

# **ProteinChip<sup>®</sup>** **System Check Kit** Instruction Manual

Catalog #C70-00081

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



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# Chapter 1

## Introduction

### 1.1 Product and Protocol Description

The ProteinChip system check kit provides tools for performing two maintenance procedures (detector calibration and high voltage conditioning) as well as prepared ProteinChip arrays for testing detector sensitivity, resolution, and mass drift. Use of this kit allows verification that the ProteinChip reader is operating within specification, and the kit can be a valuable tool that allows discrimination between assay and instrumentation problems. It is recommended that this kit be used after periods of instrument inactivity greater than 4 weeks and when instrument problems are suspected.

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

- 1 CD containing the system check instrument protocols and the ProteinChip system check form
- 1 ProteinChip detector calibration array
- 1 ProteinChip detector qualification array
- 1 ProteinChip peptide standard array
- 1 ProteinChip system check kit instruction manual

The system check protocol (Table 1) involves two maintenance procedures (detector calibration and high voltage conditioning) followed by two tests (detector sensitivity and mass drift and resolution).

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

**Table 1. Summary of procedures and tests.**

	<b>Title</b>	<b>Consumable Used</b>	<b>Estimated Time Required</b>	<b>Description</b>
<b>Initial Setup</b>	File and protocol setup		20 min	Complete this procedure the first time you use the ProteinChip system check kit. This procedure creates the file structure necessary for storing the protocols and creates the routines for high voltage conditioning.
<b>Maintenance Procedure 1</b>	High voltage (HV) conditioning	N/A	1–2 hr depending on instrument	HV conditioning helps to decontaminate the surfaces in the instrument.
<b>Maintenance Procedure 2</b>	Detector calibration	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.
<b>Test 1</b>	Detector sensitivity	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 immunoglobulin (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).**

	<b>Test</b>	<b>Consumable Used</b>	<b>Estimated Time Required</b>	<b>Description</b>
<b>Test 2</b>	Mass drift and resolution at 5.96 kD	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.

## **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 System Check Kit**

### **2.1 Overview**

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

- Export data acquired during testing to the ProteinChip SELDI system check form using the export instructions described in Appendix A
- 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 here
- 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 use the ProteinChip system check kit. This procedure sets up the file structure necessary for storing the protocols and creates the routines for high voltage conditioning (maintenance procedure 1).

<b>Task</b>	
<p><b>Step 1</b></p> <p><b>Import Protocols From CD</b></p>	<p>Import the protocols supplied on the CD into the <b>Protocols</b> folder of ProteinChip data manager software:</p> <ol style="list-style-type: none"> <li>1. Insert the CD into the computer and launch ProteinChip data manager software.</li> <li>2. Select <b>Protocols &gt; Protocols &gt; Import Protocols</b>.</li> <li>3. Use the <b>Import Protocols</b> dialog to import all protocols (files with a .ptx extension) from the CD to the <b>Protocols</b> folder.</li> </ol> <p><b>Note:</b> The protocols are NOT write-protected. Do not alter them.</p>
<p><b>Step 2</b></p> <p><b>Create the File Structure</b></p> <p><b>Note:</b> Create new folders each time the 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.</p>	<p>Create a new folder for each test (1–2) to be run:</p> <ol style="list-style-type: none"> <li>1. In the Explorer pane of ProteinChip data manager software, open the <b>Projects</b> folder and select <b>File &gt; New &gt; Folder</b>.</li> <li>2. Create a folder, <i>system check_####</i>, where <i>####</i> is the serial number of the instrument.</li> <li>3. Click on this folder and select <b>File &gt; New &gt; Folder</b>. Create a separate folder for the date of the test.</li> <li>4. Click on the folder created in step 3 and select <b>File &gt; New &gt; Folder</b>. Create a separate folder for each of the following tests: <ul style="list-style-type: none"> <li>• Test 1 Detector sensitivity</li> <li>• Test 2 Mass drift and resolution 5.96 kD</li> </ul> </li> </ol>



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## 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 eight 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.
-

## 2.3 System Check 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 system check 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.

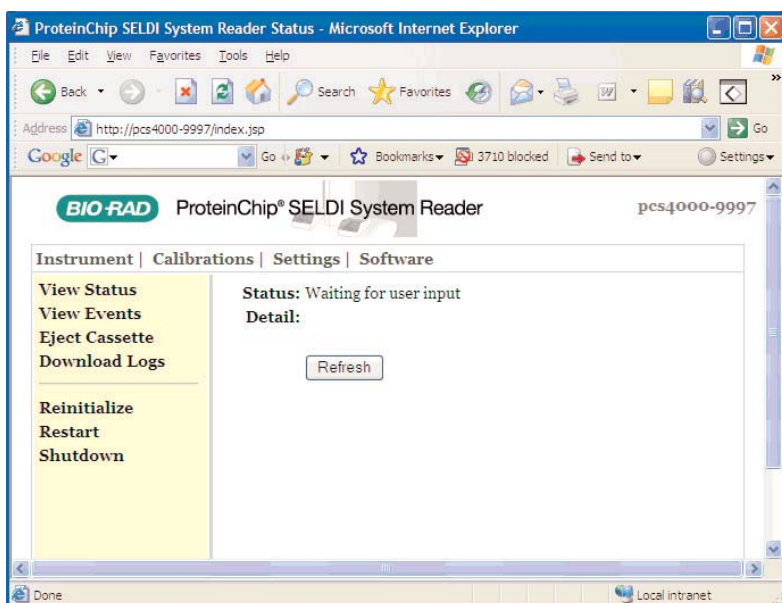
The procedure requires 45 min to 4 hr to complete, depending on the state of the instrument.

**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.

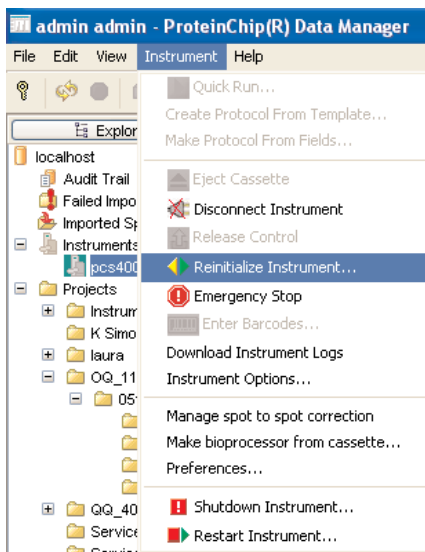
**Note:** For the Enterprise Edition instrument, place the array into slot 1 of a cassette and fill the rest of the slots with blank or used ProteinChip arrays.

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 system check form.


## 2.4 System Check Tests

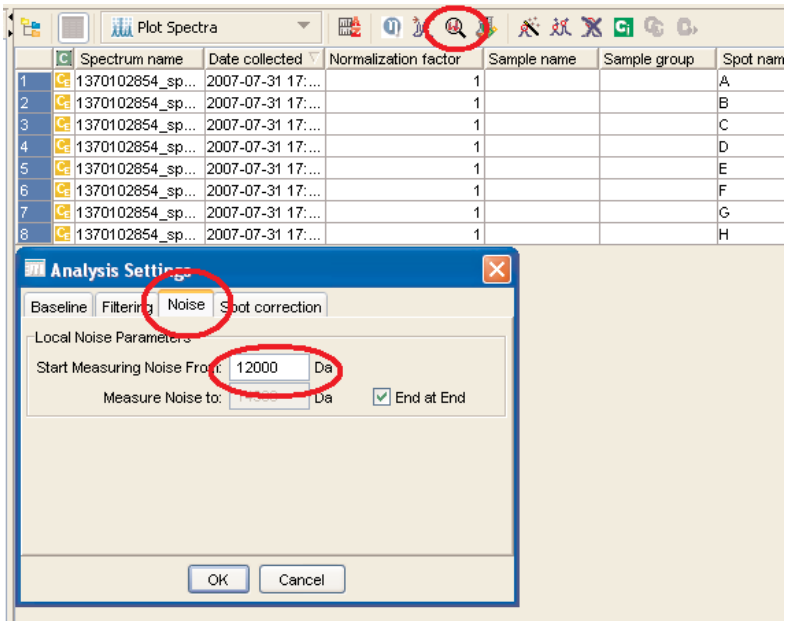
### 2.4.1 Test 1 — Detector Sensitivity

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

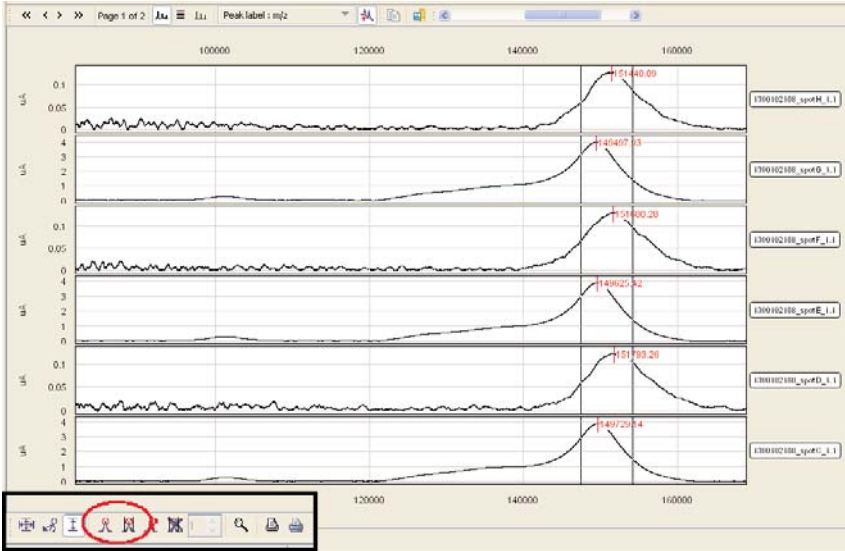
<b>Array Type</b>	ProteinChip Detector qualification array
<b>Usage Information</b>	Use once and discard; do not open until ready to use.
<b>Expected Testing Time</b>	30 min to 1 hr
<b>Pass/Fail Specification</b>	High concentration: pass if S/N is greater than 1,100 Low concentration: pass if S/N is 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 > system check\_####/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 according to the instructions in Appendix A 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 system check 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 2), 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.

<b>Array Type</b>	ProteinChip peptide standard array
<b>Usage Information</b>	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 package. Laser optimization steps use 1 of 15 partitions, and these reads do not need to be recorded or monitored.
<b>Expected Testing Time</b>	20 min to 1 hr
<b>Pass/Fail Specification</b>	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 > system check\_####/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  $\mu$ A. This optimization step does not need to be repeated on subsequent runs of test 2 unless the peak intensity falls too low.

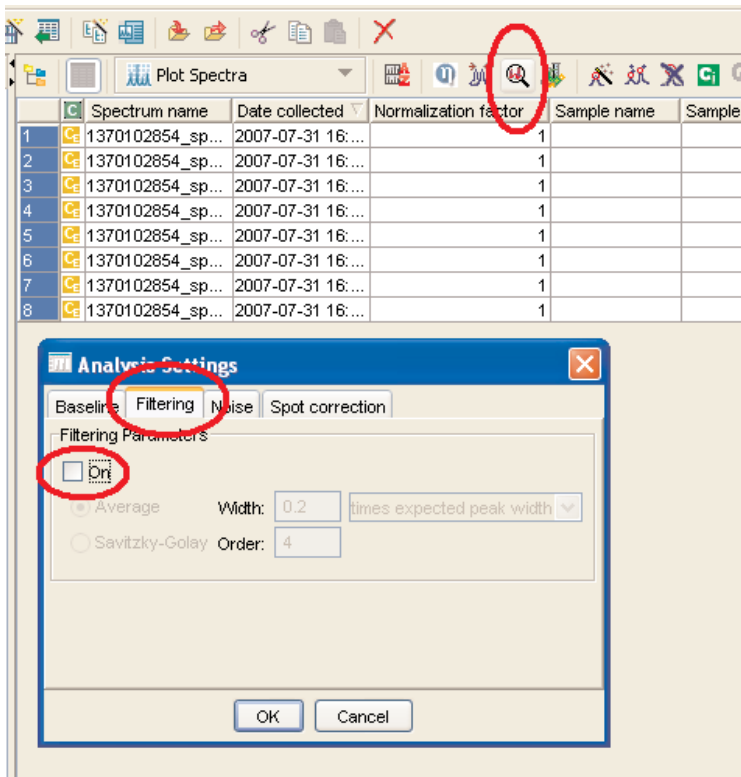


6. Run the same protocol on partition “n” of 4 for all 8 spots. Record the partition number on the usage chart provided with the product packaging.

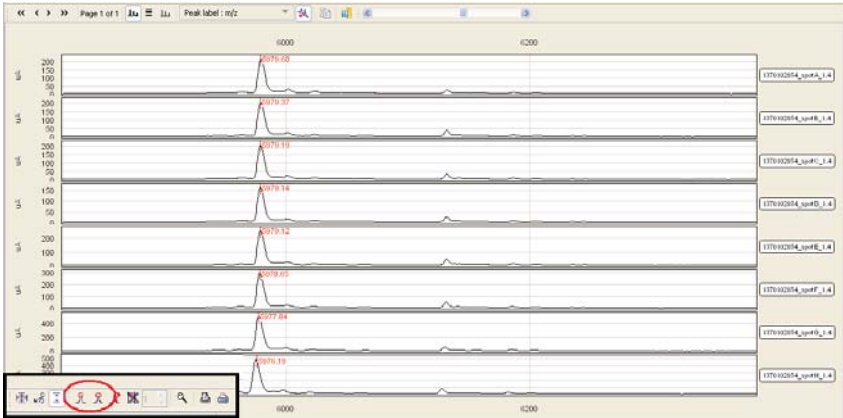
Partition #	Reads	Initial
1 of 4	1	
	2	
	3	
	4	
	5	
2 of 4	1	
	2	
	3	
	4	
	5	
3 of 4	1	
	2	
	3	
	4	
	5	
4 of 4	1	
	2	
	3	
	4	
	5	

7. Make sure that a peak intensity of 50–500  $\mu\text{A}$  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.



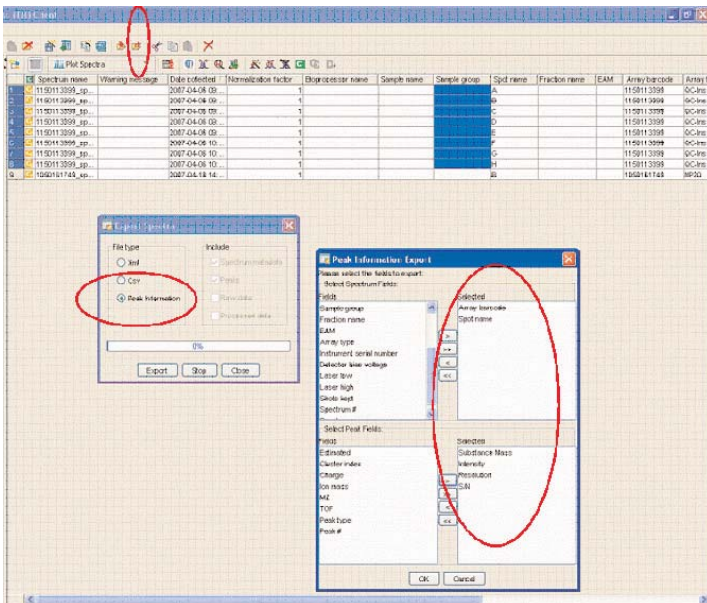
10. Export the data according to the instructions in Appendix A and selecting the parameters for export in the following order:
- Array bar code
  - Spot name
  - Substance mass
  - Intensity
  - Resolution
  - S/N
11. Paste the data into the ProteinChip SELDI system check form, data sheet “Test 2 Mass 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.

# Appendix A

## Data Export Procedure

Use this procedure to export the data obtained during testing to the Microsoft Excel spreadsheet provided with the kit (ProteinChip SELDI system check 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 the 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 each test.)
4. Click **OK**. The **Save File** dialog box opens. Enter the file name and click **OK**.



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