.0 ml fractions.
4. Count 10 µl of each fraction in an appropriate scintillation cocktail.
5. Plot CPM against fraction number to determine which fractions to pool (see Figure 5.2).
6. Discard the column and unpooled fractions in an appropriate radioactive waste container.

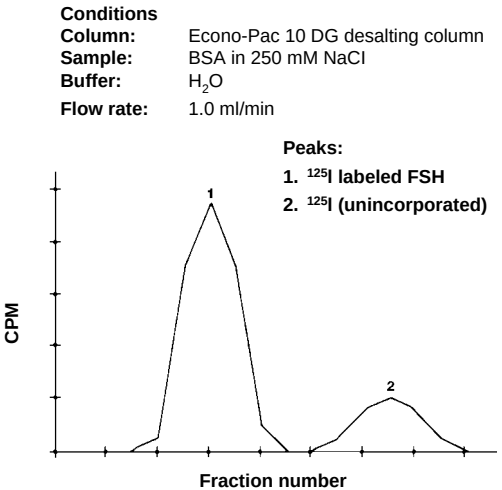

Fig. 5.2. Separation of radiolabeled protein from unincorporated radiolabel.

5.3 Application 2: Removal of Affi-Gel® or Affi-Prep® MAPS® II Binding Buffer from Antibody Solutions

1. Follow steps 1 through 3 in the general desalting procedure. (Note: The equilibration buffer should contain a minimum of 10 mM NaCI for IgG monoclonal antibodies, or 50 mM NaCI for IgM monoclonal antibodies.)
2. Load up to 3.0 ml of the elution fraction collected from the Affi-Gel or Affi-Prep protein A column.
3. Add 8.0 ml of a suitable buffer to elute the antibody. Collect 1.0 ml fractions.
4. Analyze the fractions to determine which fractions to pool.

Section 6
Storage Conditions

Econo-Pac 10DG columns should be stored at room temperature in 0.02% sodium azide.

Section 7
Product Information

Catalog Number	Product Description
732-2010	**Econo-Pac 10DG Desalting Columns,** 50
732-6000	**Bio-Spin 6 Chromatography Columns,** 10
732-6002	**Bio-Spin 6 Chromatography Columns,** 25
732-6004	**Bio-Spin 30 Chromatography Columns,** 10
732-6006	**Bio-Spin 30 Chromatography Columns,** 25
150-0738	**Bio-Gel P-6DG Gel,** 100 G
150-0739	**Bio-Gel P-6DG Gel,** 1 KG
732-1010	**Empty Econo-Pac 10 Columns** (50), include caps and bed support
732-1015	**Econo-Pac 10 Rack,** 12 place acrylic rack
731-7005	**Poly Column Rack,** holds 10 Econo-Pac columns
732-8102	**2-way Stopcock,** 10

Section 8
Technical Information

For additional information and technical assistance contact your local Bio-Rad representative or in the U.S. call Technical Service at 1-800-4BIORAD.

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