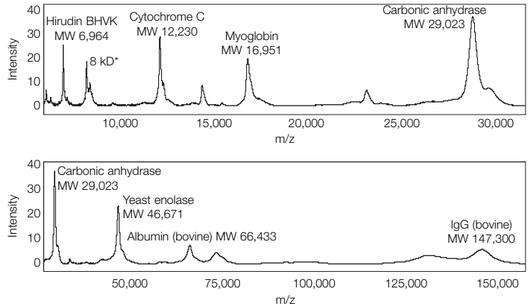

Fig. 1. Representative spectra for ProteinChip all-in-one protein standard II. Top panel, additional peak at approximately 8 kD* is a contaminant peak from carbonic anhydrase; it does not interfere with calibration using this standard.

Appendix: Addition of 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 by simply double-clicking its row, and editing the information directly.
6. Click OK to save the changes.

ProteinChip Software:

1. Click 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.

Ordering Information

Catalog #	Description
C10-00007	**ProteinChip All-in-One Protein Standard II**, lyophilized, 100
C57-30043	**ProteinChip NP20 Arrays**, A–H format, 12
C30-00002	**ProteinChip SPA Energy Absorbing Molecules (EAMs)**, 5 mg/vial, 20

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ProteinChip® All-in-One Protein Standard II

Instruction Manual

Catalog #C10-00007

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

BIO-RAD

Introduction

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

The ProteinChip all-in-one protein standard II is used to calibrate the ProteinChip SELDI reader in the low to high mass range with 7 proteins ranging in molecular weight (MW) from 6,964 to 147,300 Da. Enough protein standard is provided for at least 100 calibrations.

The ProteinChip all-in-one protein standard II is optimized for use with ProteinChip NP20 arrays (catalog #C57-30043).

Materials

Materials Included

- 1 tube containing lyophilized ProteinChip all-in-one protein standard II (lyophilized from an original volume of 25 µl)

Table 1. Composition of the ProteinChip all-in-one protein standard II.

Protein	MW, Da
Hirudin, recombinant	6,964
Cytochrome C (bovine)	12,230
Myoglobin (equine)	16,951
Carbonic anhydrase (bovine red blood cells (RBC))	29,023
Enolase (*S. cerevisiae*)	46,671
Albumin (bovine)	66,433
IgG (bovine)	147,300

Materials Needed But Not Included

- 25 µl of 20 mM ammonium acetate (add 0.0154 g of ammonium acetate to 10 ml of deionized (DI) water and mix thoroughly)
- 100 µl 1% trifluoroacetic acid (TFA)
- 5 mg ProteinChip sinapinic acid (SPA) energy absorbing molecules (EAMs) in a microcentrifuge tube (catalog #C30-00002)
- 100 µl HPLC grade acetonitrile
- ProteinChip NP20 array

Storage

The ProteinChip all-in-one protein standard II should be stored between –20°C and –50°C upon arrival. The protein standard is provided lyophilized; after reconstitution the product is stable for 3 months at –20°C. Avoid freeze-thaw cycles by aliquoting reconstituted standards into smaller quantities.

Detailed Use Protocol

Step 1: Reconstituting the ProteinChip All-in-One Protein Standard II

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. Pipet 10 µl of 20 mM ammonium acetate and add to the bottom of the vial, being careful not to touch the bottom with the pipet tip.
3. Replace the cap and flick mix (hold the top of the tube 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 proteins.
4. Briefly centrifuge the tube to ensure that all the liquid is at the bottom of the tube.
5. Transfer the 10 µl volume to an Eppendorf tube.
6. Add another 15 µl of 20 mM ammonium acetate to the original glass vial. Allow to sit for 2 minutes at room temperature.
7. Flick mix the liquid and combine with the 10 µl in the Eppendorf tube.
8. You should have a final volume of 25 µl in the Eppendorf tube.
9. Pipet this mix into appropriate size aliquots and store at –20°C for future use.

Step 2: Preparing the EAM and Protein Standard Mix

1. Add 100 µl acetonitrile and 100 µl 1% TFA to a tube of ProteinChip SPA EAMs.
2. Mix the tube vigorously for at least 15 minutes to dissolve the EAMs.
3. Spin in a microcentrifuge to pellet any undissolved EAMs.
4. Pipet 4 µl of EAM solution into a tube; add 1 µl of the ProteinChip all-in-one protein standard II.
5. Mix the protein standard and EAM mixture with a pipet by drawing the mixture in and out a few times.

Step 3: Applying the Protein Standard and SPA EAMs to a ProteinChip Array

1. Pipet 3 µl of DI water onto the spots of a new ProteinChip NP20 array.
2. Using a clean, folded laboratory wipe, working at the side of the spot, blot the DI water off, being careful not to touch the actual spot area.
3. Pipet 1 µl of the protein standard and EAM mixture on to each prewetted spot, being careful to avoid touching the surface of the spot with the pipet tip.
4. Air-dry the ProteinChip array completely (about 15 minutes).

Step 4: Reading Arrays With the ProteinChip SELDI System

After the ProteinChip array has dried, read the array in the ProteinChip SELDI reader.

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 Protein Standard II

After reading the ProteinChip array and obtaining the average mass spectra for the proteins, refer to the ProteinChip SELDI System Applications Guide, Volume I (or to the ProteinChip Software Operation Manual if using a PBS reader) for recommendations on how to calibrate the reader. Calibrate the ProteinChip SELDI reader based on the MW for the proteins that best correspond to the mass range to be measured.

It is recommended that for best calibration results, the mass range be split up into high and low mass. The recommended low mass range should be 6–29 kD, and the high mass range to be 29–150 kD (see Figure 1).