ProteinChip®
SEND ID Array

Instruction Manual

Catalog #C57-30081

For Technical Support, contact your
local Bio-Rad office, or in the U.S.,
call 1-800-4BIORAD (1-800-424-6723).

BIO-RAD

Use

- Peptide analysis

Introduction

Fundamental to most applications of laser desorption/ionization mass spectrometry (LDI-MS) is the addition of matrix to the analyte. Matrix signals interfere in the low molecular weight (MW) range of the spectra, rendering matrix-assisted laser desorption/ionization (MALDI)-MS and surface-enhanced laser desorption/ionization (SELDI)-MS techniques problematic for peptide analysis. Surface-enhanced neat desorption (SEND) technology is unique in that the matrix is integral to the ProteinChip array surface. The chemical noise in the spectra from the matrix is significantly reduced when compared to addition of matrix on-spot (particularly in the region 600 to 1,500 Da). This allows the use of SELDI for lower MW species analysis with a reduced amount of interfering peaks in the spectra.

The ProteinChip SEND ID array has C-18 as a functional group, allowing the use of the array for on-chip cleanup, removing salt and denaturant (such as urea) prior to analysis by SELDI.

The primary application of the ProteinChip SEND ID array is peptide analysis. Successful mass determination of molecules lower than 600 Da will be determined by how well these molecules are ionized, desorbed, and detected by the mass spectrometer. If laser intensity has to be increased above a certain level to detect the molecule, the background peaks below the 600 Da range may interfere with detection of analyte peaks.

Storage and Packaging

Store ProteinChip SEND ID arrays at room temperature. They should be stored in the foil pouch in which they are supplied to limit their exposure to light.

ProteinChip SEND ID arrays are packaged in a 12-array ProteinChip cassette. A bioprocessor reservoir is included in the package to protect the arrays during shipment (see Figure 1). The spare ProteinChip cassette included to separate the reservoirs from the arrays should be removed before use in the ProteinChip cassette-compatible bioprocessor (catalog #C50-30011). As the recommended protocols are rarely done with more than a few microliters of sample, this reservoir is not needed and should be discarded.

The arrays can be used in the cassette, or individual arrays can be removed for processing. Take care to avoid touching the arrays. A pair of ProteinChip array forceps (catalog #C20-10002) helps to remove the arrays from the cassette (see Figure 2).

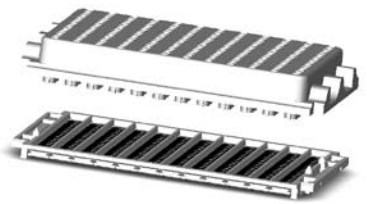


Fig. 1. ProteinChip cassette and reservoir.

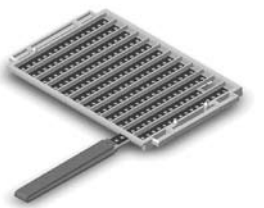


Fig. 2. Removal of ProteinChip arrays from the cassette using array forceps.

Technical Considerations

- If your sample has salt or urea contamination, proceed directly to Protocol 2. Otherwise, perform Protocol 1 first. If there is insufficient signal after reading the arrays, a cleanup step is recommended: Add 5 µl of 0.1% trifluoroacetic acid (TFA) to the spot; do not agitate; remove the 0.1% TFA droplet after 30 sec; and add 5 µl 25% acetonitrile (ACN) and 0.1% TFA to the spot. Allow to dry, and read the arrays again
- With ProteinChip SEND ID arrays, it is essential that you mix your sample with a solution of 50% ACN and 0.2% TFA at a 1:1 (v/v) ratio before adding to the spot
- Ideally, the final concentration of ACN after dilution is 25%; a final concentration of greater than 40% ACN is not compatible with the ProteinChip SEND ID array

Factors That Can Result in Weak Signal

- We do not recommend washing on-spot (by pipetting the sample up and down) because this can reduce signal
- Weak signal can be improved by adding ACN to your sample as described in Protocol 1
- A predominant peak at mass-to-charge ratio (m/z) 211 indicates sodium contamination, and you should perform sample cleanup as described in Protocol 2, step 5. This contamination can suppress sample peaks and result in weak signal

Chemical Noise

Because ProteinChip alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix is integral to the array surface, chemical noise from matrix peaks are significantly reduced with the ProteinChip SEND ID array when compared to standard MALDI or SELDI analysis. Some chemical noise will be seen; the amount seen is affected by two main factors (Figure 3):

- Increased laser intensity will increase chemical noise
- The higher the concentration of sample, the lower the intensity of chemical noise peaks

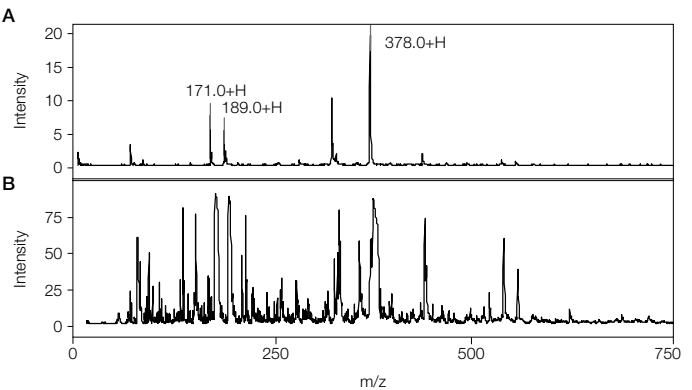


Fig. 3. Comparison of chemical noise peaks due to variation in sample concentration and laser intensity. A, minimal chemical noise is seen when concentration of sample is high and laser intensity is low (1 pmol of sample applied directly to the array); **B**, increased chemical noise is seen below 600 Da when sample concentration is decreased to 50 fmol and increased laser intensity is necessary. m/z, mass-to-charge ratio.

Protocol 1

The following method can be used to apply a sample directly to the ProteinChip SEND ID array for peptide analysis.

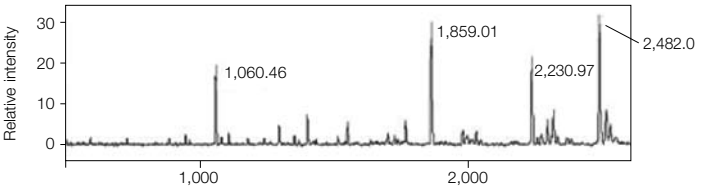
1. Mix your sample 1:1 (v/v) with 50% ACN (v/v) and 0.2% TFA in deionized (DI) water.
2. Add 1–2 µl of sample to each spot on the ProteinChip SEND ID array.
3. Allow the spots to air-dry.
4. Read the arrays in the ProteinChip SELDI reader.

Protocol 2

The following method can be used to clean up a sample on the ProteinChip array to remove salt and urea contamination (see Figure 4). The C-18 chemistry on the surface selectively retains peptides through their hydrophobic regions while impurities are washed away.

1. Mix sample with 0.2% TFA at a 1:1 (v/v) ratio.
2. Apply 5 µl of 0.1% TFA to the ProteinChip SEND ID array. Remove within 30 sec; do not agitate. Repeat.
3. Spot 2 µl of sample onto the array.
4. Incubate the samples for 10 min in a humidity chamber at room temperature. Remove the samples.
5. Add 5 µl of 0.1% TFA to each spot. Remove within 30 sec; do not agitate.
6. Add 2 µl of 25% ACN (v/v) and 0.1% TFA in DI water to each spot.
7. Allow the array to air-dry.
8. Read the array in the ProteinChip SELDI reader.

ProteinChip SEND ID array



ProteinChip NP20 array with CHCA matrix

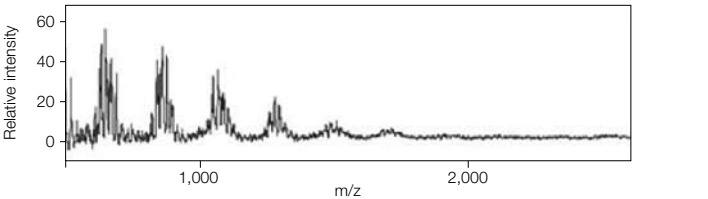


Fig. 4. Clearly visible peptide peaks with the ProteinChip SEND ID array. Upper panel, shown after on-chip cleanup of a bovine serum albumin (BSA) digest contaminated with 2 M urea; bottom panel, only matrix peaks are visible when the same sample is applied to a ProteinChip NP20 array with no cleanup and CHCA added separately.

Ordering Information

Catalog #	Description
C57-30081	**ProteinChip SEND ID Arrays**, A–H format, 12
C20-10002	**ProteinChip Array Forceps**, 1 pair

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

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