
ProteinChip® CM10 Array (Weak Cation Exchanger)

Instruction Manual

Catalog #C57-30075

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 CM10 array incorporates a carboxylate chemistry (negatively charged) and thus acts as a weak cationic exchanger. The ProteinChip CM10 array surface binds proteins that are positively charged at a given pH. To generate selectivity, the pH of the binding buffer is increased or decreased, depending on the need. By decreasing the pH of the binding and washing buffers, an overall net positive charge is imparted on a greater number of proteins within the sample (therefore more binding). By increasing the pH of the binding and washing buffers, an overall net negative charge is imparted on the proteins, resulting in less binding (i.e., more specificity).

Binding of proteins to the ProteinChip CM10 array can also be affected by changing the ionic strength of the buffer. By increasing the ionic strength, 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).

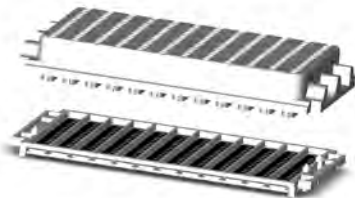


Fig. 1. ProteinChip cassette and reservoir.



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

Technical Considerations

- Increasing the ionic concentration of buffer or raising 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 a desalting spin column
- Choose a binding buffer that can buffer at least one pH unit below 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

- ProteinChip CM low-stringency buffer (catalog #K20-00003)
0.1 M sodium acetate, pH 4.0
- ProteinChip CM high-stringency buffer (catalog #K20-00004)
50 mM HEPES, pH 7.0
- Sodium/ammonium acetate buffer (10–100 mM), pH 4–6
- Sodium/ammonium phosphate buffer (10–100 mM), pH 6–8
- Tris-HCl buffer (10–100 mM), pH 7.5–9.0

Fractionation for Serum Profiling

Fractionation of serum prior to profiling is recommended. The fractionation procedure produces 6 fractions containing proteins separated on the basis of their pI. Fractionation allows segregation of highly abundant proteins into a limited number of fractions, thereby reducing signal suppression effects on lower-abundance proteins.

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 μ l 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 μ l sample to each well. Recommended concentration is 50–2,000 μ g/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 μ l binding buffer for 5 minutes, with agitation. Repeat two more times.

4. Remove the binding buffer from the wells, and add 150–250 μ l 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 energy absorbing molecules (EAM) 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 μ l of ProteinChip EAM in solution to each spot. Air-dry for 5 minutes and apply another 1 μ l of EAM in solution. Allow to air-dry.
9. Analyze the array using the ProteinChip SELDI system.

Protocol 2: Serum Profiling On-Spot

1. Prewet the spots with 5 μ l of binding buffer for 5 minutes.
Repeat once.
2. Remove the prewetting solution and replace with 5 μ l 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 μ l of binding buffer with shaking and remove buffer. Repeat two more times.
5. Wash each spot with 5 μ l of DI water. Repeat once.
6. Air-dry the array for 15–20 minutes.
7. Apply 1 μ l of EAM in solution to each spot. Air-dry for 5 minutes and apply another 1 μ l of EAM in solution. Allow to air-dry.
8. Analyze the array using the ProteinChip SELDI system.

Ordering Information

Catalog #	Description
C57-30075	ProteinChip CM10 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
C30-00001	ProteinChip CHCA Energy Absorbing Molecules (EAM) , 5 mg/vial, 20
C30-00002	ProteinChip SPA Energy Absorbing Molecules (EAM) , 5 mg/vial, 20
C30-00003	ProteinChip EAM-1 Energy Absorbing Molecules (EAM) , 5 mg/vial, 20
K20-00003	ProteinChip CM Low-Stringency Buffer , 200 ml
K20-00004	ProteinChip CM High-Stringency Buffer , 200 ml

MicroMix is a trademark of Diagnostic Products Corporation.

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.

BIO-RAD

**Bio-Rad
Laboratories, Inc.**

*Life Science
Group*

Web site www.bio-rad.com **USA** 800 4BIORAD
Australia 02 9914 2800 **Austria** 01 877 89 01
Belgium 09 385 55 11 **Brazil** 55 21 3237 9400
Canada 905 712 2771 **China** 86 21 6426 0808
Czech Republic 420 241 430 532 **Denmark** 44 52 10 00
Finland 09 804 22 00 **France** 01 47 95 69 65
Germany 089 318 84 0 **Greece** 30 210 777 4396
Hong Kong 852 2789 3300 **Hungary** 36 1 455 8800
India 91 124 4029300/5013478 **Israel** 03 963 6050
Italy 39 02 216091 **Japan** 03 5811 6270 **Korea** 82 2 3473 4460
Mexico 55 5200 05 20 **The Netherlands** 0318 540666
New Zealand 64 9415 2280 **Norway** 23 38 41 30
Poland 48 22 331 99 99 **Portugal** 351 21 472 7700
Russia 7 095 721 14 04 **Singapore** 65 6415 3188
South Africa 27 0861 246 723 **Spain** 34 91 590 5200
Sweden 08 555 12700 **Switzerland** 061 717 95 55
Taiwan 886 2 2578 7189/2578 7241
United Kingdom 020 8328 2000