ProteinChip® OQ Kit

Instruction Manual and Documentation

Catalog #C70-00080

For use with the ProteinChip SELDI system, Personal or Enterprise Edition, with embedded system processor (ESP) version 1.1.15 or higher

BIO-RAD

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

Notices

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Chapter 1
Introduction

Qualification of analytical instruments is a formal process of documenting that an instrument is fit for its intended use and that it is maintained and calibrated.

- Installation qualification (IQ) checks that the correct system or instrument was received and that it was properly installed. IQ is performed by a Bio-Rad service engineer following installation of the ProteinChip® SELDI system. The engineer will provide an IQ certificate upon completion (see Appendix A for an example of this certificate. If you do not have a copy of this certificate, contact technical support). IQ should also be performed by a service engineer when the ProteinChip SELDI system is moved to a new location and when the software is upgraded.
- Operational qualification (OQ) demonstrates that the ProteinChip SELDI system operates in accordance with Bio-Rad's requirements. The ProteinChip SELDI OQ kit is used to qualify the installation and operation of the ProteinChip SELDI system, Personal or Enterprise Edition. Bio-Rad recommends that the OQ protocols be performed in total upon installation (following IQ), on a regular basis to confirm that the system is performing to specifications, and whenever it is suspected that the instrument is not performing to specifications.

For audit review, maintain records of IQ and OQ of the ProteinChip SELDI system in a single notebook or folder.

1.1 Product and Protocol Description

The ProteinChip OQ kit contains all the ProteinChip arrays and instrument protocols required to perform OQ on a ProteinChip SELDI system, Personal or Enterprise Edition. The kit is designed to qualify the operating specifications of the ProteinChip instrument and is a valuable tool that allows discrimination between assay and instrumentation problems.

The components of each ProteinChip OQ kit support maintenance and testing for a three month period. Each kit contains the following:

1 CD containing the OQ instrument protocols and the ProteinChip SELDI OQ form

2 ProteinChip detector calibration arrays

6 ProteinChip detector qualification arrays

2 ProteinChip peptide standard arrays

1 ProteinChip OQ kit instruction manual

The OQ protocol involves two maintenance procedures (detector calibration and high voltage conditioning) followed by a series of four tests of instrument resolution, mass accuracy and drift, and sensitivity (Table 1).

Completion of each maintenance procedure and test is tracked using the ProteinChip SELDI OQ form, a Microsoft Excel spreadsheet supplied on the CD. Test data are exported to the ProteinChip SELDI OQ form, which then calculates whether the test passed or failed.

Upon completion of the OQ procedure, save, print, sign, date, and place copies of the following documentation into a notebook or folder for audit review:

- Protocol acceptance page (Appendix B)
- ProteinChip SELDI OQ form summary

Table 1. Summary of procedures and tests.

	Title	Recommended Frequency	Consumable Used	Estimated Time Required	Description
Initial Setup	File and protocol setup			20 min	Complete this procedure the first time you use the ProteinChip OQ kit. This procedure creates the file structure necessary for storing the protocols and creates the routines for high voltage conditioning and mass calibration.
Maintenance Procedure 1	High voltage (HV) conditioning	Weekly	N/A	1–2 hr depending on instrument	HV conditioning helps to decontaminate the surfaces in the instrument.
Maintenance Procedure 2	Detector calibration	Weekly	ProteinChip detector calibration array	45 min to 4 hr depending on instrument	This procedure uses the ProteinChip detector calibration array to adjust the detector voltage. These adjustments are based on a rolling average that stabilizes the gain, improving spectral reproducibility over the lifetime of the detector.
Test 1	Detector sensitivity	Biweekly	ProteinChip detector qualification array	30 min to 1 hr	This test uses the ProteinChip detector calibration array to measure the signal-to-noise ratio (S/N) of immunoglobubulin (IgG) at two different concentrations (10 fmol and 140 fmol). Measurements are compared to a specification, and a pass/fail disposition is obtained.

Table 1. Summary of procedures and tests (*continued*).

	Test	Recommended Frequency	Consumable Used	Estimated Time Required	Description
Test 2	Mass drift and resolution at 5.96 kD	Weekly	ProteinChip peptide standard array	20 min to 1 hr	This test uses the ProteinChip peptide standard array to measure the mass drift and resolution of insulin. These measurements are compared to a specification, and a pass/fail disposition is obtained.
Test 3	Resolution at 1 kD	Weekly	ProteinChip peptide standard array	20 min to 1 hr	This test uses the ProteinChip peptide standard array to measure the resolution of Arg-vasopressin. These measurements are compared to a specification, and a pass/fail disposition is obtained. The test is run in higher-resolution (lower source voltage) mode.
Test 4	Mass accuracy	Weekly	ProteinChip peptide standard array	30 min to 1 hr	This test evaluates the mass accuracy when compared to internal and external calibrations. These measurements are compared to a specification, and a pass/fail disposition is obtained.

1.2 Storage and Handling

Store the arrays supplied in the kit in the original packaging at room temperature and in a dry and dark location. Each array is individually wrapped. Do not open the array packaging until ready to use. Take care when handling the ProteinChip arrays to avoid contaminating or disturbing the matrix crystals on the surface of the arrays.

Chapter 2
Use of the ProteinChip OQ Kit

2.1 Overview

This chapter outlines the protocols used to perform the procedures and tests that comprise the ProteinChip OQ kit. Please note the following:

- Export data acquired during testing to the ProteinChip SELDI OQ form using the export instructions described in Appendix C
- Protocol transfer from the CD to ProteinChip data manager software needs only to occur once; however, new files must be created each time a test is run
- Neither spreadsheets nor protocols are write-protected. Overwriting may result in erroneous results and calculations
- Always perform the two maintenance procedures before running any of the tests
- Maintenance procedure 2 (detector calibration) is designed to standardize and stabilize detector performance over time, by resetting any drift in the response due to natural component aging. First or delayed use of this procedure may alter system response after calibration. Do not run this procedure within a series of related experiments if it has been more than two weeks since the last detector calibration
- All tests require peak measurements that are compared to specification. Incorrect peak selection may lead to erroneous results. While selecting peaks for measurement, zoom in on them so that they resemble the peaks in the examples provided
- When running single ProteinChip arrays on the ProteinChip instrument, Enterprise Edition, always fill empty locations in the cassette with blank, expired, or used arrays. Blank arrays are available as components of the ProteinChip cassette-compatible bioprocessor (catalog #C50-30011)
- The ProteinChip detector calibration array and ProteinChip peptide standard array can be used multiple times. After opening, store these arrays in a dry and dark place and in their original packaging
- When running protocols, you specify how many sections (partitions) the spot is divided into for sampling. You also specify which of these partitions should be used. For spectra intended for analysis, no more than four ("of 4") partitions should be used, with 10 shots/pixel

2.2 File and Protocol Setup

Complete this procedure the first time you run the ProteinChip OQ kit. This procedure sets up the file structure necessary for storing the protocols and creates the routines for high voltage conditioning (procedure 1) and mass accuracy (test 4).

	Task
Step 1 **Import Protocols From CD**	Import the protocols supplied on the CD into the **Protocols** folder of ProteinChip data manager software: 1. Insert the CD into the computer and launch ProteinChip data manager software. 2. Select **Protocols > Protocols > Import Protocols**. 3. Use the **Import Protocols** dialog to import all protocols (files with a .ptx extension) from the CD to the **Protocols** folder. **Note**: The protocols are NOT write-protected. Do not alter them.
Step 2 **Create the File Structure** **Note**: Create new folders each time the OQ tests are run. The instructions in this manual assume you are following the convention described here. However, any file structure and naming convention may be used.	Create a new folder for each test (1–4) to be run: 1. In the Explorer pane of ProteinChip data manager software, open the **Projects** folder and select **File > New > Folder**. 2. Create a folder, *OQ_####,* where *####* is the serial number of the instrument. 3. Click on this folder and select **File > New > Folder**. Create a separate folder for the date of the test. 4. Click on the folder created in step 3 and select **File > New > Folder**. Create a separate folder for each of the following tests: • Test 1 Detector sensitivity • Test 2 Mass drift and resolution 5.96 kD • Test 3 Resolution 1 kD • Test 4 Mass accuracy

Step 3

Set Up the High Voltage (HV) Conditioning Routine

1. Insert a blank ProteinChip array into the reader. If blank arrays are not available, use expired arrays or arrays that have already been used.

    a. For the Enterprise Edition instrument: place a cassette filled with 12 blank or used arrays into the instrument.

    b. For the Personal Edition instrument: insert a blank ProteinChip array.

2. In ProteinChip data manager software, select the instrument and click **Start**.

3. In the **Protocol Mode** tab, select **Instrument > Quick Run**.

4. Select all 8 spots, then set up a Quick Run protocol as shown below:

    a. For the Enterprise Edition instrument:

    RC3 HV pos 15 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 20 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 25 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 30 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV neg 15 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV neg 20 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV neg 25 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV neg 30 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 15 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 20 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 25 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 30 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 25 kV conditioning, partition 1 of 8, all 8 spots

    b. For the Personal Edition instrument:

    RC3 HV pos 15 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 20 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 25 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 30 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 15 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 20 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 25 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 30 kV conditioning, partition 1 of 8, all 8 spots
    RC3 HV pos 25 kV conditioning, partition 1 of 8, all 8 spots

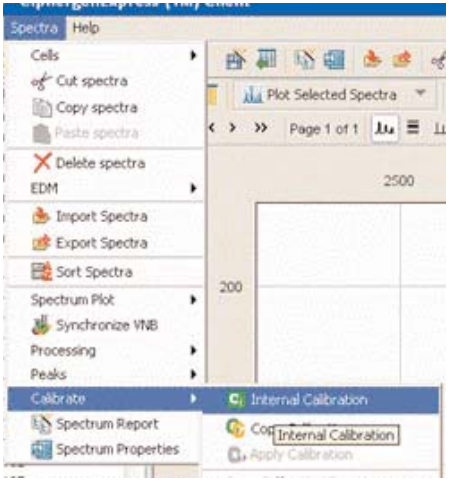
5. Click **Save** in the Quick Run dialog, and save the protocol as ***SoftHV*** in the Quick Run directory (C:\\XXXXXXX). The Quick Run directory location is defined by the user. The default location, "My Documents", will work as well as any other location that is easy to remember.

6. In the Explorer pane of ProteinChip data manager software, select **Projects** and click on any spectrum file (for example, the spectrum obtained during installation).

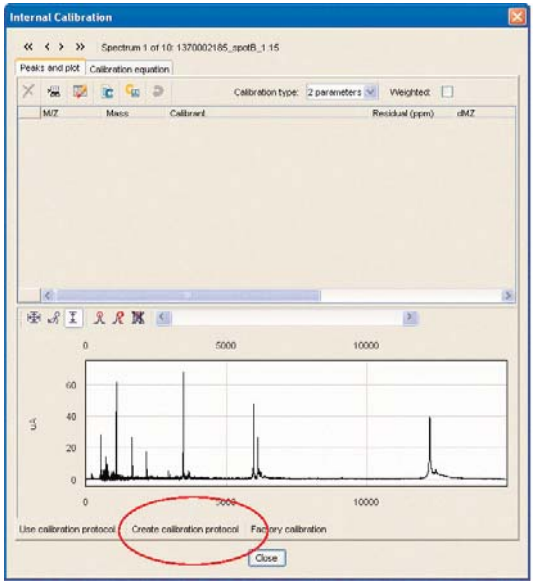
Step 4

Create a Standard Mass Calibration Routine

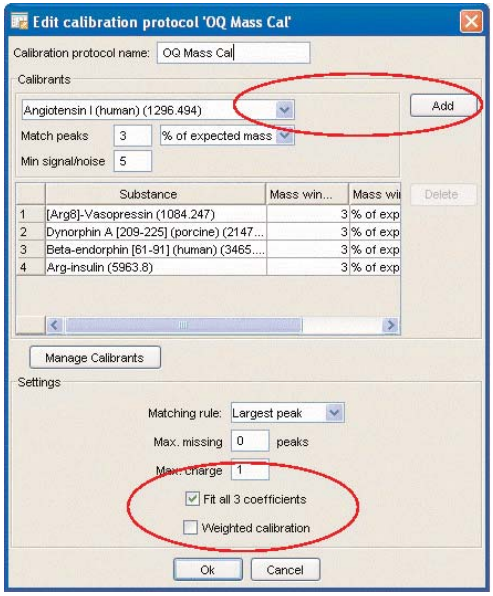
1. Select **Spectra > Calibrate > Internal Calibration.**



2. Select **Create calibration protocol**.



3. In the **Calibration protocol name** field, enter ***OQ Mass Cal.***



4. Select [Arg8]-Vasopressin (1084.247) from the drop-down list, and click **Add** (keep the default values of 3% of expected peaks and minimum signal/noise of 5).
5. Select Dynorphin A (2147.5) from the drop-down list, and click **Add** (keep the default value of 3% of expected peaks and signal/noise of 5).
6. Select Beta-endorphin (3465.0) from the drop-down list, and click **Add** (keep the default value at 3% of expected peaks and signal/noise of 5).
7. Select Arg-insulin (5963.8) from the drop-down list, and click **Add** (keep the default value at 3% of expected peaks and signal/noise of 5).
8. Select **Fit all 3 coefficients**. Click **OK**.

2.3 OQ Maintenance Procedures

2.3.1 Maintenance Procedure 1 — High Voltage (HV) Conditioning

This procedure helps to decontaminate the surfaces of the ProteinChip instrument and is essential for obtaining correct test results. Run this procedure weekly using any blank ProteinChip array (or an array that has already been used). The procedure takes 1–2 hr to complete. Therefore, to save time, we recommend running this protocol at the end of the day prior to running the remaining tests. There is no need to stay with the instrument while the protocol is running.

1. Launch ProteinChip data manager software, select the instrument, and click **Start**.
2. Insert a blank ProteinChip array into the instrument. If blank arrays are not available, use an expired or used ProteinChip array.

   Note: If using an Enterprise Edition instrument, place a cassette filled with used ProteinChip arrays into the instrument. Blank arrays maybe used as long as at least one array in the cassette has a bar code.
3. Open the **Protocol Mode** tab and select **Instrument > Quick Run**.
4. The **Quick Run Protocol(s)** dialog opens. Select the ***SoftHV*** protocol you saved during file and protocol setup, step 3 and click **Run**.
5. After completion of the protocol, an “Acquisition Error” message appears. This is expected. Click **OK**.
6. Record the operator name and date in the ProteinChip SELDI OQ form.

2.3.2 Maintenance Procedure 2 — Detector Calibration

This procedure uses the ProteinChip detector calibration array to adjust the detector voltage. These adjustments are based on a rolling average that stabilizes the gain, improving spectral reproducibility over the lifetime of the detector. This procedure is essential for obtaining correct test results and, when run on a weekly basis, is designed to improve reproducibility as the detector ages.

Each run uses a single spot on the array, and each spot can only be used once. Store the array in its original packaging and in a dry, dark location. Record usage data directly on the packaging.

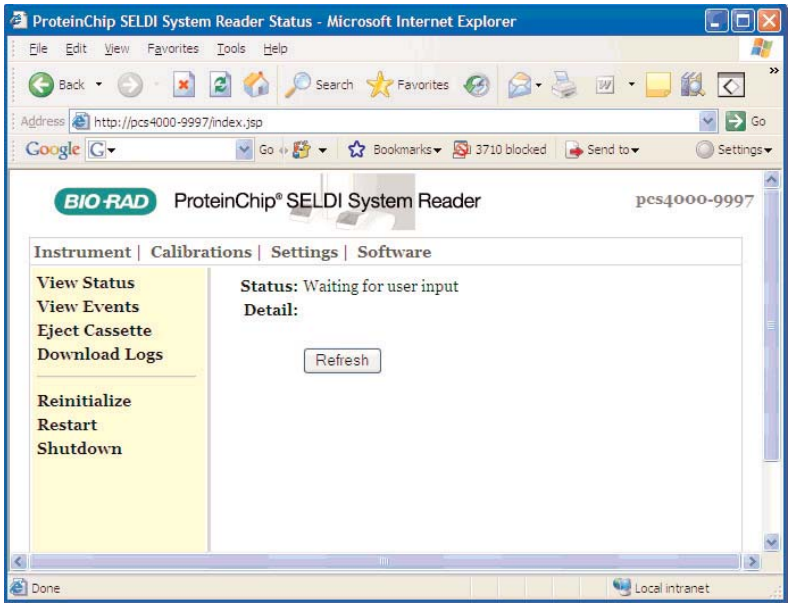
The procedure requires 45 min to 4 hr to complete, depending on the state of the instrument.

Note: The detector calibration procedure is intended to standardize instrument performance over time. First-time use may alter system response and is not recommended within a series of experiments. It is, however, possible to manually set the voltage back to its original state through the instrument’s web page.

1. Insert the ProteinChip detector calibration array into the instrument.

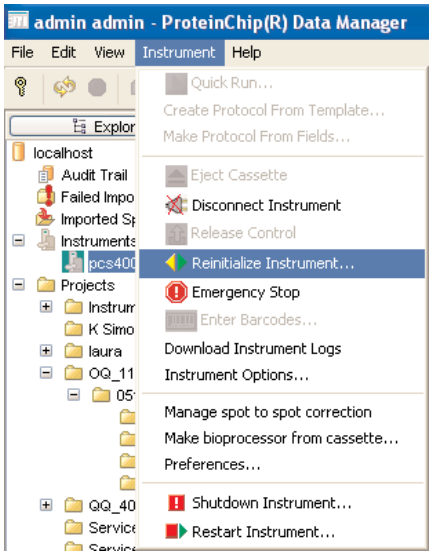
Note: For the Enterprise Edition instrument, place the array into slot 1 of a cassette and fill the rest of the slots with blank or used ProteinChip arrays.

2. Open the instrument's interactive web page (http://pcs4000-####/index.jsp, where #### is the instrument serial number). If the instrument is under local control, the web page will only be on the local computer. If it is installed on an intranet, it will be available on all intranet computers.



3. Select **Calibrations > Automatic Detector Gain**. The **Detector Gain** page opens. Check that **Automatic and NOT manual** is selected.
4. If using the Enterprise Edition instrument, select **Array number 1** (slot 1 in cassette).
5. If this is the first calibration performed on the instrument, or if the last calibration was performed more than two weeks ago, select two previously unused spots. If this is a routine, weekly calibration, select one unused spot.
6. Click **Start**.

7. The time required to complete this procedure is variable, and it may require up to several hours as the instrument continually collects data of a specific intensity. If the calibration routine does not complete, run the procedure again with unused spots.
8. Once the procedure is complete, open ProteinChip data manager software, select the instrument, and select **Instrument > Reinitialize Instrument**.



9. The maintenance procedure is complete. Record the operator name and date in the ProteinChip SELDI OQ form.

2.4 OQ Tests

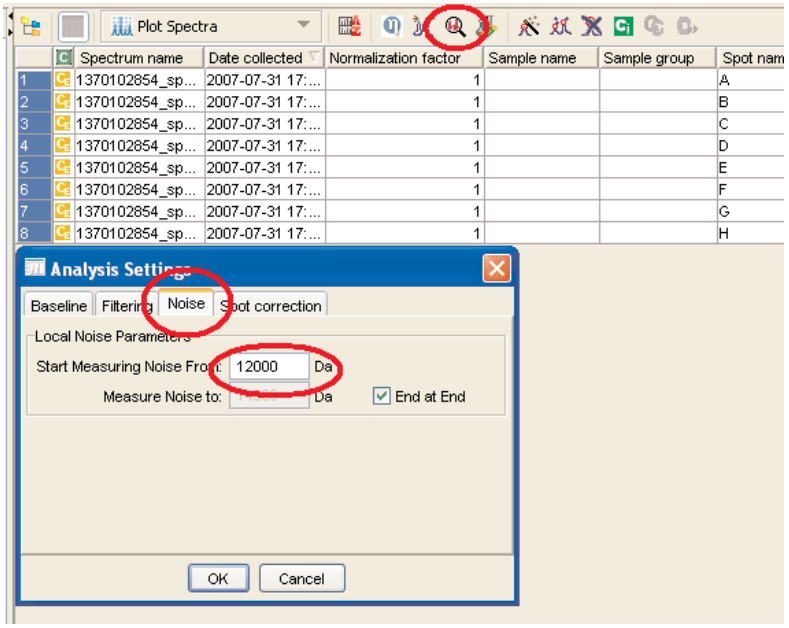
2.4.1 Test 1 — Detector Sensitivity

This test uses the ProteinChip detector qualification array to measure the signal-to-noise ratio (S/N) of immunoglobubulin (IgG) at two different concentrations (10 fmol and 140 fmol). Measurements are compared to a specification, and a pass/fail disposition is obtained.

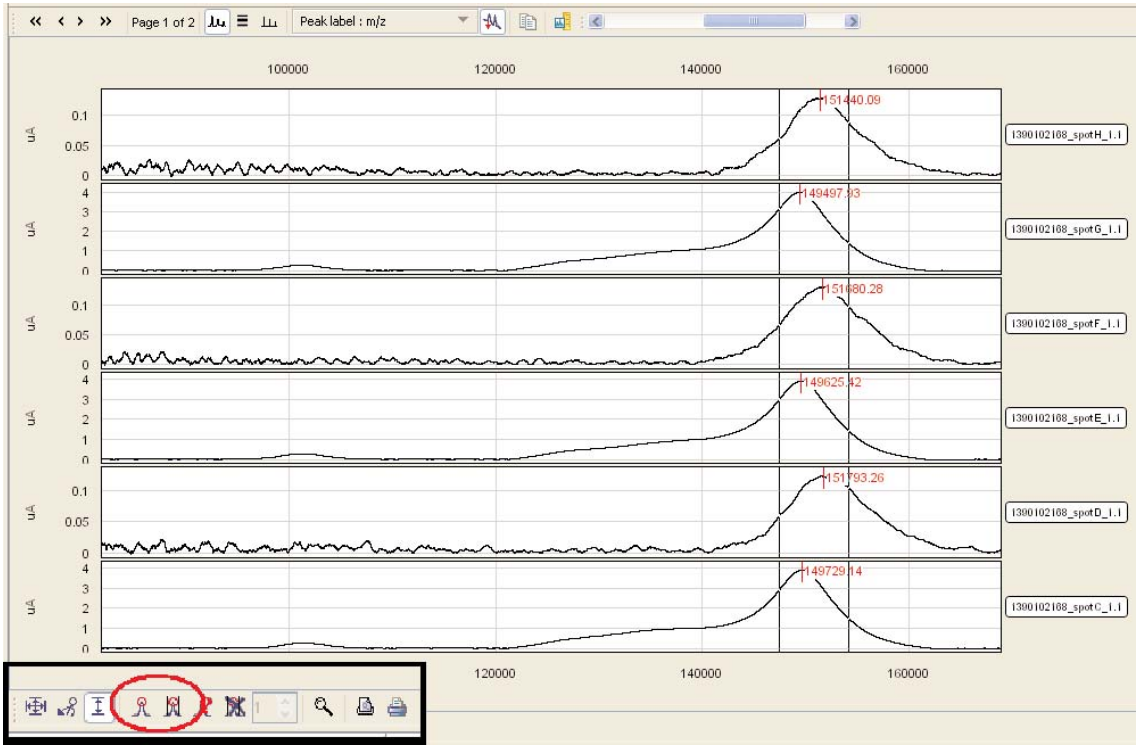
Array Type	ProteinChip detector qualification array
Usage Information	Use once and discard; do not open until ready to use.
Recommended Testing Frequency	Every other week
Expected Testing Time	30 min to 1 hr
Pass/Fail Specification	High concentration: pass if S/N is greater than 1,100 Low concentration: pass if S/N is greater than 5

1. Insert the ProteinChip detector qualification array into the instrument. If you are using an Enterprise Edition instrument, place the array into any location within the 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 **Projects > OQ_####/*Date*/Test 1 Detector Sensitivity**, where #### is the serial number of the instrument.
5. Run protocol "Test 1 Detector Sensitivity " on partition 1 of 1 for all 8 spots on the array. **Do not change any values**.

6. Plot the peaks with noise set to 12,000 Da:
   a. Select all 8 spectra.
   b. Click on the data analysis icon  . The **Analysis Settings** dialog opens.
   c. Open the **Noise** tab and set **Start Measuring Noise From** to 12,000 Da. Click **OK**.



7. Plot all 8 spectra and select the IgG peak using the peak selection tool.



Note: Spots with high concentrations (spots A, C, E, and G) alternate with those with low concentrations (spots B, D, F, and H). Due to the different concentrations on different spots, it is critical to export the peak data with the spots in alphabetical order from A to H.

8. Export the data following the instructions in Appendix C and selecting parameters for export in the following order:
   - Array bar code
   - Spot name
   - Substance mass
   - Intensity
   - Resolution
   - S/N
9. Paste the data into the ProteinChip SELDI OQ form, data sheet “Test 1 Detector Sensitivity”.
10. The spreadsheet indicates if the test passed or failed.
11. If test 1 fails, run the detector calibration procedure (maintenance procedure), and repeat the test. If the test fails a second time, contact technical support.

2.4.2 Test 2 — Mass Drift and Resolution 5.96 kD

This test uses the ProteinChip peptide standard array to measure the mass drift and resolution of insulin. These measurements are compared to a specification, and a pass/fail disposition is obtained.

Array Type	ProteinChip peptide standard array
Usage Information	Each test uses 1 partition on all 8 spots on the array. The array can be used up to 20 times (5 times on each of 4 partitions). Store the array in the original packaging and record usage information in the usage chart provided on the packaging. Laser optimization steps use 1 of 15 partitions, and these reads do not need to be recorded or monitored.
Recommended Testing Frequency	Weekly
Expected Testing Time	20 min to 1 hr
Pass/Fail Specification	Mass drift: pass if less than 7 Da Resolution: pass if average is greater than 750

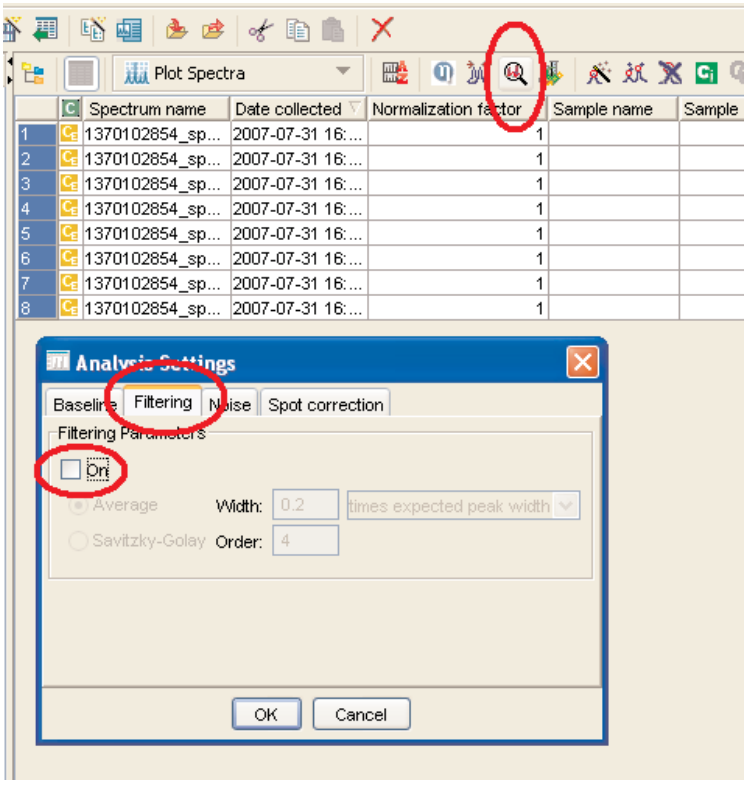
1. Insert the ProteinChip peptide standard array into the instrument. If you are using an Enterprise Edition instrument, place the array into a cassette, fill the remaining slots in the cassette with blank or used arrays, and place the cassette into the instrument.
2. In ProteinChip data manager software, click **Start** and open the **Protocol Mode** tab.
3. Select **Instrument > Preferences**. In the **Preferences** dialog, click "…". The **Select Folder** dialog opens.
4. Select **Projects > OQ_####/*Date*/Test 2 Mass Drift and Resolution 5.96 kD**, where #### is the serial number of the instrument.
5. Optimize the laser intensity. Run protocol "Test 2 Mass Drift and Resolution 5.96 kD" on any partition ("n") of 15 on spot D (for example, 1 of 15, 2 of 15, 3 of 15, etc). Adjust the laser energy until the height of the insulin peak at 5,963 Da is 100–200 µA. This optimization step does not need to be repeated on subsequent runs of test 2 unless the peak intensity falls too low.

6. Run the same protocol on partition “n” of 4 for all 8 spots. Record the partition number on the usage chart provided with the product packaging.

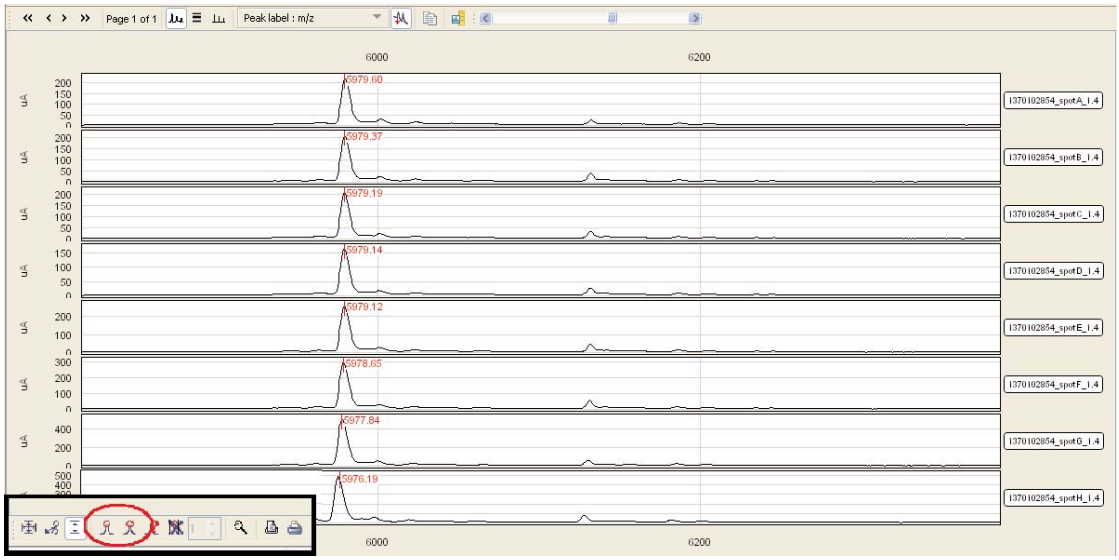
Partition #	Reads	Initial
1 of 4	1	
	2	
	3	
	4	
	5	
2 of 4	1	
	2	
	3	
	4	
	5	
3 of 4	1	
	2	
	3	
	4	
	5	
4 of 4	1	
	2	
	3	
	4	
	5	

7. Make sure that a peak intensity of 50–500 µA is visible on each spot. If peak intensities appear out of this range, change the laser energy and run the test again.

8. Plot the peaks with the filtering option turned off:
    a. Select all 8 spectra. (Do not select the first laser optimization spectrum.)
    b. Click the analysis settings icon. The **Analysis Settings** dialog opens.
    c. Open the **Filtering** tab and deselect the **On** checkbox.
    d. Click **OK**.



9. Plot all 8 spectra. Using the peak selection tool, select the insulin peak (5.963 kD) in all spectra.



10. Export the data following the instructions in Appendix C and selecting the parameters for export in the following order:

    - Array bar code
    - Spot name
    - Substance mass
    - Intensity
    - Resolution
    - S/N

11. Paste the data into the ProteinChip SELDI OQ form, data sheet “Test 2 Mass Drift and Resolution”.

12. The spreadsheet indicates whether the test passed or failed.

13. If the test fails, run the HV conditioning procedure (maintenance procedure 1) and repeat the test. Contact technical support if the test fails a second time.

2.4.3 Test 3 — Resolution at 1 kD

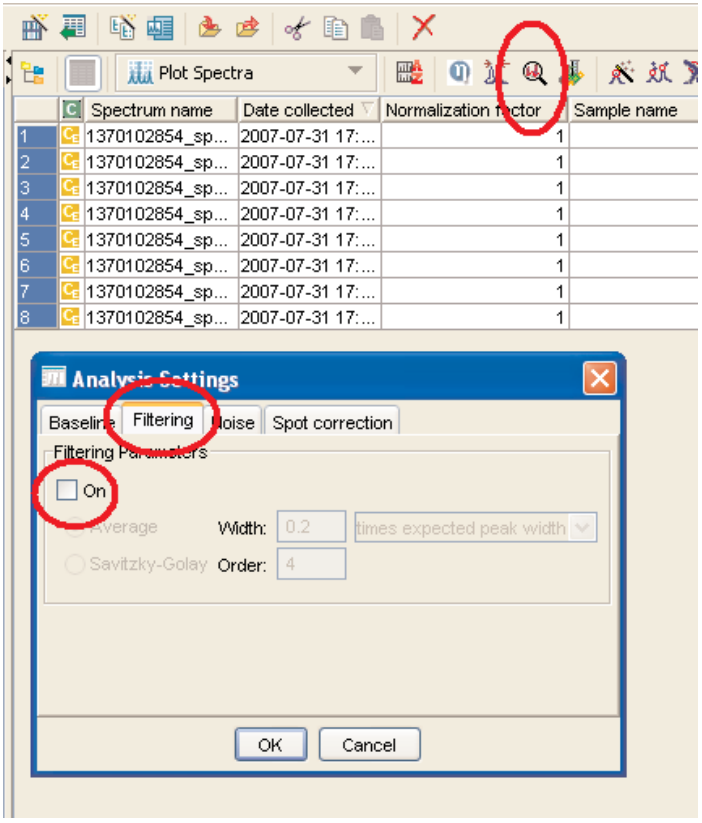
This test uses the ProteinChip peptide standard array to measure the resolution of Arg-8-vasopressin. To do this, a higher-resolution, lower source mode (15 kV) is employed rather than the default high-sensitivity source mode (voltage 25 kV).These measurements are compared to a specification, and a pass/fail disposition is obtained.

Caution: Spectra taken using one source mode should not be compared either qualitatively or quantitatively to spectra taken using other source modes. Spectra are only comparable within modes.

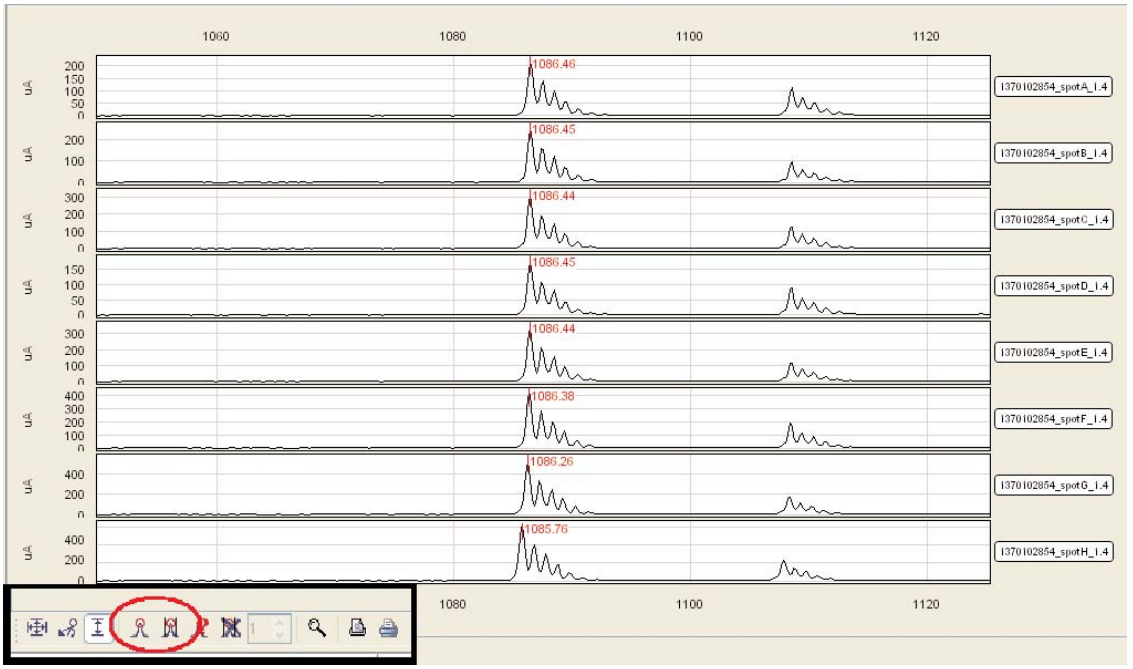
Array Type	ProteinChip peptide standard array
Usage Information	Each test uses 1 partition on all 8 spots on the array. The array can be used up to 20 times (5 times on each of 4 partitions). Store the array in the original packaging and record usage information in the usage chart provided and on the packaging. Laser optimization steps use 1 of 15 partitions, and these reads do not need to be recorded or monitored.
Recommended Testing Frequency	Weekly
Expected Testing Time	30 min to 1 hr
Pass/Fail Specification	Pass if resolution is greater than 1000

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 **Projects > OQ_####/*Date*/Test 3 Resolution 1 kD**, where #### is the serial number of the instrument.
5. Optimize the laser intensity. Run protocol "Test 3 Resolution 1 kD" on any partition ("n") of 15 on spot D. Adjust the laser energy until the height of the insulin peak at 5.96 kD is 100–200 µA. This optimization step does not need to be repeated on subsequent runs of test 3 unless the peak intensity falls too low.
6. Run the same protocol on partition "n" of 15 on spot D. Change the laser energy until the height of the Arg-8-vasopressin peak at 1 kD is 100–200 µA.
7. Ensure that a peak intensity of 50–500 µA is visible on each spot. If peak intensities appear out of this range, change the laser energy and run the test again.

8. Plot the peaks with the filtering option turned off:
   a. Select all 8 spectra.
   b. Click the data analysis icon . The **Analysis Settings** dialog opens.
   c. Open the **Filtering** tab and deselect the **On** checkbox.
   d. Click **OK**.



9. Plot all 8 spectra (do not select the first optimization spectrum). Using the peak selection tool, select the Arg-8-vasopressin (1,084.247 Da) peak. **Zoom in to ensure correct peak selection**.



10. Export the data following the instructions in Appendix C and selecting the parameters for export in the following order:
    - Array bar code
    - Spot name
    - Substance mass
    - Intensity
    - Resolution
    - S/N
11. Paste data into the ProteinChip SELDI OQ form, data sheet “Test 3 Resolution 1 kD”.
12. The spreadsheet indicates if the test passed or failed. If the test fails, run the HV conditioning procedure (maintenance procedure 1) and repeat the test. Contact technical support if the test fails a second time.

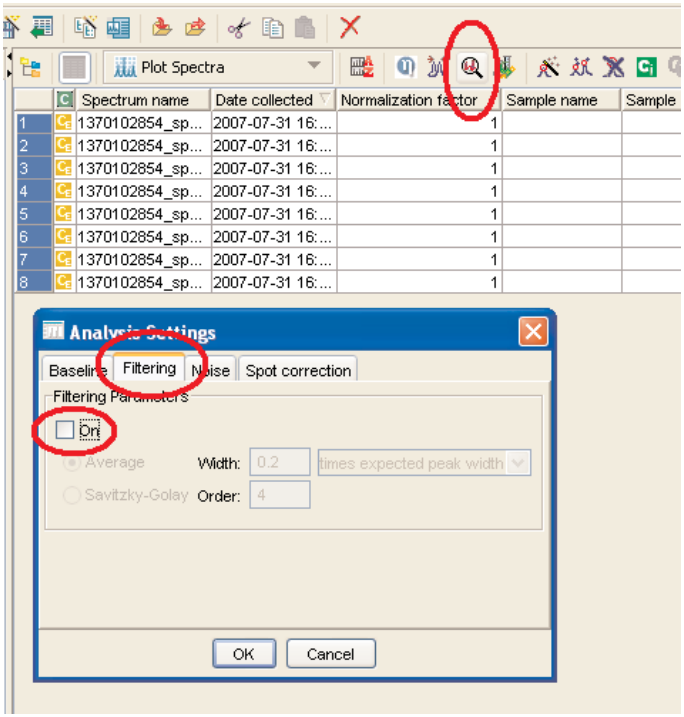
2.4.4 Test 4 — Mass Accuracy

This test uses the ProteinChip peptide standard array to test the mass accuracy of the system's internal and external calibrations. These measurements are compared to a specification, and a pass/fail disposition is obtained.

Array Type	ProteinChip peptide standard array
Usage Information	Each test uses 1 partition on all 8 spots on the array. The array can be used up to 20 times (5 times on each of 4 partitions). Store the array in the original packaging and record usage information in the usage chart provided and on the packaging. Laser optimization steps use 1 of 15 partitions, and these reads do not need to be recorded or monitored.
Recommended Testing Frequency	Weekly
Expected Testing Time	30 min to 1 hr
Pass/Fail Specification	External calibration: Pass if average mass within 0.1% of calibrant mass and pooled CV of <0.05 Internal calibration: Pass if average mass within 0.01% of calibrant mass and pooled CV of <0.01

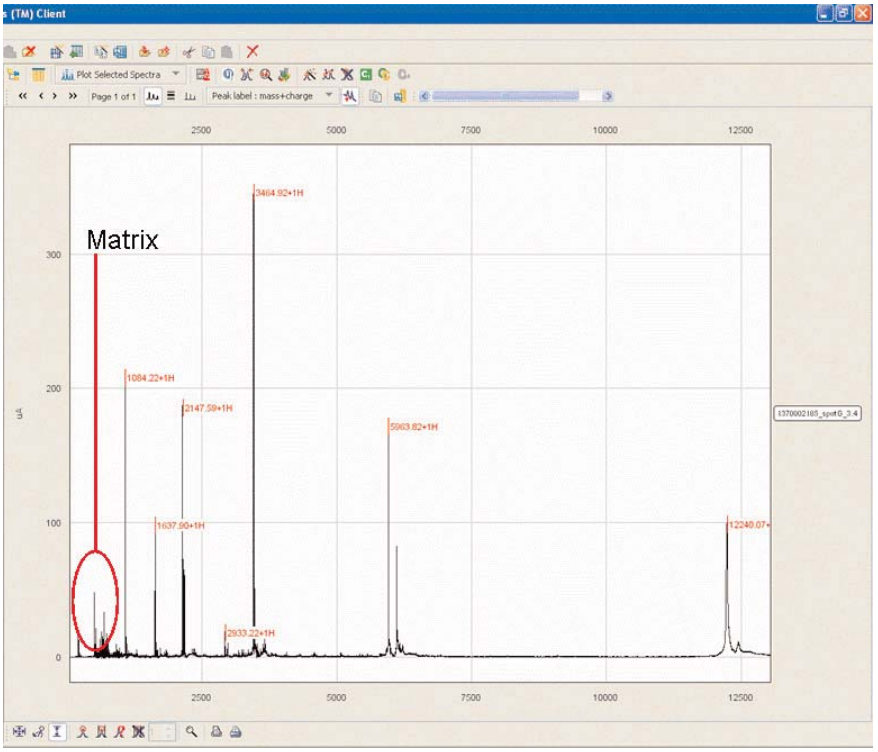
1. Insert the ProteinChip peptide standard array into the instrument. If you are using an Enterprise Edition instrument, place the array into a cassette, fill the remaining slots in the cassette with blank or used arrays, and place the cassette into the instrument.
2. In ProteinChip data manager software, click **Start** and open the **Protocol Mode** tab.
3. Select **Instrument > Preferences**. In the **Preferences** dialog, click "…". The **Select Folder** dialog opens.
4. Select **Projects > OQ_####/*Date*/Test 4 Mass Accuracy**, where #### is the serial number of the instrument.
5. Optimize the laser intensity. Run protocol "Test 4 Mass Accuracy" on any partition ("n") of 15 on spot D. Adjust the laser energy until the height of the insulin peak at 5.96 kD is 100–200 µA. This optimization step does not need to be repeated on subsequent runs of test 4 unless the peak intensity falls too low.
6. Run protocol "Test 4 Mass Accuracy". Run on partition 1 of 1 for all 8 spots on chip. **Do not change any values**.

7. Plot the peaks with the filtering option turned off:
   a. Select all 8 spectra.
   b. Click the data analysis icon . The **Analysis Settings** dialog opens.
   c. Open the **Filtering** tab and deselect the **On** checkbox.
   d. Click **OK**.

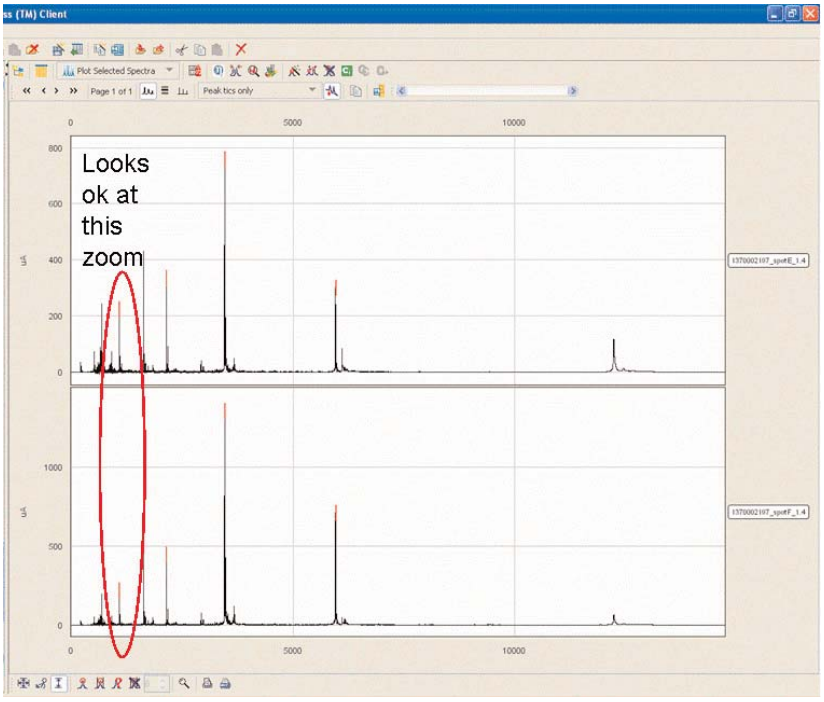


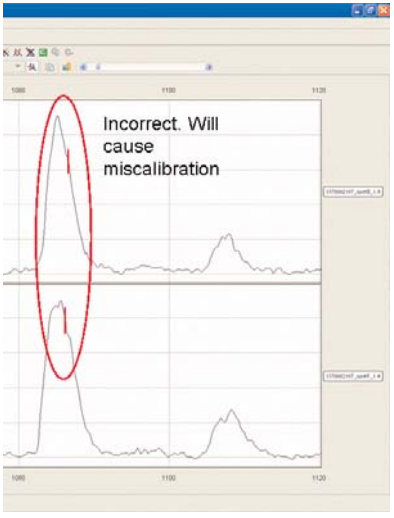
8. Plot all 8 spectra. Using the peak selection tool, select all 7 peaks. Each spectrum should resemble that shown below (intensities relative to each other may vary). Additional peaks other than those indicated may consist of matrix, doubly-charged peaks, dimers or other multimers, salt peaks, etc., as for any mass spectrum.

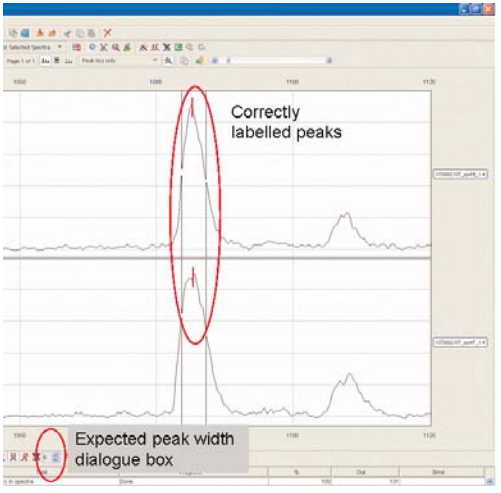
Peak	Name	Molecular Weight (Da)
1	Arg-8-vasopressin	1084.247
2	Somatostatin	1637.903
3	Dynorphin A	2147.5
4	ACTH	2933.5
5	Beta endorphin	3465
6	Arg-insulin	5963.8
7	Cytochrome C	12230.92



Note: To ensure correct placement of the peak marker, zoom in on each peak during selection. Though some plots appear acceptable when zoomed out, zooming in may reveal incorrect placement of peak markers. This is more accurate when the expected peak width dialog box option is set to 5.


Looks ok at this zoom


Incorrect. Will cause miscalibration

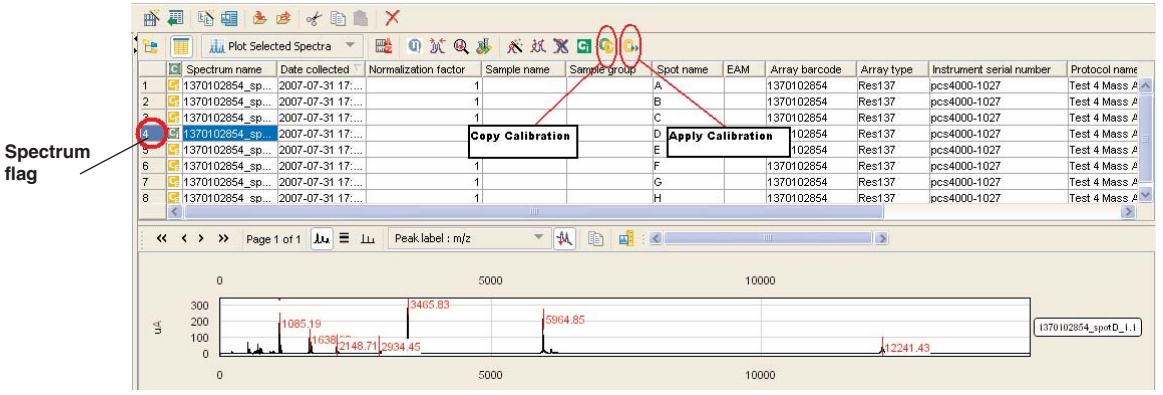

Correctly labelled peaks
Expected peak width dialogue box

9. Zoom in and check that all 7 peaks in all 8 spectra are marked correctly. Failure to do so may result in incorrect results. Close the plot.

Mass Accuracy — External Calibration Test

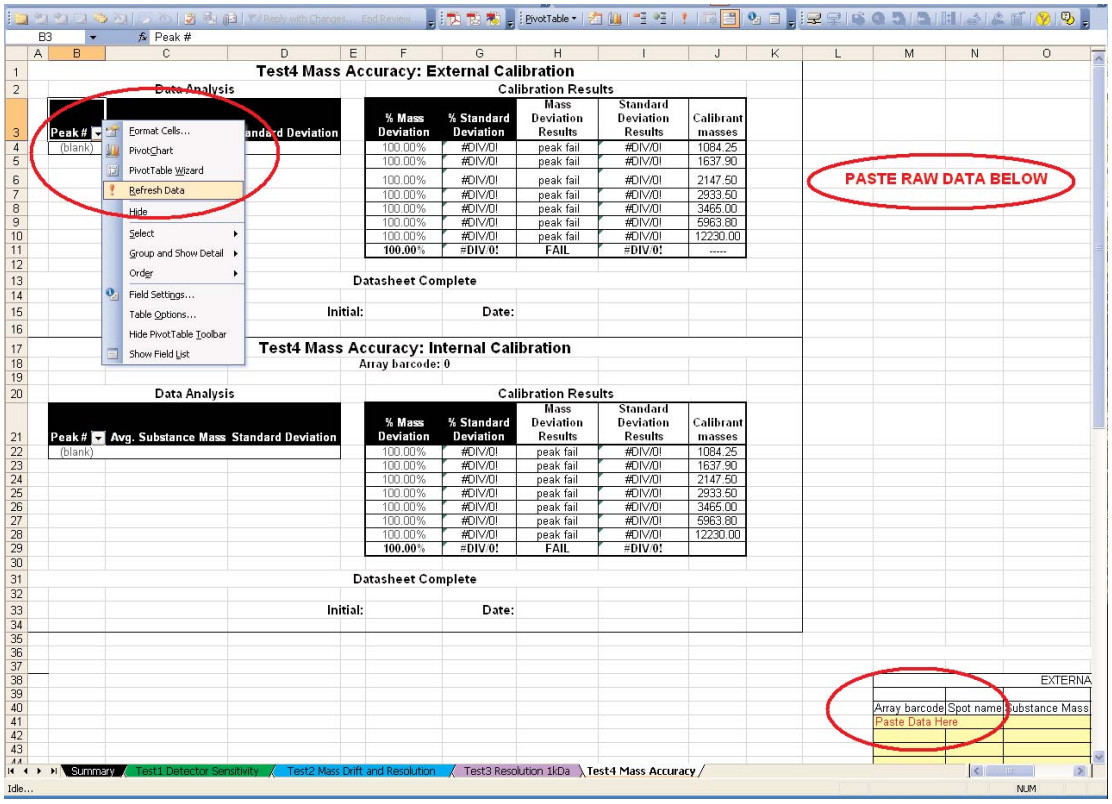
Perform this test BEFORE the internal calibration test.

1. Click on the spectrum for spot D.
2. Select **Spectra > Calibrate > Apply Calibration Protocol**.
3. Select **OQ Mass Cal**.
4. Click **OK**. The spectrum flag changes from yellow to green.
5. In the main window, make sure that only the spectrum from spot D is selected. Click the **Copy Calibration** button. The spectrum flag on spot D reverts to yellow.



6. Select all 8 spectra and click the **Apply Calibration** button. (Do not select the first optimization spectrum.)
7. Export the data following the instructions in Appendix C and selecting the parameters for export in the following order:
   - Array bar code
   - Spot name
   - Substance mass
   - Intensity
   - Resolution
   - S/N
   - Peak #
8. Paste the data into the ProteinChip SELDI OQ form, data sheet “Test 4 Mass Accuracy”. Paste the data into cell M41.

9. In the External Table, right-click on cell B3 and select **Refresh Data**.



10. The spreadsheet indicates if the test passed or failed. The test passes if the following specifications are achieved:
    - Peptide average mass within 0.1% of calibrant mass
    - % Standard deviation (pooled CV) of <0.05
11. If the test fails, run the HV conditioning procedure (maintenance procedure 1), and repeat the test. If the test fails a second time, contact technical support.

Mass Accuracy — Internal Calibration Test

Perform this test AFTER the external calibration test (above).

1. Check that all peaks have been selected and that all peaks are marked correctly (not on shoulders, etc.).
2. Select all 8 spectra.
3. Select **Spectra > Calibrate > Apply Calibration Protocol**.
4. Select **OQ Mass Cal**.

12. Click **OK**. The spectrum flag changes from yellow to green for all 8 spectra, once calibration is complete.
13. Export the data following the instructions in Appendix C and selecting the parameters for export in the following order:
    - Array bar code
    - Spot name
    - Substance mass
    - Intensity
    - Resolution
    - S/N
    - Peak #
14. Paste the data into the ProteinChip SELDI OQ form, data sheet “Test 4 Mass Calibration”. Paste the data into cell V41.
15. In the Internal Table, right-click on cell B21 and select **Refresh Data**.
16. The spreadsheet indicates if the test passed or failed. The test passes if the following specifications are achieved:
    - Peptide average mass within 0.01% of calibrant mass
    - % Standard deviation (pooled CV) of <0.01
17. If the test fails, run maintenance procedure 1 (HV conditioning) and repeat the test. Contact technical support if the test fails a second time.

Appendix A

ProteinChip® SELDI System

Installation Qualification (IQ) Certificate

Site Information

Site	Date
Address	Case Number
	Contact Name:
	Primary Investigator

System Information

Instrument Serial Number	
Configuration (Personal/Enterprise)	

Description	Parameter	Specification	Results
Site Conditions	Local Voltage	Record	
	UPS (Uninterruptible Power System)	Yes / No	
Computer	Computer supplier (Bio-Rad/Customer)	Record	
	Computer Manufacturer	Record	
	Computer Service Tag Number	Record	
	Computer Service Code	Record	
	Windows Operating System	Record	
	Bio-Rad Data Manager Software Installed	Record Version	
	Time & Date	Set to Local	

Appendix B
Protocol Acceptance Form

Bio-Rad Laboratories recommends that the operational qualification (OQ) protocols be performed in total on a regular basis to confirm that the ProteinChip SELDI system is performing to specifications, or whenever it is suspected that the instrument is not performing to specifications.

I have reviewed the ProteinChip OQ kit instruction manual and agree that it provides the appropriate procedures for the operational qualification of the ProteinChip SELDI system, Personal or Enterprise Edition.

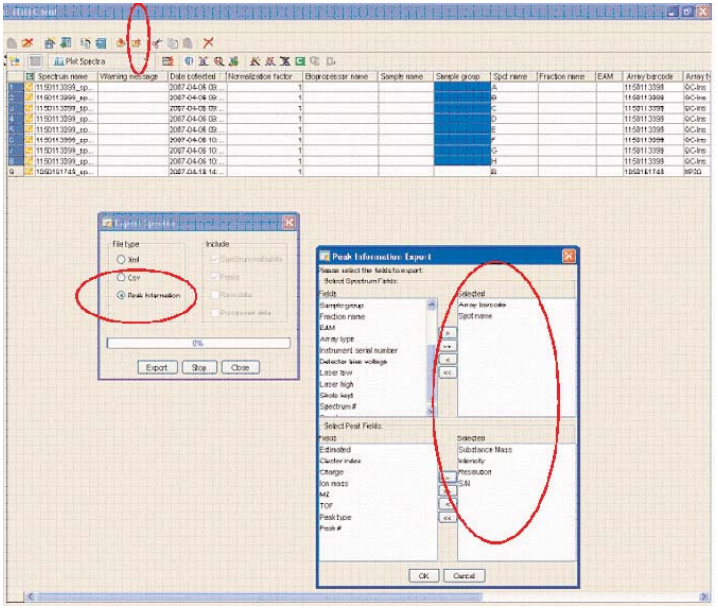
Customer Name (print)_____ Signature_____Date_____

Reviewer Name/Title (print)_____ Signature_____ Date_____

Appendix C
Data Export Procedure

Use this procedure to export the data obtained during testing to the Microsoft Excel spreadsheet provided with the kit (ProteinChip SELDI OQ form). Following export, open the resulting .csv file using the Excel program and copy and paste the contents into a COPY of the spreadsheet provided. Retain this copy for your records.

1. In the Explorer pane, open the **Projects** folder and select the spectra to be exported. Click the **Export Spectra** button in the toolbar. The **Export Spectra** dialog opens.
2. In the **Export Spectra** dialog, select **Peak Information** and click **Export**. The **Peak Information Export** dialog opens.



3. Select parameters and the order in which they are to be exported. (These parameters are specific to the test and are described in the instructions for the test.)
4. Click **OK**. The **Save File** dialog box opens. Enter the file name and click **OK**.

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Italy *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500*
Norway *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723*
Spain *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

Sig 1106

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