Example Protocol: Antibody–Antigen Capture Using Protein G

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

1. Apply 2 µl of 0.5 mg/ml protein G and 2 µl 50 mM $NaHCO_3$ buffer, pH 9.2 to spot. Incubate the array for 1 hour at RT in a humid chamber.
2. Remove protein G and wash with 5 µl 1x PBS for 2 minutes on shaker.
3. Remove buffer and apply 2 µl 100 µM BSA and 2 µl of 1x PBS, pH 7.2 for 1 hour at RT to block any remaining active sites.
4. Remove BSA and add 5 µl of 1x PBS. Shake for 2 minutes on a shaker and then remove buffer from spot. Repeat wash step.
5. Remove the buffer and apply 2 µl of 0.1 mg/ml antibody and 2 µl 50 mM $NaHCO_3$ buffer, pH 9.2 to spot. Incubate the array for 2 hours at RT in a humid chamber.
6. Remove antibody and wash with 5 µl 1x PBS for 2 minutes on shaker.
7. Remove buffer. Prepare the antigen in 50% human serum diluted in a 0.1% Triton X-100 in 1x PBS solution, pH 7.2. Apply 2 µl of antigen and incubate for 2 hours at RT.
8. Remove antigen and add 5 µl of 1x PBS and wash for 30 seconds on shaker.
9. Remove buffer and add 5 µl urea CHAPS buffer (50 mM Tris + 1 M urea + 0.1% CHAPS + 0.5 M NaCl, pH 7.2); wash buffer for 30 seconds on shaker.
10. Remove buffer and add 5 µl of 1x PBS, pH 7.2; wash for 2 minutes on shaker.
11. Quickly bulk-rinse the array with 5 mM HEPES, pH 7.2 in a 15 ml tube for 10 seconds.
12. Allow the array surface to air-dry (5 minutes).
13. Add 1 µl of 50% saturated SPA EAM solution to each spot. Allow to air-dry. Repeat addition of SPA solution and allow to dry.
14. Analyze the array using the ProteinChip SELDI system.

Ordering Information

Catalog #	Description
C55-30082	**ProteinChip RS100 Arrays**, A–H format, 12
C30-00002	**ProteinChip SPA Energy Absorbing Molecules (EAMs)**, 5 mg/vial, 20

Triton is a trademark of Union Carbide.

The SELDI process is covered by US patents 5,719,060, 5,894,063, 6,020,208, 6,027,942, 6,124,137, 6,225,047, 6,528,320, 6,579,719, and 6,734,022. Additional US and foreign patents are pending.

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10008240 US/EG Rev C *06-0675 1106 Sig 1205*

ProteinChip® RS100 Array (Reactive Surface)

Instruction Manual

Catalog #C55-30082

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

How It Works

The ProteinChip RS100 array is preactivated with carbonyl diimidazole chemistry that reacts with amine 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 RS100 arrays in sealed tubes are vacuum-packaged and protected from moisture. Store the arrays between –20°C and –50°C in the original packaging. Open the array tube just prior to use. Once opened, the arrays have limited stability.

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 7.2–9.2, 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 for on-spot application and up to 180 µl for bioprocessor application
- Include denaturants, salts, and chaotropic agents in binding and wash buffers, as required to modify binding stringency and reduce nonspecific binding
- Prepare energy absorbing molecule (EAM) solution according to instructions in EAM product insert

Recommended Buffers

Coupling Buffers

- PBS or sodium bicarbonate, pH 7.2–9.2
- 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
- Bovine serum albumin (BSA) (100 µM in 1x PBS), pH 7.2

Washing Buffers

- Post-blocking, use buffers and additives more stringent than binding conditions. Repeated washing and pH cycling may be necessary
- Post-binding, use buffers containing 0–2.0 M salt, 0–2.0 M denaturant (urea), and 0.1–0.5% detergent (Triton X-100), prepared in PBS, pH 7–7.5 or 50 mM Tris
- Include nonionic detergent, salts, and chaotropic agents as required
- A final rinse (10–20 seconds) with 5 mM HEPES is often required to remove salts and detergents from washing buffers

Binding Buffers

- PBS, pH 7–7.5 or buffer of choice
- Include nonionic detergent (e.g., 0.1–0.5% Triton X-100 or CHAPS) as needed
- Include salt (0.15–1.0 M), other modifiers (e.g., ethylene glycol), and carrier protein (e.g., 1% BSA), if necessary

Example Protocol: Antibody–Antigen Capture Assay

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

1. Apply 2 µl of 0.25 mg/ml antibody and 2 µl 50 mM $NaHCO_3$ buffer, pH 9.2.
2. Incubate the array for 1 hour at room temperature (RT) in a humid chamber.
3. Remove antibody and apply 2 µl 100 µM BSA and 2 µl of 1x PBS, pH 7.2 for 1 hour at RT to block any remaining active sites.
4. Remove the blocking solution and add 5 µl of 1x PBS. Shake for 2 minutes on a shaker and then remove buffer from spots. Repeat wash step.
5. Prepare the antigen in 50% human serum diluted in 0.1% Triton X-100 in 1x PBS solution, pH 7.2. Apply 2 µl of antigen and incubate for 2 hours at RT.
6. Remove antigen and add 5 µl of 1x PBS and wash for 30 seconds on a shaker.
7. Remove buffer and add 5 µl urea CHAPS buffer (50 mM Tris + 1 M urea + 0.1% CHAPS + 0.5 M NaCl, pH 7.2); wash buffer for 30 seconds on shaker.
8. Remove buffer and add 5 µl of 1x PBS, pH 7.2; wash for 2 minutes on shaker.
9. Quickly bulk-rinse the array with 5 mM HEPES, pH 7.2 in a 15 ml tube for 10 seconds.
10. Allow the array surface to air-dry (5 minutes).
11. Add 1 µl of 50% saturated sinapinic acid (SPA) EAM (catalog #C30-00002) solution to each spot. Allow to air-dry. Repeat addition of SPA solution and allow to dry.
12. Analyze the array using the ProteinChip SELDI system.