ProteinChip® Peptide Mass Calibration Kit

Catalog #C70-00070

General Recommendations

- Store the array in the original packaging and do not open the packaging until ready to use
- Prepared arrays can be used multiple times, though the peak features will degrade over time. After opening, store arrays in a dry and dark location and their original packaging. Record the usage dates
- The ProteinChip premade array supplied with this kit has been prepared with a mixture of peptides and matrix. Avoid exposing these arrays to vibration, moisture, or light
- ProteinChip array spots contain chemically treated surfaces. Avoid contamination of these surfaces: always handle ProteinChip arrays by their edges, and never touch the spots
- When running single ProteinChip arrays on the ProteinChip instrument, Enterprise Edition, always fill empty locations in the cassette with blank, expired, or used arrays.
- To ensure best results, evaluate the performance of the ProteinChip system using the ProteinChip system check kit (catalog #C70-00081) or ProteinChip OQ kit (catalog #C70-00080), or as included in service contracts

Introduction

Mass calibration is the process by which the time-of flight data obtained by mass spectrometry are converted to molecular mass data. Data collected using standards (known peptides or proteins) are used to derive a calibration equation, which is then applied to the spectra of experimental samples. In the following protocol, you use the spectra obtained from the ProteinChip® peptide standard array to perform the mass calibration procedure. The procedures for importing protocols and performing mass calibration are illustrated in a tutorial video included on the ProteinChip Peptide Mass Calibraiton Kit CD. The tutorial video is also available online at www.bio-rad.com/proteinchip/starterkit.

Import the Pep Std Array Protocol

The ProteinChip reader uses data collection protocols to collect data from arrays. The protocol used with this kit is provided with the CD. Import the protocol into the Protocols folder of the ProteinChip data manager software.

1. Ensure that the ProteinChip reader is turned on and connected to the computer. (This is usually done during installation.)
2. Insert the ProteinChip Peptide Mass Calibration Kit CD and launch the ProteinChip data manager software.
3. In the Explorer pane, select the **Protocols** folder. In the toolbar, select **Protocols>Import Protocol**. The **Choose File(s) to Import** dialog opens.
4. Select the file named **Pep Std Array.ptx** and click import. Click **OK** at the prompt.

Read the Peptide Standard Array

1. Insert the ProteinChip peptide standard array into the instrument. If you are using an Enterprise Edition instrument, place the array into a cassette, fill the remaining slots in the cassette with blank or used arrays, and place the cassette into the instrument.
2. In ProteinChip data manager software, click **Start** and open the **Protocol Mode** tab.
3. Select **Instrument > Preferences**. In the **Preferences** dialog, click "…". The **Select Folder** dialog opens.
4. Select **New Folder**, name the new folder **Peptide Mass Calibration** and click **OK**.
5. Optimize the laser intensity. Run protocol **Pep Std Array** on any partition ("n") of 10 on spot A. Adjust the laser energy until the height of the insulin peak at 5.96 kD is 100–200 µA.
6. Run protocol **Pep Std Array**. Run on partition 1 of 4 on spot A of chip. Record usage on the label provided on the package. Each spot on the array may be read up to 5 times on each of 4 partitions (1 of 4, 2 of 4, 3 of 4 and 4 of 4).

Perform Mass Calibration

1. Open the spectrum obtained using the peptide standard array, and label the 4 peaks indicated in the table below.

    Note: Label each peak at its highest point; if not, the mass will be recorded incorrectly, reducing the accuracy of the calibration.

    Mass standards included in the ProteinChip peptide standard array. Use the four peaks marked "yes" in the table below.

Label Peak for Calibration	Peak	Name	Molecular Mass (Da)
Yes	1	Arg8-vasopressin	1,084.25
No	2	Somatostatin	1,637.90
Yes	3	Dynorphin A	2,147.50
No	4	ACTH	2,933.50
Yes	5	Beta endorphin	3,465.00
No	6	Arg-insulin	5,963.80
Yes	7	Cytochrome C	12,230.92

2. From the toolbar, select **Spectra > Calibrate > Internal Calibration** (or click the **Internal Calibration** icon). The I**nternal Calibration** dialog opens. The spectrum appears in the bottom half of the window with existing peak labels appearing in red. A table of calibrants and peaks appears in the top half of the window; the calibrant names are blank.

3. Click the **Match Calibrants to Peak** icon . Confirm that the peaks are labeled correctly by comparing the peak names to those in the table.

4. Select 3 parameters from the **Calibration type** drop-down list and select the **Weighted** check box. Click the **Calibrate** icon . The labels now appear in the spectra.

5. Open the **Calibration equation** tab. The residual plot displays the error for each calibrant in parts per million (ppm).

6. Select 300 from the **Scale (ppm)** drop-down list. If the error of a calibrant is greater than ±300 ppm, double-check the peak label or name to ensure proper labeling. Close the window.

7. Click the **Toggle Spectrum Table** button to view all the spectrum files within your folder. Note that the **Calibration type** icon has changed to green , indicating that the spectrum has been calibrated.

Save the Calibration Equation

1. Select a calibrated spectrum (green icon) from within your **Pep Std Array** project folder.

2. Save the equation by selecting **Spectra > Calibrate > Save Calibration Equation**. The **Save Calibration Equation** dialog opens.

3. Enter a name for the calibration equation. Include the date of creation in the name. Click **OK**.

    Note: A calibration equation can be used to externally calibrate other spectra. For best results, use the calibration equation within 1 day for mass ranges between 1 kD and 25 kD. For more information on external calibration, refer to the ProteinChip Data Manager Software Manual.

Ordering Information

C70-00080   **ProteinChip OQ Kit**, includes 2 detector calibration arrays, 6 detector qualification arrays, 2 peptide standard arrays, CD with protocols, instructions

C70-00081   **ProteinChip System Check Kit**, includes 1 detector calibration array, 1 detector qualification array, 1 peptide standard array, CD with protocols, instruction manual

C50-30013   **ProteinChip Cassettes**, empty, hold 12 ProteinChip arrays, 5

C10-00001   **ProteinChip Protein Calibrant Kit**, includes protein MW standards (2 sets of 10 standards)

C10-00002   **ProteinChip Peptide Calibrant Kit**, includes peptide MW standards (2 sets of 7 standards)

C10-0006   **ProteinChip Human Serum Control**, 500 µl

C10-0005   **ProteinChip All-In-One Peptide Standard**, lyophilized, 100 spots

C10-0007   **ProteinChip All-In-One Protein Standard II**, lyophilized, 100 spots

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