ProteinChip® Q10 Array (Strong Anion Exchanger)

Instruction Manual

Catalog #C57-30080

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

BIO-RAD

Uses

- Protein profiling and biomarker discovery
- Rapid protein analysis to determine purity, mass confirmation, or both

How It Works

The operating mechanism of ionic-exchange ProteinChip arrays is the reversible binding of charged molecules to the surface, and the property of a peptide or protein that governs its binding is its net surface charge. Since surface charge is the result of weak acidic and basic amino acids within the protein, binding of the protein to the array is highly pH dependent.

The ProteinChip Q10 array incorporates quaternized ammonium groups (positively charged) and thus acts as a strong anion exchanger. The ProteinChip Q10 array surface binds peptides and proteins that are negatively charged at a given pH. By maintaining the pH of the binding or washing buffer at alkaline conditions (e.g., pH 8), an overall net negative charge is imparted on a greater number of proteins within the sample (therefore more binding). By decreasing the pH of the binding or washing buffer, an overall net positive charge is imparted on the proteins, resulting in less binding (i.e., more specificity).

Binding of proteins to the ProteinChip Q10 array can also be affected by changing the ionic strength of the buffer. By increasing the ionic strength, a competition is generated between the charged protein on the surface and the buffer ions, causing weakly bound proteins to elute from the array surface (i.e., more specificity).

Packaging and Storage

Store the arrays at room temperature.

ProteinChip arrays are packaged in a 12-array cassette. A bioprocessor reservoir is included in the package (see Figure 1). The spare ProteinChip cassette included to separate the reservoir from the arrays should be removed before use in the ProteinChip cassette-compatible bioprocessor (catalog #C50-30011). It is not necessary to remove the arrays when using the cassette-compatible bioprocessor; however, individual arrays can be removed if needed. To do this, remove the bioprocessor reservoir before taking any arrays out of the cassette. Be careful not to touch the spots on the array. A pair of ProteinChip array forceps (catalog #C20-10002) helps effectively remove the arrays from the cassette (see Figure 2).

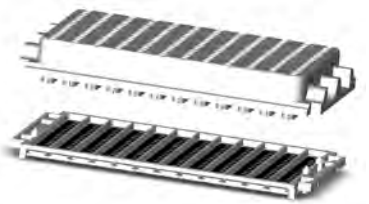

Figure 1. ProteinChip cassette and reservoir.

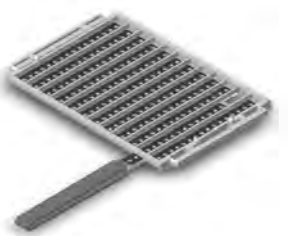

Figure 2. Removal of ProteinChip arrays from cassette using array forceps.

Technical Considerations

- Increasing the ionic concentration of buffer and/or lowering the pH of the binding and washing buffers will increase the selectivity of the surface
- If salts are present in the sample (>50 mM), remove with spin column
- Choose a binding buffer that can buffer at least one pH unit above the isoelectric point (pI) of the target protein(s)
- When using a bioprocessor, make sure there are no air bubbles in the wells. To avoid introducing bubbles, lower the pipet tip very close to the spot surface while dispensing sample. Empty the wells completely between washes

Recommended Binding and Washing Buffers

- Tris-HCl buffer (10–100 mM), pH 7.5–9.0
- Sodium/ammonium phosphate buffer (10–100 mM), pH 6–8
- HEPES buffer (20–100 mM), pH 6.8–8.2
- Sodium/ammonium acetate buffer (10–100 mM), pH 4–6

Protocol 1: Serum Profiling Using the Bioprocessor

Note: These protocols are intended as a guideline; you may need to optimize the method for your particular sample type and experimental design.

Note: This protocol is for the 8-spot array in the ProteinChip cassette-compatible bioprocessor. For processing a single array, use a ProteinChip 8-well bioprocessor (catalog #C50-30008).

1. Place the ProteinChip array cassette in the bioprocessor and add 150–250 µl binding solution to each well. Incubate for 5 minutes at room temperature with vigorous shaking (e.g., 250 rpm, or on MicroMix shaker setting 20/7). Repeat once.

2. Remove the buffer from the wells. Immediately add 50–150 µl sample to each well. Recommended concentration is 50–2,000 µg/ml total protein, diluted in binding buffer. Incubate with vigorous shaking for 30 minutes.
3. Remove the samples from the wells and wash each well with 150–250 µl binding buffer for 5 minutes, with agitation. Repeat two more times.
4. Remove the binding buffer from the wells and add 150–250 µl deionized (DI) water to each well, remove immediately. Repeat once.
5. Remove the reservoir from the bioprocessor base clamp assembly.
6. Air-dry the arrays for 15–20 minutes.
7. Add ProteinChip sinapinic acid (SPA) energy absorbing molecues (EAM) (catalog #C30-00002) after removing the reservoir; use the cassette hold-down frame provided with the cassette-compatible bioprocessor to keep the cassette flat during EAM addition.
8. Apply 1 µl of ProteinChip SPA EAM in solution to each spot. Air-dry for 5 minutes and apply another 1 µl of EAM in solution. Allow to air-dry.
9. Analyze the arrays using the ProteinChip SELDI system.

Protocol 2: Serum Profiling On-Spot

1. Prewet the spots with 5 µl of binding buffer for 5 minutes. Repeat once.
2. Remove the prewetting solution and replace with 5 µl of sample. Do not allow the spot to air-dry during sample application.
3. Incubate in a humid chamber for 30 minutes with shaking (on MicroMix shaker setting 20/4).
4. Wash each spot with 5 µl of binding buffer with shaking, and remove buffer. Repeat two more times.
5. Wash each spot with 5 µl of DI water. Repeat once.
6. Air-dry the array for 15–20 minutes
7. Apply 1 µl of ProteinChip SPA EAM in solution to each spot. Air-dry for 5 minutes and apply another 1 µl of EAM in solution. Allow to air-dry.
8. Analyze the array using the ProteinChip SELDI system.

Ordering Information

Catalog #	Description
C57-30080	**ProteinChip Q10 Arrays**, A–H format, 12
C50-30011	**ProteinChip Cassette-Compatible Bioprocessor**, includes ProteinChip array forceps, cassette hold-down frame, 12 blank ProteinChip arrays
C50-30008	**ProteinChip 8-Well Bioprocessor**, A–H format
C50-30012	**ProteinChip Cassette-Compatible Bioprocessor Reservoirs**, 5
C20-10002	**ProteinChip Array Forceps**, 1 pair
C30-00002	**ProteinChip SPA Energy Absorbing Molecules (EAM)**, 5 mg/vial, 20
C54-00017	**ProteinChip Q Spin Columns**, 20
C54-00018	**ProteinChip Q Filtration Plate**, 1 x 96-well

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The SELDI process is covered by US patents 5,719,060, 6,225,047, 6,579,719, 6,818,411, and other issued patents and pending applications in the US and other jurisdictions.

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