Ordering Information

Catalog #	Description
C54-00018	**ProteinChip Q Filtration Plate**, 1 x 96-well
C57-30075	**ProteinChip CM10 Arrays**, A–H format, 12
C55-30033	**ProteinChip Gold Array**, A–H format
C57-30028	**ProteinChip H4 Arrays**, A–H format, 12
C57-30065	**ProteinChip H50 Arrays**, A–H format, 12
C57-30078	**ProteinChip IMAC30 Arrays**, A–H format, 12
C57-30043	**ProteinChip NP20 Arrays**, A–H format, 12
C55-30058	**ProteinChip PG20 Array**, A–H format
C55-30044	**ProteinChip PS10 Array**, A–H format
C57-30045	**ProteinChip PS20 Arrays**, A–H format, 12
C55-30082	**ProteinChip RS100 Arrays**, A–H format, 6
C57-30081	**ProteinChip SEND ID Arrays**, A–H format, 12
C30-00001	**ProteinChip CHCA Energy Absorbing Molecules (EAMs)**, 5 mg/vial, 20
C30-00002	**ProteinChip SPA Energy Absorbing Molecules (EAMs)**, 5 mg/vial, 20
C30-00003	**ProteinChip EAM-1 Energy Absorbing Molecules (EAMs)**, 5 mg/vial, 20

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ProteinChip® Q Filtration Plate

Instruction Manual

Catalog #C54-00018

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

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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 a cation exchange support. At pH values above the protein's pI, that protein acquires a net negative surface charge and will adsorb to an anion exchange support. 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.

The 96-well ProteinChip Q filtration plate contains Q ceramic HyperD F sorbent, an anion exchange chromatography support that has been dried to allow extended storage in the filtration plate. The sorbent 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. The ProteinChip Q filtration plate is 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 on ProteinChip arrays.

Storage

Store ProteinChip Q filtration plates at 4°C.

Technical Considerations

- Mix adequately during the rehydration and sample incubation stages to ensure that the sorbent is well suspended
- Recommended sample binding time is 20–40 minutes
- Each well containing Q ceramic HyperD F sorbent has a minimal binding capacity of 7.1 mg

Recommended Buffers

Equilibration Buffer

Q ceramic HyperD F sorbent requires buffer equilibration prior to use. This equilibration buffer should be the same as the intended sample binding buffer. The salt concentration should be <100 mM, pH ~9.0 to ensure that most proteins will bind to the sorbent. Recommended equilibration buffer — 50 mM Tris HCl, pH 9.0.

Rehydration of Q Ceramic HyperD F Sorbent

The ProteinChip Q filtration plate should be rehydrated directly before use:

1. Tap the filtration plate on the workbench several times to make sure that all of the dry Q ceramic HyperD F sorbent is settled to the bottom of the plate.
2. Take the filtration plate out of the pouch and carefully remove the top seal on the filtration plate.

Note: If using only part of the filtration plate, with a sharp blade remove only enough of the foil seal to reveal the columns needed. While processing samples, only the wells in use should be uncovered. After using part of the plate, reseal those wells with microplate sealing strips and mark those columns as used. Place the plate back in the foil pouch; seal and store at 4°C.

3. With an 8-channel pipet, add 200 µl of deionized (DI) water to each well that you plan to use.
4. Vortex the filtration plate on a MicroMix 5 shaker (Diagnostic Products Corp.) (set at 20, 7, 60) for 60 minutes at room temperature (RT). The settings for the MicroMix 5 may need to be adjusted for your particular piece of equipment. During mixing, check that the sorbent is fully suspended in the DI water.
5. Apply vacuum to remove the rehydration solution from the filtration plate.
6. Wash with DI water four times by adding 200 µl of DI water, then applying the vacuum to remove it. The wash step will remove the protective reagent from the dry Q ceramic HyperD F sorbent.
7. Wash with equilibration buffer four times by adding 200 µl to each well, then applying the vacuum to remove it.
8. Add sample to the ProteinChip Q filtration plate.