ProteinChip® H4 Array (Reverse Phase)

Instruction Manual

Catalog #C57-30028

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

BIO-RAD

Uses

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

How It Works

The ProteinChip H4 array binds proteins by reverse-phase interactions. The active surface contains chains of 16 methylene groups that can bind proteins through reverse-phase chemistry via alanine, valine, leucine, isoleucine, phenylalanine, tryptophan, or tyrosine. 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 wash 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.

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).

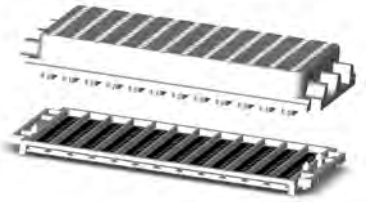

Fig. 1. ProteinChip cassette and reservoir.

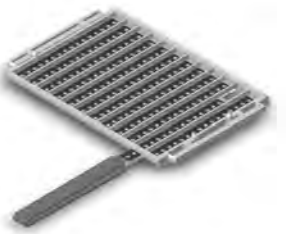

Fig. 2. Removal of ProteinChip arrays from cassette using array forceps.

Technical Considerations

- Increasing the concentration of organic solvent in the binding and 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
- 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

- 0–60% acetonitrile/methanol ± 0.1% trifluoroacetic acid (TFA); a reasonable starting buffer is phosphate buffered saline (PBS) + 10% ACN + 100 mM NaCl
- 0.1% TFA may be added to the binding solution to increase binding

Related Products

The ProteinChip H50 array (catalog #C53-70065) is an advanced array for reverse-phase retentate chromatography*. The array shows high reproducibility and has a hydrophobic barrier coating for sample containment. The array is recommended for:

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

Note: We do not recommend the ProteinChip H50 array for peptide analysis. For this application, the ProteinChip SEND ID array is preferred.

The ProteinChip SEND ID array (catalog #C53-70081) enables protein identification by either peptide mass fingerprinting or MS/MS sequencing using SELDI/MALDI MS. Surface enhanced neat desorption (SEND) technology is unique in that the EAM is integral to the ProteinChip array surface. The chemical noise in the spectrum from the EAM is significantly reduced when compared to addition of EAM on-spot. SEND ID has C18 as a functional group, allowing the use of the array for cleanup on-chip for desalting and denaturant (such as urea) removal prior to analysis by SELDI.

* US patent 7,112,453.

Protocol 1: 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 uses the ProteinChip cassette-compatible bioprocessor; for processing a single array, use an 8-well bioprocessor (catalog #C50-30008).

1. Place the ProteinChip array cassette in the bioprocessor and prewash the ProteinChip arrays by adding 50 µl methanol or acetonitrile for five minutes. Repeat once. (Use the same percentage that will be used for binding/washing steps.)
2. Remove the prewash solution 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 50–250 µl sample to each well.
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. Repeat once.
6. Remove the reservoir from the bioprocessor base clamp assembly.
7. Air-dry the arrays for 5–10 minutes.
8. Using a hydrophobic pen, draw a circle around each spot.
9. 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.
10. 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.
11. Analyze the arrays using the ProteinChip SELDI system.

Protocol 2: Profiling On-Spot

1. Outline each spot using a hydrophobic pen.
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 solution 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.

Protocol 3: Analysis of a Trypsin Digest

1. Outline each spot using a hydrophobic pen.
2. Apply 1 µl of the trypsin digest to a spot on a ProteinChip H4 array and air-dry for 5 minutes.
3. If the starting protein concentration is low, reapply 1 µl aliquots (2–3 times) to the same spot (allow to air-dry between applications) to increase signal response.
4. Prepare a spot with solution from a no-protein control to establish the baseline, and identify background signal from trypsin autolysis.
5. Apply 0.5 µl of 10% saturated ProteinChip alpha-cyano-4-hydroxy cinnamic acid (CHCA) EAM (catalog #C30-00001) in solution (saturated CHCA solution diluted 10-fold with 50% acetonitrile, 0.5% TFA) to each spot. Air-dry for 5 minutes and apply another 0.5 µL of EAM solution. Allow to air-dry.
6. Analyze the array using the ProteinChip SELDI system.

Ordering Information

Catalog #	Description
C57-30028	**ProteinChip H4 Arrays**, A–H format, 12
C57-30065	**ProteinChip H50 Arrays**, A–H format, 12
C57-30081	**ProteinChip SEND ID Arrays**, A–H format, 12
C50-30008	**ProteinChip 8-Well Bioprocessor**, A–H format
C50-30011	**ProteinChip Cassette-Compatible Bioprocessor**, includes ProteinChip array forceps, cassette hold-down frame, 12 blank ProteinChip arrays
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

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*

10008232 US/EG Rev F *06-0668 1006 Sig 1205*