ProteinChip® H50 Array (Reverse Phase)

Instruction Manual

Catalog #C57-30065

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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Uses

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

How It Works

The ProteinChip H50 array surface binds proteins through reverse-phase or hydrophobic interaction chromatography and has binding characteristics similar to that of a C6 to C12 alkyl chromatographic resin. In reverse-phase interactions, proteins within the sample are partitioned between the lipophilic phase of the array surface and the sample buffer. Proteins less hydrophobic relative to the binding buffer will not bind to the array surface, while proteins more hydrophobic will bind to the array surface.

By increasing the organic content of the washing buffer, the hydrophobic nature of the buffer increases. Proteins that had previously bound to the array will repartition into the washing buffer and be washed away if their hydrophobicity is less than that of the washing buffer. Only the most hydrophobic proteins will be retained with wash buffers containing a high organic solvent.

Hydrophobic interaction chromatography is characterized by binding of proteins to a hydrophobic surface at high salt concentrations (salt precipitation of proteins). Typically conditions are nondenaturing, and since no organic solvent is used, biological activity has a much higher probability of being retained. Proteins are sequentially washed from the array surface by decreasing the salt concentration of the wash buffers.

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).

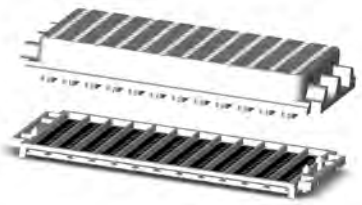

Fig. 1. ProteinChip cassette and reservoir.

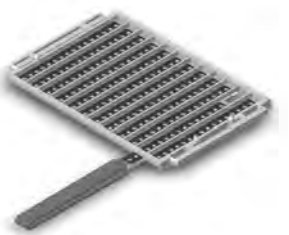

Fig. 2. Removal of ProteinChip arrays from cassette using array forceps.

Technical Considerations

- Increasing the concentration of organic solvent in the binding/washing solution will increase the selectivity of the surface (only the most hydrophobic proteins will be retained with higher organic solvent concentrations). Use a shorter wash time (2 minutes or less) during the wash step after sample binding if the washing solution contains more than 20% organic solvent
- Increasing the salt concentration will increase hydrophobic interactions and therefore can be included in the binding buffer. Suggested salt concentration range is 50–1000 mM. Higher salt concentrations are likely to adversely affect reproducibility
- Prewashing the ProteinChip array in 50% acetonitrile or methanol before sample binding may increase spot-to-spot reproducibility
- To obtain optimum performance, prewet the spot with 5 µl of binding buffer before applying sample
- 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 Solutions

- ProteinChip H50 buffer (catalog #K20-00001) (10% acetonitrile, 0.1% trifluoroacetic acid (TFA))
- 0–50% methanol or acetonitrile ± 0.1–1% TFA
- 0.1% TFA may be added to the binding solution to increase binding

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 isoelectric point. 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 the ProteinChip 8-well bioprocessor (catalog #C50-30008).

1. Place the ProteinChip array cassette in the bioprocessor, and prewash the arrays by adding 50 µl 50% methanol or acetonitrile for five minutes. Repeat once.
2. Remove the prewash solution from the wells 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.
3. 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, in binding buffer. Incubate with vigorous shaking for 30 minutes.
4. 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.
5. Remove the binding buffer from the wells and add 150–250 µl deionized (DI) water to each well. Remove immediately.
6. Remove the reservoir from the bioprocessor base clamp assembly.
7. Air-dry the arrays for 5–10 minutes.
8. Add ProteinChip energy absorbing molecules (EAM) after removing the reservoir; use the cassette hold-down frame provided with the ProteinChip cassette-compatible bioprocessor to keep the cassette flat during EAM addition.
9. Apply 1 µl of ProteinChip EAM in solution to each spot. Air-dry for 5 minutes, and apply another 1 µl of EAM in solution. Allow to air-dry.
10. Analyze the arrays using the ProteinChip SELDI system.

Protocol 2: Serum Profiling On-Spot

1. Optional: Bulk-wash the ProteinChip array with 50% methanol or acetonitrile for five minutes. Repeat once. Dry the array for an hour after bulk wash to minimize any spot-to-spot cross-contamination.
2. Prewet the spots with 5 µl of binding buffer for 2 minutes. Repeat once.
3. Remove the prewetting solution and replace with 5 µl of sample. Do not allow the spot to air-dry during sample application.
4. Incubate in a humid chamber for 30 minutes with shaking (on MicroMix shaker setting 20/4).
5. Wash each spot with 5 µl binding buffer for 2 minutes with shaking, and remove buffer. Repeat two more times.
6. Optional: If binding buffer contains salt, wash each spot with 5 µl of DI water.
7. Air-dry the array for 5–10 minutes.
8. Apply 1 µl of ProteinChip 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 array using the ProteinChip SELDI system.

Ordering Information

Catalog #	Description
C57-30065	**ProteinChip H50 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
K20-00001	**ProteinChip H50 Buffer**, 200 ml
K20-00005	**ProteinChip H50 Buffer**, 1 L

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