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A Geno Technology, Inc. (USA) brand name

Anionic & Cationic Columns

For Ion Exchange Chromatography

(Cat. # 786-184A, 786-184C)

INTRODUCTION

Ionic interaction is the basis of protein purification by the Ion Exchange Chromatography (IEC). Protein contains regions of charged groups on the surface that interact with the ion-exchange group immobilized on the stationary phase (column). Immobilized proteins are eluted with a salt gradient.

ITEM(S) SUPPLIED:

Cat. #	Description	Size
786-184A	Anionic Resin Column, 1.5ml resin	6 Columns
786-184C	Cationic Resin Column, 1.5ml resin	6 Columns

ADDITIONAL ITEM(S) REQUIRED

- Loading Buffer-I (0.5M Tris, 20mM NaCl, pH 6.5)
- Loading Buffer-II (0.5M Tris, 20mM NaCl, pH 7.5)
- Loading Buffer-III (0.5M Tris, 20mM NaCl, pH 8.5)
- NaCl [4M]

NOTE: *The kit is supplied with either anion or cation chromatography columns and three sample loading buffers. Use any one column at a time and perform chromatography with any one sample loading buffer at a time. You would be able to run six chromatography runs with anion columns, using Loading Buffers I, II, & III and six chromatography runs with cation columns, using Loading Buffers I, II, & III. In total you would be able to run 6-separate chromatography runs either for anionic or cationic exchange.*

PROTOCOL

Prepare A Column

Select one anionic column (or a cationic column) and mark it Column-1, Position the column in a collection tube. Prepare column equilibration buffer, as follows:

Equilibration Buffer

1. To make Equilibration Buffer I, combine 5ml Loading Buffer-I with 5ml Extraction Buffer of choice that contains >10mM salt.
   NOTE: See the section on the preparation of protein extracts for information on extraction buffers.
2. Equilibrate the Column-1 with 10ml Equilibration Buffer-1 (e.g., apply 3-4 ml buffer at a time and allow the buffer to drip until the column is empty of buffer). After Equilibration Buffer is completely drained out of the column, replace the bottom closure on the column.

Column Centrifugation:

For elution step the protocol requires a brief centrifugation of the column. Centrifugation should not be too severe to dry the column. Centrifugation should be at such a moderate speed (~200-300xg for 30-40 seconds) that it removes only 60-70% of the buffer from the column, leaving behind in the column 30-40% buffer. If necessary, make a trial run (before loading the protein sample) to determine an appropriate centrifugation condition. Make a note of the centrifugation condition (centrifugation speed and duration) and use the same condition at each step, unless specified otherwise.

Preparation of Elution Buffer:

Mix Sample Loading Buffer-1 with increasing amounts of 4M NaCl, as follows:

Elution Buffer #	Loading Buffer-I (ml)	4M NaCl (ml)	Elution Buffer Composition
Elution Buffer-1	0.897	0.013	0.5M Tris, pH 6.5, 50mM NaCl
Elution Buffer-2	0.975	0.025	0.5M Tris, pH 6.5, 100mM NaCl
Elution Buffer-3	0.950	0.050	0.5M Tris, pH 6.5, 200mM NaCl
Elution Buffer-4	0.925	0.075	0.5M Tris, pH 6.5, 300mM NaCl
Elution Buffer-5	0.90	0.1	0.5M Tris, pH 6.5, 400mM NaCl
Elution Buffer-6	0.850	0.15	0.5M Tris, pH 6.5, 600mM NaCl
Elution Buffer-7	0.750	0.250	0.5M Tris, pH 6.5, 1M NaCl

Prepare Samples for Loading on the columns:

For the best result, use the crude extract that has been subjected to ammonium sulfate fractionation, as described above. The samples must be first dialyzed 3-4 hours in extraction buffer (containing >20mM NaCl) before running IEC-chromatography. Mix the appropriate sample with the Loading Buffer-1 as follows.

Loading Sample-I

Mix 0.25ml sample with 0.25ml Loading buffer-I [0.5M Tris, pH 6.5, 20mM NaCl].

Apply Sample

Apply the sample (0.1-0.5ml) (containing 0.4-.6mg total protein) on the column. Incubate for 5 minutes.

Remove the bottom closure and allow the column to drain. Allow the column to drip until there is no buffer dripping from the column. Collect the eluent in a collection tube and mark the tube as IE Eluent-IA.

Position the column on a clean collection tube and centrifuge the column for a brief 30-40 seconds (see note above on column centrifugation). Mark the tube and the eluent as IE Eluent-IB.

Elution

Elute the protein from the column by applying 0.25ml of each of the following elution buffers, one after another in the following order and collect eluent, as follows.

1. Apply 0.25 ml of Elution Buffer-1 and collect the fraction and label it IEC-Fraction-1
2. Apply 0.25 ml of Elution Buffer-2 and collect the fraction and label it IEC-Fraction-2
3. Apply 0.25 ml of Elution Buffer-3 and collect the fraction and label it IEC-Fraction-3
4. Apply 0.25 ml of Elution Buffer-4 and collect the fraction and label it IEC-Fraction-4
5. Apply 0.25 ml of Elution Buffer-5 and collect the fraction and label it IEC-Fraction-5
6. Apply 0.25 ml of Elution Buffer-6 and collect the fraction and label it IEC-Fraction-6
7. Apply 0.25 ml of Elution Buffer-7 and collect the fraction and label it IEC-Fraction-7

Analyze Fractions

Analyze each fraction for protein concentration, biological activity, and if possible run electrophoresis.

Protein Purification Analysis

1. Protein Concentration (mg protein/ml)
2. Gel Electrophoresis Profile
3. Protein Activity Assay
4. (Optional) Specific Activity i.e. units of activity /mg protein

Repeat the procedure described above with the remaining columns (anionic and cationic) using the Loading Buffer-II and -III, respectively. Use the same scheme for preparing the Equilibration Buffer and Elution Buffer.

TROUBLESHOOTING

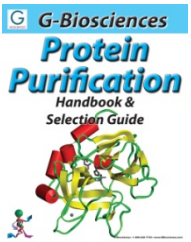
If you notice that the protein of your interest is detected in the fractions eluting out of the column immediately after loading the sample on the column as well as in the fractions eluted off the column with the elution buffer - most probably you are loading too much protein on the column. Cut down the total protein or sample volume loaded on the column.

RESULTS AND CONCLUSIONS

By comparing the results of Ion Exchange Chromatography (IEC)-Fractions, with (anionic or cationic) columns and under different loading and elution buffer conditions, it would be possible to determine how your protein behaved during IEC-chromatography. You would be able to find chromatographic loading and elution conditions for obtaining effective enrichment of your protein.

RELATED PRODUCTS

Download our Protein Purification Handbook.



http://info.gbiosciences.com/complete-protein-purification-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

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