4. Centrifuge the spin column at ~80 x g (1,000 rpm)* for 30 seconds to remove the buffer.
5. Replace the bottom cap of the spin column.
6. Add 200 µl 50 mM Tris buffer, pH 9 to the column, then replace the top cap on the column.
7. Vortex the column to mix the sorbent with the buffer.
8. Remove the top and bottom caps of the spin column; set the column upright in a new 1.5 ml microcentrifuge tube.
9. Centrifuge the spin column for 30 seconds to remove the buffer.
10. Repeat steps 5–9 twice, for a total of three buffer washes.
11. Replace the bottom cap on the spin column. The sample can now be added to the spin column.
12. If the column is not used right away, add 0.5 ml buffer, pH 9 to prevent dehydration.

* Recommended speeds for an Eppendorf 5417R centrifuge.

Ordering Information

Catalog #	Description
C54-00017	**ProteinChip Q Spin Columns,** 20
C57-30080	**ProteinChip Q10 Arrays,** A-H format, 12

Bio-Rad Laboratories, Inc.

Life Science Group

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ProteinChip® Q Spin Columns

Instruction Manual

Catalog #C54-00017

For technical support, call your local Bio-Rad office, or in the US, call **1-800-4BIORAD (1-800-424-6723)**.

Introduction

Ion exchange chromatography (IEC) is the most frequently used chromatography technique for the separation of proteins, peptides, and other charged biomolecules. IEC achieves separation by taking advantage of the difference in net surface charge of these elements in a complex biological mixture.

Surface charge is determined by the presence of weak acidic and basic groups on a protein and as such is highly pH dependent. Typically, at pH values below a protein's isoelectric point (pI), the protein acquires a net positive surface charge and will adsorb to cation exchange media. At pH values above the protein's pI, the protein acquires a net negative surface charge and will adsorb to anion exchange media. Variations in mobile phase pH are most often used to selectively bind a molecule of interest to an IEC support. The ideal pH value is one that creates a large net charge difference among the different sample components.

Desorption of bound proteins from IEC media is achieved by either altering the pH or increasing the salt concentration (introducing competing ions) of the mobile phase. The higher the surface charge of the protein, the higher the ionic strength that is needed to bring about desorption. Variations in the mobile phase ionic strength are used that take advantage of the charge differences in species bound to the IEC support.

ProteinChip Q (quaternary amine) spin columns contain Q ceramic HyperD F sorbent, an anion exchange chromatography support. This material has been specifically designed to rapidly capture and separate proteins and peptides from crude biological mixtures under physiological conditions of ionic strength.

Applications

Crude biological samples contain thousands of proteins and peptides. Their complexity presents a significant analytical challenge for researchers evaluating in vitro and in vivo experiments from cell lysates and plasma samples. Strategies to reduce the complexity of these samples are essential to gaining a better understanding of cellular mechanisms and disease states.

IEC is a powerful approach to prefractionation of biological samples. This technology can reduce the presence of abundant species, greatly increase the number of less abundant species, and enrich samples in elements of interest. ProteinChip Q spin columns are designed to facilitate the analysis of crude biological samples by fractionating proteins on the basis of net surface charge. Biological samples can be further simplified by fractionation with ProteinChip arrays.

Q ceramic HyperD F sorbent mimics the binding and elution characteristics of ProteinChip Q10 arrays. ProteinChip Q spin columns can be used for small-scale purifications of proteins discovered on array surfaces.

Storage

Store columns at 4°C.

Technical Considerations

- Recommended sample binding time is 20–40 min
- Each spin column containing Q ceramic HyperD F sorbent has a minimal protein binding capacity of 7.7 mg

Column Equilibration

ProteinChip Q spin columns contain a sorbent that requires buffer equilibration prior to use. This equilibration buffer should be the same as the intended sample binding buffer. To ensure that most proteins will bind to the column, the salt concentration should be <100 mM, pH ~9.

1. Tap the spin column lightly to settle the sorbent to the bottom (near the tapered end) of the column.
2. Remove the caps on the top and bottom of the spin column.
3. Set the column upright in a 1.5 ml microcentrifuge tube.

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