1. For each assay, add 5 µl (packed) ProteinChip affinity beads to an Eppendorf tube. Wash beads extensively 6–7 times with 500 µl DI water per wash.

Note: Do not use coupling buffer for this wash step.

2. Add 2 µg antibody in 50 µl 50 mM sodium acetate buffer, pH 5.0 to the beads in a 500 µl tube. The pH of this coupling reaction can be adjusted depending on your specific antibody. Sodium phosphate, pH 7.0 or 50 mM sodium bicarbonate, pH 9.0 are also suitable coupling buffers. Incubate overnight at 4°C in an end-over-end mixer.
3. Remove the supernatant (antibody solution). Wash the beads once with 200 µl of 50 mM sodium acetate buffer, pH 5.0 or coupling buffer selected in step 2.
4. Add 25 µl of 2 mg/ml bovine serum albumin (BSA) in 0.5 M Tris-HCl, pH 9.0, 0.1% Triton X-100 to end-cap residual reactive groups. Incubate for 2 hours in an end-over-end mixer at RT.
5. Wash the beads with 200 µl 1x PBS, 0.1% Triton X-100 followed by 500 µl 1x PBS.
6. Add 25 µl sample to the beads and incubate for 1 hour in an end-over-end mixer at RT.
7. Following incubation, wash the beads with 500 µl 1x PBS.
8. Wash twice with 200 µl 50 mM Tris buffer, pH 7.2, 1 M urea, 0.1% CHAPS, 0.5 M sodium chloride for 5 minutes.
9. Wash twice with 500 µl 1x PBS for 5 minutes.
10. Rinse with 500 µl DI water.
11. Add 10 µl 50% acetonitrile, 1 M acetic acid to each tube and gently vortex for 5–60 minutes.

Note: Alternative extraction buffers may be used. 50% acetonitrile/0.3% trifluoroacetic acid (TFA) is suitable for antigens that have a high affinity for the antibody. 50% ethanol/1 M acetic acid can be used to reduce the signal from nonspecifically bound proteins.

Note: The length of extraction time should be optimized for your particular experiment. We suggest monitoring after 5 minutes; if the signal is weak, increase extraction up to 30 minutes or more.

12. Prerinse ProteinChip H50 arrays (catalog #C57-30065) in bulk with 50% acetonitrile for 5 minutes and allow to dry before use.

13. Apply 1 µl extract onto an array spot. Allow to dry.
14. Wash the spot with 2 µl DI water and allow to dry.
15. Apply 0.8 µl 20% ProteinChip CHCA energy absorbing molecules (EAMs) once or 1 µl 50% ProteinChip SPA EAMs twice to each spot. Allow arrays to dry.
16. Read the arrays in the ProteinChip SELDI reader.

Protocol 4: Coupling of Streptavidin to ProteinChip Affinity Beads

This protocol describes the coupling of streptavidin directly to ProteinChip affinity beads. Pure biotin can be used to measure the binding capacity of streptavidin beads.

Note: Biotin is not soluble in water. Dissolve in 2 N NaOH (50 mg/ml), and then dilute with 20 mM Tris, pH 7.2 to the required concentration.

1. Add 100 µl (packed) ProteinChip affinity beads to a 1.5 ml Eppendorf tube. Wash beads extensively 6–7 times with 1 ml of DI water per wash. Carefully remove the supernatant.

Note: Do not use coupling buffer for this wash step.

2. Add 200 µl streptavidin (4 mg/ml) in acetate buffer, pH 5.0 and incubate overnight in an end-over-end mixer at RT.
3. Remove the supernatant and wash the beads twice with 1 ml of incubation buffer (acetate, pH 5.0).
4. Add 600 µl of 0.5 M Tris-HCl, pH 9.0, 0.1% Triton X-100 to end-cap residual reactive groups. Incubate for 2 hours in an end-over-end mixer at RT.
5. Wash the beads with 1 ml of 1x PBS and incubate for 15 minutes in an end-over-end mixer at RT. Repeat 3 times or more until there is no foaming after a few seconds of vortexing. The beads are ready for use, or storage at 2–8°C in 1x PBS, 0.02% sodium azide.

Sample Results

Streptavidin — 60 µg/ml binding capacity for biotin in 1x PBS.

Binding capacity was determined by repeated injections of 0.1 mg/ml biotin solution (10 µl) to the streptavidin beads packed in a 200 µl column.

Ordering Information

Catalog #	Description
C54-00019	**ProteinChip Affinity Beads,** 2 ml, approximately 200 reactions
C57-30065	**ProteinChip H50 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

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Poland *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3188*
South Africa *27 861 246 723* ***Spain*** *34 91 590 5200*
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ProteinChip® Affinity Beads

Instruction Manual

Catalog #C54-00019

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

BIO-RAD

Uses

Immobilization of:

- Antibody directly to beads
- Proteins A or G for subsequent antibody adsorption
- Streptavidin for capture of biotinylated molecules
- Protein or DNA, for capture of binding proteins

Protein Interaction Analysis

Protein interaction analysis on the ProteinChip surface-enhanced laser desorption/ionization (SELDI) system includes an integrated set of tools that use the combination of biospecificity with SELDI time-of-flight mass spectrometry for the discovery of protein interactions, elucidation of biological pathways, and rapid assay development.

Product Description

ProteinChip affinity beads are zirconia-based porous beads coated with a hydrophilic polysaccharide and activated with carbonyldiimidazole (CDI). A major advantage of CDI activation is the immobilization of primary amino-containing ligands (proteins, peptides) within a very broad pH range of 4–10. This is particularly advantageous in applications which involve pH-sensitive proteins that irreversibly lose biological activity when exposed to conditions outside their physiological range. After reacting with the imidazole-ester reactive groups of ProteinChip affinity beads, the amino groups of the ligand to be immobilized form a neutral carbamate linkage which is stable for long-term storage. Another advantage of the zirconia skeleton is its high density. This speeds up all steps involving settlement of the solid phase in test tubes, and in most cases renders centrifugation unnecessary. The opacity conferred by the mineral skeleton helps to visualize the solid phase in test tubes and pipet tips and therefore helps to accurately estimate bead volume for further calculations (see Protocol 1 for reproducible sampling of ProteinChip affinity beads).

Storage

ProteinChip affinity beads should be stored at 2–8°C. The beads are supplied in a solution of 0.1% acetic acid in dry dimethyl sulfoxide (DMSO) and should be washed extensively with deionized (DI) water before use. DMSO will solidify at 4°C. Bring the beads to room temperature for approximately 30 minutes before use to thaw the DMSO.

Technical Considerations

ProteinChip affinity beads are very dense; therefore, the mixer used needs to be carefully selected. You should ensure that the beads and the solution mix thoroughly, and that the beads do not remain lying at the side or bottom of the tube. An end-over-end mixer or a Vortex-Genie mixer is recommended depending on the volume used. Mixing should not be too vigorous because it can cause damage to the beads.

Protocol 1: Reproducible Sampling of ProteinChip Affinity Beads in Microtubes

To achieve consistent results, it is important to use precise bead volumes. This is particularly true when the biological target you are aiming to capture is in excess, or when running many replicates.

The following method takes advantage of the density of ProteinChip affinity beads to achieve improved precision. A standard 20 µl micropipet tip marked at 2 µl and 10 µl is used (see Figure 1).

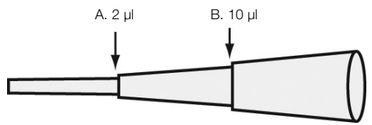

Fig. 1. Micropipet tip used for reproducible sampling of beads.

1. Choose mark A or B on a 20 µl tip, according to the desired volume. You can also make your own mark if a different volume is required. This is particularly useful for multiple sampling of the same volume of beads.
2. Mix the ProteinChip affinity bead slurry in the tube for a few seconds with the end of the tip. Draw up a 50% excess of the slurry and immediately remove the tip from the tube (see Figure 2).
3. Let the beads settle in the tip for at least 15 seconds. The slurry level must be above the chosen mark A or B. Make sure there are no air bubbles trapped at the end of the tip.

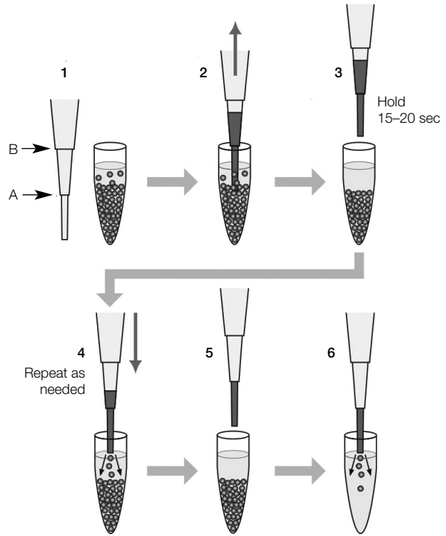

Fig. 2. Reproducible sampling of ProteinChip affinity beads.

4. Decrease the amount of beads until you reach mark A or B by touching the pipet tip to the liquid in the tube. The beads are dense enough that they will immediately fall into the liquid.
5. Precisely adjust bead volume by repeating step 4, touching the pipet tip to the liquid as many times as necessary. Wait 15–20 seconds between each contact to allow bead level in the pipet to stabilize.
6. Release the precise volume of beads into the experiment tube. Rinse the tip with working buffer in this tube.

Protocol 2: Direct Coupling of Protein A or G to ProteinChip Affinity Beads

This protocol describes the coupling of protein A or G directly to ProteinChip affinity beads. This method can be modified to increase the amount of protein A or G when higher binding capacity is required for the target antibody. Human IgG can be used to measure the binding capacity of protein A or G beads.

1. Add 100 µl (packed) ProteinChip affinity beads to a 1.5 ml Eppendorf tube. Wash beads extensively 6–7 times with 1 ml of DI water per wash. Carefully remove the supernatant.

Note: Do not use coupling buffer for this wash step.

2. Add 200 µl protein A (4 mg/ml) or protein G (2 mg/ml) in acetate buffer, pH 5.0 to the beads and incubate overnight in an end-over-end mixer at room temperature (RT).
3. Remove the supernatant and wash the beads twice with 1 ml of acetate buffer, pH 5.0.
4. Add 600 µl of 0.5 M Tris-HCl, pH 9.0, 0.1% Triton X-100 to end-cap residual reactive groups. Incubate for 2 hours in an end-over-end mixer at RT.
5. Wash the beads with 1 ml of 1x PBS and incubate for 15 minutes in an end-over-end mixer at RT. Repeat 3 times or more until there is no foaming after a few seconds of vortexing. The beads are ready for use or storage at 2–8°C in 1 M sodium chloride, 20% ethanol.
6. After storage in this solution, wash the beads extensively in 1x phosphate buffered saline (PBS) before use.

Sample Results

Protein A beads — 10 mg/ml binding capacity for human IgG in 1x PBS.

Protein G beads — 5 mg/ml binding capacity for human IgG in 1x PBS.

Protocol 3: Coupling of an Antibody to ProteinChip Affinity Beads and Subsequent Capture and Elution of Antigen

This protocol describes a generic method for the coupling of antibody directly to the ProteinChip affinity beads, the subsequent capture of an antigen, and its detection by SELDI. The protocol is intended as a guideline; you may need to optimize the method for your particular sample type and experimental design.

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