Ordering Information

Catalog #	Description
C10-00005	**ProteinChip All-in-One Peptide Standard**, lyophilized, 100
C57-30043	**ProteinChip NP20 Arrays**, A-H format, 12
C57-30028	**ProteinChip H4 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

Appendix: Adding a Calibrant Into ProteinChip Data Manager Software and ProteinChip Software

ProteinChip Data Manager Software

1. Select any spectrum to enable the **Internal calibration** button.
2. Click **Internal calibration**.
3. In the Internal Calibration dialog box, click **Calibrants...** to open the Calibrant list dialog box.
4. At the Calibrant list dialog box, you can add a new calibrant by clicking **New row**, then entering the mass (in Da) for the new calibrant.
5. You may also edit any calibrant simply by double-clicking its row and editing the information directly.
6. Click **OK** to save the changes.

ProteinChip Software

1. Select **Calibration**, then **Calibrants** from the main toolbar menu.
2. Enter the calibrant name and mass (in Da) in the two fields at the bottom of the dialog box.
3. Click **Add** to add the new calibrant to the list.

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Bio-Rad Laboratories, Inc.

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Web site *www.bio-rad.com* ***USA*** *800 4BIORAD*
Australia *61 02 9914 2800* ***Austria*** *01 877 89 01*
Belgium *09 385 55 11* ***Brazil*** *55 21 3237 9400*
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Taiwan *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

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ProteinChip® All-in-One Peptide Standard

Instruction Manual

Catalog #C10-00005

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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Introduction

The ProteinChip SELDI reader should be routinely calibrated. Calibration optimizes the performance of the instrument, enabling users to obtain more reliable results.

The ProteinChip all-in-one peptide standard is used to calibrate the ProteinChip SELDI reader in the low mass range, with 7 peptides ranging in molecular weight (MW) from 1,084.25 to 6,963.52. Enough ProteinChip all-in-one peptide standard is provided for 100 calibrations.

The ProteinChip all-in-one peptide standard is optimized for use with the ProteinChip NP20 array. The ProteinChip H4 array has also shown good results using this standard. Use of other ProteinChip arrays may result in loss of one or more peaks due to retention by the surface.

Materials

Materials Included

- 1 tube containing lyophilized ProteinChip all-in-one peptide standard

Table 1. Composition of the all-in-one peptide standard.

Peptide	Average MW
Arg^8-vasopressin	1,084.25
Somatostatin	1,637.90
Dynorphin (porcine)	2,147.50
ACTH (1–24) (human)	2,933.50
Bovine insulin β-chain	3,495.94
Human insulin	5,807.65
Hirudin, recombinant	6,963.52

Materials Needed but Not Included

- Resuspension solution, 50 µl (final concentration is 10 mM ammonium acetate, 25% acetonitrile, 1.25% trifluoroacetic acid (TFA)). Add 25 µl acetonitrile into an Eppendorf tube containing 25 µl of 5% TFA. Add 50 µl of 20 mM ammonium acetate to make 100 µl resuspension solution.

- 1% TFA, 100 µl
- ProteinChip sinapinic acid (SPA) energy absorbing molecules (EAMs) in a microcentrifuge tube, 5 mg (catalog #C30-00002), or ProteinChip alpha-cyano-4-hydroxycinnamic acid (CHCA) EAMs in a microcentrifuge tube, 5 mg (catalog #C30-00001)
- HPLC grade acetonitrile, 100 µl
- ProteinChip NP20 array

Shipping and Storage

The ProteinChip all-in-one peptide standard is usually shipped at room temperature but should be stored between –20°C and –50°C upon arrival. After reconstitution, the standard should be stored in single-use aliquots (e.g., 5–10 µl) at –20°C or lower.

Do not store in a frost-free freezer.

Protocol

Step 1: Reconstituting the ProteinChip All-In-One Peptide Standard

1. Remove the vial from the freezer and allow it to warm to room temperature, either on the benchtop or in your hands. Wipe any condensation off the outside of the vial before opening.
2. Remove the metal ring and discard.
3. Remove but do not discard the rubber septum.
4. Pipet 25 µl of resuspension solution into the bottom of the vial, being careful not to touch the bottom of the V-shape with the pipet tip.
5. Replace the rubber septum and flick mix (hold the top of the vial loosely in one hand and flick or tap the bottom of the vial with the other hand) the liquid in the vial for at least 30 seconds to redissolve the lyophilized peptides. Shake the vial down vigorously or tap the bottom several times on the benchtop to get all liquid into the bottom of the V-well.
6. Transfer this 25 µl of liquid to a microcentrifuge tube. Pipet an additional 25 µl resuspension solution into the glass vial. Repeat the mixing steps. Allow vial to stand at room temperature for 10 minutes. Add this liquid to the microcentrifuge tube.

7. Flick mix the pooled standard solutions, microcentrifuge briefly if necessary, and divide into single-use aliquots. Store remaining aliquots at –20°C or lower.

Step 2: Preparing the EAM and Peptide Standard Mix

1. Add 100 µl acetonitrile and 100 µl 1% TFA to the tube of SPA or CHCA EAMs.
2. Mix the tube vigorously in a Tomy mixer (Tomy Seiko Co., Ltd.) for at least 15 minutes to dissolve the EAMs.
3. Spin in a microcentrifuge to pellet any undissolved EAMs.
4. For CHCA only: Transfer 50 µl of solution into a new microcentrifuge tube. To this tube, add 100 µl acetonitrile and 100 µl 1% TFA. This solution is 20% saturated CHCA EAMs.
5. Pipet 10 µl of EAM solution into a microcentrifuge tube.
6. Add 10 µl of the resuspended ProteinChip all-in-one peptide standard, flick mix, and microcentrifuge briefly. The sample is now ready to be applied to a ProteinChip array.

Step 3: Applying the EAM and Peptide Standard Mix to a ProteinChip Array

1. Pipet 3 µl of water onto the spots of a new ProteinChip NP20 array (prewetting is optional).
2. Using a clean, folded laboratory wipe and working at the side of the spot, blot the water off, being careful not to touch the actual spot area.
3. Pipet 1 µl of the EAM and ProteinChip all-in-one peptide standard mix onto each spot, being careful to avoid touching the surface of the spot with the pipet tip.
4. Air-dry the ProteinChip array completely (about 10 minutes) before reading in the ProteinChip SELDI reader.

Step 4: Reading Arrays With the ProteinChip SELDI Reader

After the ProteinChip NP20 array has dried, read the array in the ProteinChip SELDI reader using the recommended spot protocol.

Note: Please refer to the ProteinChip Data Manager Software Operation Manual (or to the ProteinChip Software Operation Manual if using a ProteinChip biology system (PBS) reader) for details on spot protocol setup.

Step 5: Calibrating the ProteinChip SELDI Reader Using the ProteinChip All-in-One Peptide Standard

After reading the ProteinChip NP20 array and obtaining the average mass spectra for the peptides, refer to the ProteinChip Data Manager Software Operation Manual to calibrate the reader.

Calibrate the ProteinChip SELDI reader based on the MW for the 5 (out of 7) peptides that best correspond to the mass range to be measured. When assigning the bovine insulin β-chain peak, be careful to distinguish between this peak (3,496 Da) and the doubly charged ion of hirudin (apparent mass 3,479.48 Da), which is sometimes present. These two peaks are close enough together to appear as almost a doublet: the peak with a higher mass-to-charge ratio (m/z) is bovine insulin β-chain, and is the one to be used for calibration.

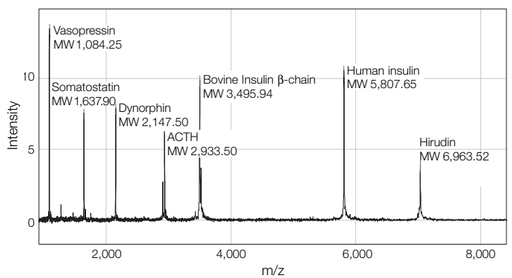

Fig. 1. Example spectra for ProteinChip all-in-one peptide standard generated on the ProteinChip biology system II (PBS II) (no longer available); laser intensity 195; sensitivity 5.