10. Wash the array 2 times with deionized water (10 seconds each).
11. Tap the array on the benchtop to remove water drops.
12. Allow the array surface to air-dry (5 minutes).
13. Add 0.5 µl of energy absorbing molecule (EAM) solution to each spot. Allow to air-dry. Repeat addition of EAM solution.
14. Analyze the array using the ProteinChip SELDI system.

Ordering Information

Catalog #	Description
C57-30045	**ProteinChip PS20 Arrays**, A–H format, 12
C55-30044	**ProteinChip PS10 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 (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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10008234 US/EG Rev F *06-0670 1106 Sig 1205*

ProteinChip® PS20 Array (Preactivated Surface)

Instruction Manual

Catalog #C57-30045

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

- Covalent immobilization of biomolecules for the subsequent capture of proteins from complex biological samples
- Recommended when goals include sensitive detection and low nonspecific binding, and when target protein is less than 1% of total protein

How It Works

The ProteinChip PS20 array is preactivated with epoxide chemistry that reacts with amino groups on the surface of biomolecules to form stable covalent linkages. In turn, these immobilized biomolecules capture proteins from biological samples through specific, noncovalent interactions.

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 reservoirs 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).

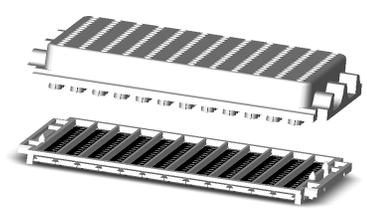

Fig. 1. ProteinChip cassette and reservoir.

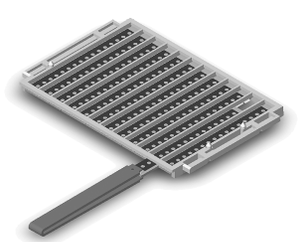

Fig. 2. Removal of ProteinChip arrays from cassette using array forceps.

Technical Considerations

- Optimization of assay parameters will be required for each specific application
- Proteins primarily couple through amine groups but will also couple through surface-exposed sulfhydryl groups
- For coupling, use pure biomolecules without carrier proteins whenever possible
- Generally, couple biomolecules at pH 7.5–9.0, using phosphate buffered saline (PBS) or sodium bicarbonate buffers
- Caution: During coupling, avoid buffers containing free amines (e.g., glycine, Tris), free sulfhydryls (e.g., dithiothreitol, β-Mercaptoethanol), and azide
- Avoid physical contact with spot surface and surrounding coating
- Array design allows sample containment of up to 5 µl per spot
- Include denaturants, salts, and chaotropic agents in binding and wash buffers, as required to modify binding stringency and reduce nonspecific binding
- In general, the ProteinChip PS20 array surface exhibits lower nonspecific binding compared to the ProteinChip PS10 array (catalog #C55-30044), and therefore will require less stringent washing conditions

Recommended Buffers

Coupling Buffers

- PBS or sodium bicarbonate, pH 7.5–9.0
- Avoid buffers containing free amines, free sulfhydryls, or azide

Blocking Buffers

- Ethanolamine (0.5 M), pH 8
- Tris-HCl or glycine (0.1–0.5 M), pH 8

Washing Buffers

- Post-blocking, use buffers and additives more stringent than binding conditions. Also, repeated washing and pH cycling may be necessary
- Post-binding, use buffers and additives as in binding conditions
- Include nonionic detergent, salts, and chaotropic agents as required
- A final water wash is often required

Binding Buffers

- PBS, pH 7.5 or buffer of choice
- Include nonionic detergent (e.g., 0.1–0.5% Triton X-100) as needed
- Include salt (0.15–1.0 M), other modifiers (e.g., ethylene glycol), and carrier protein (e.g., 1% bovine serum albumin (BSA), if necessary

Example Protocol: On-Spot

Note: This protocol is intended as a guideline; you may need to optimize the method for your particular sample type and experimental design.

1. Prepare bait molecule: Buffer exchange and protein G beads can be used to purify antibodies; dilute or dissolve the biomolecule in coupling buffer (0.1–1.0 mg/ml protein).
2. Load 2–5 µl of bait molecule solution per spot. Place the array in a humid chamber.
3. Incubate the array at room temperature for 1–4 hours or at 4°C overnight.
4. Block with blocking buffer.
5. Dilute or dissolve the sample in binding buffer.
6. Load 2–5 µl of sample per spot and place the array in a humid chamber.
7. Incubate the array at room temperature for 1–4 hours or at 4°C overnight.
8. Wash each spot with 5 µl of washing buffer by pumping in and out 5 times using a pipet.
9. Repeat washing 1–2 times as above. Or, wash 1–2 times by submerging the array in a tube with washing buffer and placing on a rocker or agitator for 5 minutes.

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