Ordering Information

Catalog #	Description
K20-00004	**ProteinChip CM High-Stringency Buffer**, 200 ml
K20-00003	**ProteinChip CM Low-Stringency Buffer**, 200 ml
C57-30075	**ProteinChip CM10 Arrays**, A-H format, 12
C50-30011	**ProteinChip Cassette-Compatible Bioprocessor**, includes ProteinChip array forceps, cassette hold-down frame, 12 blank ProteinChip arrays
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

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ProteinChip® CM High-Stringency Buffer

Instruction Manual

Catalog #K20-00004

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

Uses

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

Introduction

ProteinChip CM high-stringency buffer is designed for use with the ProteinChip CM10 array. The ProteinChip CM10 array incorporates a carboxylate chemistry (negatively charged) that acts as a weak cation exchanger. The carboxymethyl (CM) surface binds proteins that are positively charged at a given pH. To control selectivity, the pH of the binding buffer is increased or decreased, depending on the need. By using the higher pH ProteinChip CM high-stringency buffer, an overall net negative charge is imparted on some of the proteins, resulting in fewer proteins binding, i.e., higher stringency. Using a low-stringency buffer imparts an overall net positive charge on a greater number of proteins within the sample, resulting in more proteins binding, i.e., lower stringency.

Storage

Store buffer at 2–8°C.

Buffer Composition

50 mM HEPES, pH 7.0, antimicrobial preservatives, 200 ml — Amount is sufficient to run 12 ProteinChip arrays in a ProteinChip cassette-compatible bioprocessor (catalog #C50-30011), using binding and elution buffer volumes as outlined in the suggested protocol below.

Suggested Protocol

1. Assemble ProteinChip arrays in the ProteinChip bioprocessor.
2. Add 150 µl of the ProteinChip CM high-stringency buffer to each well. Shake for 5 minutes at room temperature.
3. Remove buffer from wells.
4. Repeat steps 2–3 for a total of two washes.
5. Add 90 µl of ProteinChip CM high-stringency buffer to each well.

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

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