# ProteinChip® IMAC30 Array (Immobilized Metal Affinity Capture)

## Instruction Manual

Catalog #C57-30078

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

- Protein profiling and biomarker discovery
- Analysis of phosphorylated peptides and proteins
- Analysis of histidine (His)-tagged proteins
- Analysis of metal-binding proteins
- On-chip optimization of purification process to be transferred to IMAC sorbent

## How It Works

The operating mechanism of the ProteinChip IMAC30 array is the reversible binding of proteins to the surface through a coordinated metal interaction. The ProteinChip IMAC30 array incorporates nitrilotriacetic acid (NTA) groups and is capable of forming stable octahedral complexes with polyvalent metal ions, including $Cu^{2+}$, $Ni^{2+}$, $Fe^{3+}$, and $Ga^{3+}$. After loading the array surface with the desired metal ion, two free sites are available from the formed octahedral complex for interaction with specific amino acid residues (such as His) or posttranslational modifications such as phosphate groups. To generate selectivity, binding and washing buffers may contain increasing concentrations of competitors, such as imidazole, which compete with the coordinated metal on the NTA group for binding to the protein or peptide.

## 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).

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## Technical Considerations

- Use ProteinChip IMAC30 arrays with $Cu^{2+}$ metal for general protein profiling, $Ni^{2+}$ metal for capture of His-tagged recombinant proteins, and $Ga^{3+}$ or $Fe^{3+}$ for phosphorylated peptide and protein capture

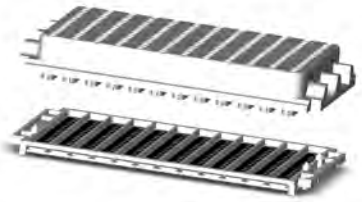

**Figure 1. ProteinChip cassette and reservoir.**

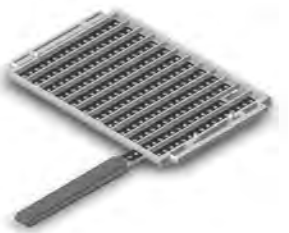

**Figure 2. Removal of ProteinChip arrays from cassette using array forceps.**

- A sodium acetate wash is necessary when charging arrays with copper. This step is not neeeded when charging with nickel or gallium
- Increasing the concentration of imidazole in binding and washing buffers will increase the selectivity of the surface
- 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 Buffers

- ProteinChip IMAC binding buffer (catalog #K20-00006) (0.1 M sodium phosphate, 0.5 M NaCl, pH 7)
- 10–100 mM phosphate buffered saline (PBS), pH 7.2 or choose alternative binding buffer of desired pH
- Include salt (0.5–1.0 M) in binding and washing buffers
- If needed, include 5–10 mM imidazole in binding buffer to increase selectivity. Increasing the concentration of imidazole beyond 10 mM may, however, disrupt low-affinity metal interactions

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### Fractionation for Serum Profiling

Fractionation of serum prior to profiling is recommended; the fractionation procedure produces 6 fractions containing proteins separated on the basis of their isoelectric point. Fractionation allows segregation of highly abundant proteins into a limited number of fractions, thereby reducing signal suppression effects on lower-abundance proteins.

### Protocol 1: Serum Profiling Using a Copper-Enriched Array and a 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 is for the 8-spot array in the ProteinChip cassette-compatible bioprocessor. For processing a single array, use a ProteinChip 8-well bioprocessor (catalog #C50-30008).

1. Place the ProteinChip array cassette in the bioprocessor and add 50 µl of 0.1 M copper sulfate solution to each well. Incubate for 10 minutes at room temperature with vigorous shaking (e.g., 250 rpm, or on MicroMix shaker setting 20/7).
2. Remove the metal solution from the wells. Immediately add 150–250 µl of deionized (DI) water to each well. Incubate for 1 minute at room temperature with vigorous shaking.
3. Remove the DI water solution from the wells. Immediately add 150–250 µl of 0.1 M sodium acetate buffer, pH 4 (neutralization buffer) to each well. Incubate for 5 minutes at room temperature with vigorous shaking.
4. Remove the buffer solution from the wells. Immediately add 150–250 µl of DI water to each well. Incubate for 1 minute at room temperature with vigorous shaking.
5. Remove the DI water solution from the wells. Immediately add 150–250 µl of binding buffer to each well. Incubate for 5 minutes at room temperature with vigorous shaking. Repeat once.
6. Remove the binding buffer from the wells. Immediately add 50–150 µl of sample to each well. Recommended concentration is 50–2,000 µg/ml total protein, diluted in binding buffer. Incubate with vigorous shaking for 30 minutes.

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7. 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.
8. Remove the binding buffer from the wells and add 150–250 µl DI water to each well; remove immediately. Repeat once.
9. Remove the reservoir from the bioprocessor base clamp assembly.
10. Air-dry the arrays for 15–20 minutes.
11. Add ProteinChip sinapinic acid (SPA) energy absorbing molecues (EAM) (catalog #C30-00002) after removing the reservoir. Use the cassette hold-down frame provided with the cassette-compatible bioprocessor to keep the cassette flat during EAM addition.
12. 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.
13. Analyze the arrays using the ProteinChip SELDI system.

**Protocol 2: Serum Profiling Using a Copper-Enriched Array On-Spot**

1. Add 5 µl per spot of 0.1 M copper sulfate and incubate for 10 minutes with shaking (on MicroMix shaker setting 20/4).
2. Remove the metal solution and replace with 5 µl of DI water and incubate for 1 minute.
3. Remove the water and replace it with 0.1 M sodium acetate buffer, pH 4 (neutralization buffer) and incubate for 5 minutes.
4. Remove the neutralization buffer and replace with 5 µl of DI water and incubate for 1 minute.
5. Remove the water and replace with 5 µl of binding buffer on each spot for 5 minutes. Repeat once.
6. Remove the binding buffer and replace with 5 µl of sample. Do not allow the spot to air-dry during sample application.
7. Incubate in a humid chamber for 30 minutes with shaking (on MicroMix shaker setting 20/4).

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8. Wash each spot with 5 µl of binding buffer with shaking, and remove buffer. Repeat two more times.
9. Wash each spot with 5 µl of DI water. Repeat once.
10. Air-dry the array for 15–20 minutes.
11. Apply 1 µl of ProteinChip SPA EAM in solution to each spot. Air-dry for 5 minutes and apply another 1 µl of EAM in solution. Allow to air-dry.
12. Analyze the array using the ProteinChip SELDI system.

## Related Products

ProteinChip IMAC spin columns containing BioSepra sorbents mimic the binding and elution conditions of ProteinChip arrays. These products can be used for small-scale purification of proteins identified on the ProteinChip IMAC30 array.

## Ordering Information

| Catalog # | Description |
|---|---|
| C57-30078 | **ProteinChip IMAC30 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 |
| C30-00002 | **ProteinChip SPA Energy Absorbing Molecules (EAM)**, 5 mg/vial, 20 |
| K20-00006 | **ProteinChip IMAC Binding Buffer**, 1 L |
| C54-00027 | **ProteinChip IMAC Spin Columns**, 20 |

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