Ordering Information

Catalog #	Description
C55-30044	**ProteinChip PS10 Arrays**, A–H format, 12
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

Bio-Rad Laboratories, Inc.

Life Science Group

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ProteinChip® PS10 Array (Preactivated Surface)

Instruction Manual

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

Uses

- Covalent immobilization of biomolecules for the subsequent capture of proteins from complex biological samples

Introduction

The ProteinChip PS10 array is preactivated with carbonyldiimidazole 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

ProteinChip PS10 arrays are packaged in sealed tubes under argon, protected from moisture. Store the arrays as shipped.

Technical Considerations

- Optimization of assay parameters will be required for each specific application
- Proteins primarily couple through amine groups
- For coupling, use pure biomolecules without carrier proteins whenever possible
- Generally, couple biomolecules at pH 7.5-9.0, using 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/or chaotropic agents in binding and wash buffers as required to modify binding stringency and reduce nonspecific binding

Recommended Buffers

Coupling Buffers

- Phosphate buffered saline (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 8.0
- Tris-HCl or glycine (0.1–0.5 M), pH 8.0

Wash 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/or 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/or carrier protein (e.g., 1% 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 may be required; protein G beads can be used to purify antibodies; dilute or dissolve the biomolecule in coupling buffer (0.1–1 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 wash buffer by drawing liquid 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.
10. Wash the array 2 times with deionized water (10 seconds each).
11. Tap the array on the workbench to remove water drops.
12. Allow the array surface to air-dry (5 minutes).
13. Add 0.5 µl of ProteinChip 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 reader.