

Ordering Information

Catalog #	Description
K20-00002	ProteinChip IMAC Buffer Set , includes 30 ml IMAC charging solution, 30 ml IMAC neutralizing solution, 200 ml IMAC binding buffer
K20-00006	ProteinChip IMAC Binding Buffer , 1 L
K20-00008	ProteinChip IMAC IMAC Charging Solution , 200 ml
K20-00009	ProteinChip IMAC IMAC Neutralizing Solution , 200 ml
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

The SELDI process is covered by US patents 5,719,060, 6,225,047, 6,579,719, and 6,818,411 and other issued patents and pending applications in the US and other jurisdictions.

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ProteinChip® IMAC Buffer Set

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).

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Uses

- Protein profiling and biomarker discovery
- Rapid protein analysis to determine purity, mass confirmation, or both

Introduction

The ProteinChip IMAC buffer set is designed for use with the ProteinChip IMAC30 array (catalog #C57-30078). The ProteinChip IMAC30 array is coated with a nitrilotriacetic acid (NTA) functional group to entrap transition metals for subsequent metal affinity binding to proteins. In profiling applications using the ProteinChip IMAC buffer set, the array is charged with copper prior to applying sample to the surface. After a neutralizing step, the sample is bound to the array in a low-stringency binding buffer to allow maximal binding of proteins to the surface. Selectivity is determined by the concentration of imidazole in the binding buffer. Increasing imidazole in the binding and wash buffer reduces the binding of proteins with weaker affinities for metal.

Storage

Store at 2–8°C.

ProteinChip IMAC Buffer Set Composition

- IMAC charging solution — 0.1 M cupric sulfate solution, antimicrobial preservatives, 30 ml
- IMAC neutralizing solution — 0.1 M sodium acetate, pH 4, antimicrobial preservatives, 30 ml
- IMAC binding buffer — 0.1 M sodium phosphate, 0.5 M sodium chloride, pH 7, antimicrobial preservatives, 200 ml

These volumes are sufficient to run 12 ProteinChip arrays in a bioprocessor using binding and elution buffer volumes as outlined in the suggested protocol below.

Suggested Protocol

1. Assemble the ProteinChip arrays in the ProteinChip cassette-compatible bioprocessor (catalog #C50-30011), and add 50 µl of IMAC charging solution to each well. Vortex for 5 minutes at room temperature.
2. Remove buffer from wells. Rinse with water.
3. Add 50 µl of IMAC neutralizing solution to each well. Vortex for 5 minutes at room temperature.
4. Remove buffer from wells. Rinse with water.

5. Add 150 µl of IMAC binding buffer to each well. Vortex for 5 minutes at room temperature.
6. Remove buffer from wells.
7. Repeat steps 5–6 for a total of two washes.
8. Add 90 µl of IMAC binding buffer to each well.
9. Add 10 µl of sample to each well. Vortex for 30 minutes at room temperature.
10. Remove samples from wells.
11. Wash each well with 150 µl IMAC binding buffer for 5 minutes, with agitation. Repeat twice for a total of three buffer washes.
12. Remove wash buffer from wells and rinse each well with deionized water.
13. Drain wells and remove arrays from the ProteinChip bioprocessor.
14. Allow arrays to air-dry.
15. Apply 1.0 µl ProteinChip energy absorbing molecule (EAM) solution per spot. Two applications of EAM solution can be used in order to increase signal intensity. Allow arrays to air-dry.
16. Analyze arrays using the ProteinChip SELDI reader.