



User Guide

# Direct Detect<sup>®</sup> Spectrometer



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# 1 Introduction

The Direct Detect® infrared (IR)-based quantification system is an innovative combination of software-controlled instrumentation and EMD Millipore Corporation's advanced membrane technology, optimized for biomolecule detection, quantification of proteins, and analysis of lipids/detergents. Biomolecules are applied directly to a card-based hydrophilic polytetrafluoroethylene (PTFE) membrane that is transparent in most of the infrared spectral region.

The Direct Detect® system measures amide bonds in protein chains, accurately quantifying an intrinsic component of every protein without relying on amino acid composition, dye binding properties, or redox potential. Protein concentrations from 0.25 to 5 mg/mL can accurately be determined from a minimal sample volume (2 µL) without bio- or immuno-chemical staining. Sample analysis takes only minutes and in most cases can be performed directly from the buffered or native solution.

Quantification of lipids and detergents requires a user-defined standard curve. Unknown mixtures can be analyzed by determining the relative IR absorbance.

The system has been validated with proteins and cell lysates representative of those currently in use in research laboratories.

## 2 Components Supplied

The Direct Detect® system includes the following components:











Figure 1. Components supplied

1A	Direct Detect® spectrometer
1B	Instrument power adapter with 4 power cord/plug configurations (EU, UK, US, Japan)
1C	Data cable (crossover, 10Base-T Ethernet with RJ45 connectors)
1D	Dell® Netbook computer with Direct Detect® Software installed
1E	Computer power adapter with world-wide plug adapter
1F	Netbook stand
1G	Direct Detect® Assay-free cards (package of 50)
1H	Direct Detect® spotting tray
	Desiccant pack replacement (1, not shown)
	Torx® TX20 screw driver for replacing the IR source (not shown)
	Direct Detect® Spectrometer Quick Start Card (not shown)

### 3 Symbols Used in this User Guide

The following symbols are used throughout this user guide and/or on product labels, and the user shall abide by indicated requirements:

Symbol	Definition
	Warning alerts you to actions that may cause personal injury or pose a physical threat.
Class I Laser Product	Class I Laser product, complies with 21 CFR Chapter 1, Subchapter J
	Read the documentation
	Catalogue number
	Serial number
	Manufacturer
	CE conformity marking. Reference Declaration of Conformity for specific directives.
	Do not discard with common solid waste at end of life. Segregate with other waste electrical and electronic equipment (WEEE) and send to an appropriate facility for recycling. For information on recycling electrical and electronic products in the European Union, please visit <a href="http://www.millipore.com/weee">www.millipore.com/weee</a> .
	Hot Surface

### 4 Product Labeling

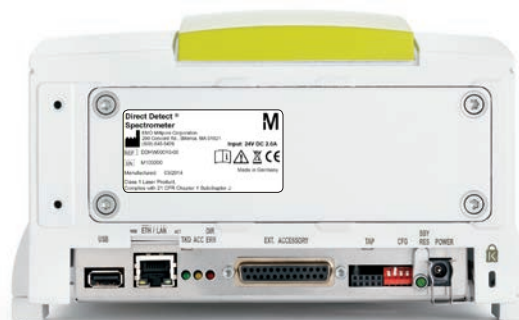
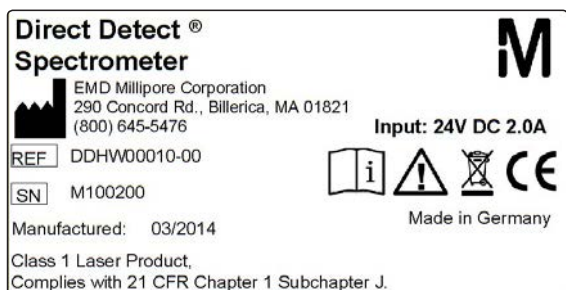


Figure 2. Product label appears on both the back and the bottom of the instrument

## 5 Safety Precautions

Review and understand the safety precautions below before installing and operating the Direct Detect® spectrometer.

### WARNING


- To avoid danger of electric shock, do not install the instrument in an area with a high humidity level. Refer to Site Requirements in section 6.2.
- Do not touch the Ethernet cable or universal power adapter plugs with wet hands.
- To avoid potential shock hazard, choose the correct plug configuration and make sure that the power adapter is plugged securely into a properly grounded AC power outlet. Make sure that the connection between the cable and the instrument is secure.
- Always ensure that the power supply input voltage matches the voltage available in your location.
- Use only the power supplies that were supplied by EMD Millipore Corporation for use with the Direct Detect® system. Use of other power supplies may damage the system.
- Minimize power draw from other instruments on the same power circuit.
- Do not use with flammable or explosive liquids. Cards that have been spotted with samples in flammable solvents must be dried prior to insertion into the instrument.

## 6 Installation

**NOTE:** For a full installation, operation, and process qualification protocol and checklist, refer to section 14.

### 6.1 Packaging

Inspect the packaging for damage upon receipt. If there are any signs of damage, contact the responsible shipping company.

 **WARNING:** Do not put a spectrometer showing signs of damage into operation as this may result in severe personal injuries and/or property damage.

Keep the original shipping boxes in case you need to return the spectrometer for servicing or repair.

**NOTE:** Instruments will not be accepted for repair if not in original packaging. EMD Millipore Corporation will provide shipping boxes for a charge if original packaging has been discarded.

## 6.2 Site Requirements

The user must provide an installation site that meets the following requirements:

Space requirement	<p>Stable and horizontal base which is able to bear the weight of at least 8 kilograms (kg)</p> <p>A clearance of at least 25 centimeters (cm) at the spectrometer rear side</p>
Environment	<p>Temperature range: 18–35 °C</p> <p>Humidity (non-condensing): less than 35% (relative humidity)</p> <p>Temperature variation: less than 1 °C/hour</p> <p>Humidity variation: less than 3%/hour</p> <p>Do not install devices such as large electric motors, heaters, welding equipment, radio transmitting equipment, units emitting pulsed NMRs, or high-powered lasers in close proximity to the Direct Detect® spectrometer. These devices can cause a spectrometer malfunction.</p> <p>Make sure that these types of devices are not connected to the same electrical circuit as the Direct Detect® spectrometer.</p>
Vibration	<p>Do not locate the Direct Detect® spectrometer near vibration sources (centrifuges, vortexes, ventilation hoods, air conditioners, etc.), or in rooms with intense floor vibrations.</p>
Inductive electrical interference	<p>Do not install the Direct Detect® spectrometer near any sources of potential inductive electrical interference (e.g., pumps, switching motors, microwave ovens, etc.), sources of high energy pulses, or sources that might cause magnetic or radio frequency interference.</p>
Power supply	<p>Voltage range: 100–240 VAC</p> <p>Frequency range: 50–60 Hz</p> <p>Protection class: I</p> <p>The power supply unit automatically adapts to most power sources commonly used.</p> <p>Make sure that the power supply is easily accessible at all times.</p> <p>Connect the Direct Detect® spectrometer only to a grounded power outlet.</p> <p>Take precautions to ensure uninterrupted power supply if there is the potential for power supply issues (e.g., brownouts, power surges, frequent thunderstorms, etc.)</p>

## 6.3 Installation Configuration

The Direct Detect® spectrometer is configured to operate as a stand-alone unit connected to the supplied Netbook computer (PC) using the factory-set standard IP address (10.10.0.1).

## 6.4 Unpacking and Setting up the Computer

Unpack the Netbook computer, power adapter, and world-wide plug adapter from the shipping box. Choose the plug adapter with the correct configuration for local requirements and attach it to the plug. Plug the low voltage (round) end of the power adapter into the power port of the PC, but do not plug the computer into a power outlet at this time.

## 6.5 Unpacking and Setting Up the Direct Detect® Spectrometer

Remove all components from shipping boxes and lay out on work surface.

### 6.5.1 Positioning and Connecting Components (Figure 3)

1. Place instrument on bench and put Netbook stand on top of instrument. The front tab of the stand fits into the slot on top of the instrument (5B in Figure 5).
2. Place Netbook computer on stand.

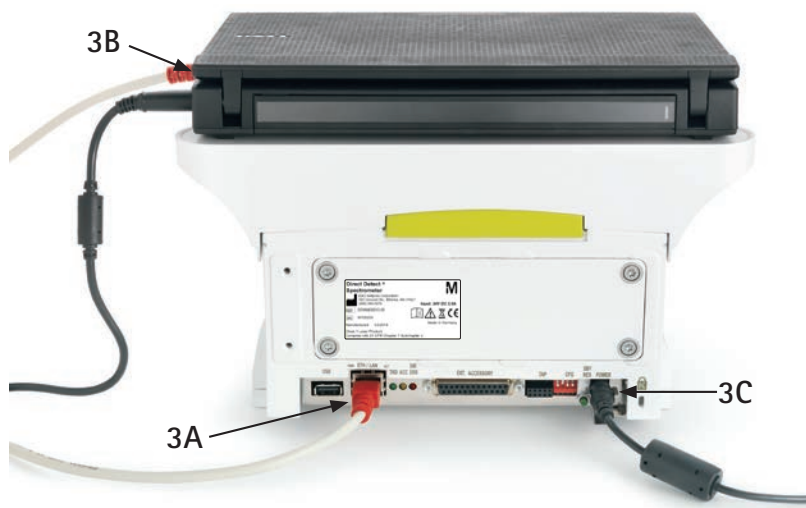



Figure 3. Rear side – Connection ports

3. Turn the instrument/computer assembly so that the rear of the instrument is facing you (Figure 3).
4. Connect one end of the provided Ethernet cable to the instrument ETH/LAN port (3A) and the other end to the PC network/LAN port (3B).

## 6.5 Unpacking and Setting Up the Direct Detect® Spectrometer, cont.

5. Connect the low voltage (round) end of the instrument power adapter to the power port of the instrument (3C). Secure the connector with the clamp.
6. Choose the instrument power cord with the correct plug configuration for local requirements and plug it into the power adapter.
7. Plug both the PC and instrument power cords into a grounded power outlet. A surge protector is recommended.

 **WARNING:** To avoid personal injuries and/or property damage caused by electrical power, connect the spectrometer and computer to a grounded power outlet. Make sure that this power outlet complies with IEC (International Electrotechnical Commission) regulations.

8. Turn on the PC.

**NOTE:** The instrument is powered through the computer and does not have a separate ON/OFF switch. It should be left on and powered up at all times.

### 6.5.2 Initializing the Direct Detect® Spectrometer

1. Refer to the Direct Detect® Software User Guide for instructions on starting up the software and logging in (section 1.2).
2. During initialization, the laser, IR source, and detector are switched on. The IR source warms up, the scanner moves forward and backward several times, and tones once. This phase takes several minutes, and the hardware diagnostics icon is yellow. After the initialization has been completed successfully, the hardware diagnostics icon turns to green, but the instrument validation icon will be yellow (Figure 4). This indicates that the PQ test has expired and needs to be run. Refer to section 13.5 for instructions on running the PQ test.

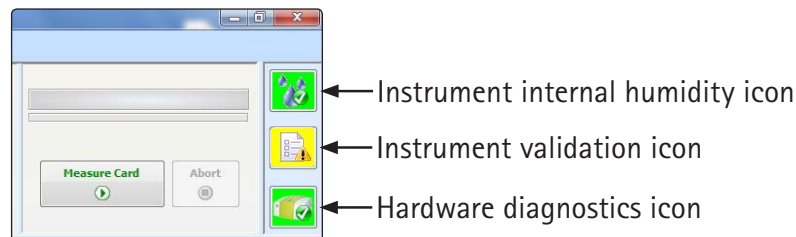


Figure 4. Instrument status icons

**NOTE:** If the status indicator on either the instrument or the software is red or remains yellow after initialization is complete and the PQ test has been run, refer to section 12, Troubleshooting.

# 7 Direct Detect® Spectrometer Parts and Functions

## 7.1 General Technical Features

### 7.1.1 The Instrument

The Direct Detect® spectrometer is a small compact FTIR spectrometer designed for measuring IR absorbance.

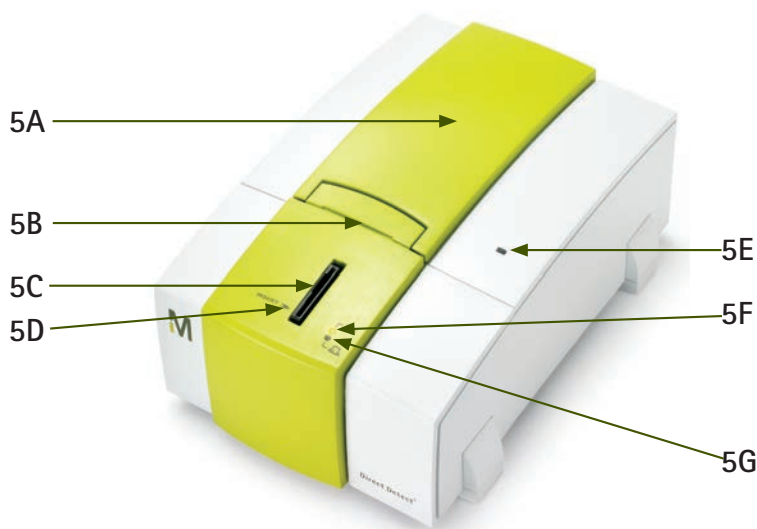


Figure 5. Direct Detect® spectrometer

#### Definition

5A	Instrument module (with integrated electronics and optics)
5B	Slot for Netbook stand tab
5C	Direct Detect® automated card holder
5D	Card insertion alignment arrow
5E	Status light
5F	Sample drying indicator light
5G	Card insertion light

### 7.1.2 The Direct Detect® Automated Card Holder

The automated card holder (5C) accepts only 4-sample Direct Detect® Assay-free cards. The top of the card holder module contains two LED lights located at the bottom right corner (5F and 5G as viewed from the top).

The bottom LED light (5G) is green whenever an Assay-free card is inserted properly.

The top LED light (5F) is yellow whenever the drying system heater and fan are active.

Periodically (default setting is 15 minutes) the software will initiate a reference measurement which must be performed without a sample card inserted in the card holder. If a card is found in the card holder, the software will prompt you to remove it.

## 7.1 Direct Detect® Spectrometer General Technical Features, continued

### 7.1.3 Connecting Ports and LEDs

The connecting ports (e.g., power, Ethernet) and the system diagnostic LEDs are on the back of the instrument.

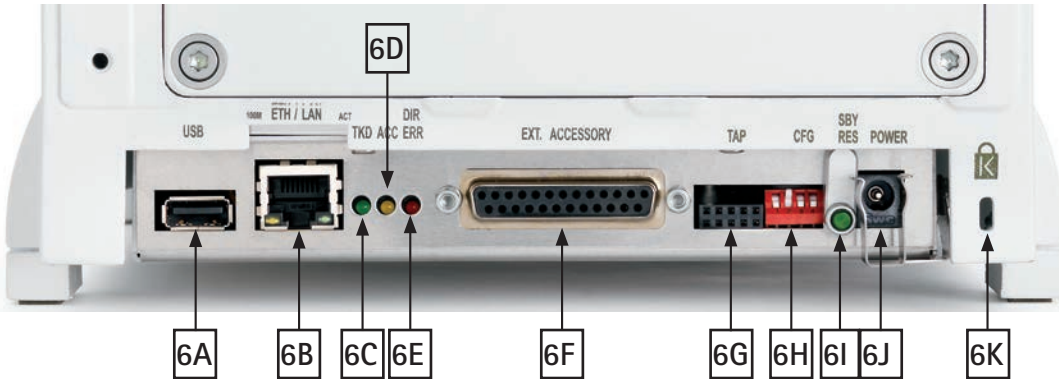


Figure 6. Direct Detect® Spectrometer rear side – Connecting ports and LEDs

Type	Definition	
6A	USB port	Not available to user. For instrument service use only.
6B	ETH/LAN port	The ETH/LAN port connects the spectrometer to the Netbook computer, using the supplied data cable (crossover, 10Base-T with RJ45 ports).
6C	TKD LED	The green TKD LED indicates that the interferometer mirror is within the data acquisition range. When data acquisition is occurring, the light is bright green. The abbreviation TKD means <i>Take Data</i> .
6D	ACC LED	The yellow ACC LED indicates that the network is accessing the optics. During transition of measuring data the LED is light yellow. The abbreviation ACC means <i>Access</i> .
6E	DIR/ERR LED	In normal operation mode the DIR/ERR LED flashes pale red and indicates scanner movement. If the LED lights permanently bright red, there is an interferometer problem, e.g., missing laser signal. When this LED is permanently red no data acquisition is possible. The abbreviation DIR means <i>Direction</i> and ERR means <i>Error</i> .
6F	External accessory port	Not available to user. For instrument service use only.
6G	TAP port	Not available to user. For instrument service use only.

## 7.1 Direct Detect® Spectrometer General Technical Features, continued

	Type	Definition
6H	CFG switch	CFG is a four-fold DIP switch: #1 ON and #2 OFF: DHCP mode - use last DHCP address #1 OFF and #2 ON: use ON-IP address 10.10.0.1 #3: stay in boot loader, do not load the application program #4: reserved The abbreviation CFG means <i>Configuration</i> .
6I	SBY/RES button	Pressing this button for approximately 2 seconds, activates/deactivates the stand-by mode. Pressing this button for more than 6 seconds resets the spectrometer to the original state. The abbreviation SBY means <i>Standby</i> and RES means <i>Reset</i> . Do not use unless instructed to do so by service personnel.
6J	POWER port	This port connects the spectrometer to the power supply via the supplied power adapter and power cord. The POWER port is for extra low-voltage connectors. Use only power cords/supplies that have been supplied by EMD Millipore Corporation.
6K	Kensington lock slot	The lock slot is an anti-theft lock for mobile hardware. Lock and cable are not supplied.

## 7.2 Direct Detect® Computer and Software

The Direct Detect® spectrometer is designed to operate with the Direct Detect® Software that is pre-installed on the Netbook computer. For computer specifications, refer to section 15.

Direct Detect® Software may be updated periodically. For information on the most up-to-date software, go to [www.millipore.com/directdetect](http://www.millipore.com/directdetect). Register your instrument in order to receive notification about relevant software upgrades.

**WARNING:** Do not install third-party software (screen savers, etc.), as this may impact the performance of the Direct Detect® system.

## 8 General Guidelines

The Direct Detect® spectrometer is recommended for use with protein samples ranging from 0.25 to 5 mg/mL. Although the instrument can measure protein concentrations from 0.1 mg/mL to around 30 mg/mL, the most accurate results are achieved within the recommended concentration range. The instrument is also capable of detecting and analyzing lipids and detergents. Analysis is performed with a user-defined calibration method. Samples to be analyzed are introduced to the system using the Assay-free card which contains a novel IR-compatible membrane. The recommended sample volume is 2 µL per spot position.

### 8.1 Buffer components

The Direct Detect® system is compatible with most commonly used buffers, detergents, and reducing agents, however, use of buffers which contain amides or amine functional groups may affect quantification.

Buffers containing more than 5% glycerol, SDS, and/or Tween® 20 surfactant should be avoided due to the excessive time required to achieve dryness.

The instrument comes with preloaded protein calibration files, prepared using National Institute of Standards & Technology (NIST) bovine serum albumin (BSA) in phosphate-buffered saline (PBS). However, preparation of a fresh calibration method with the buffer used for protein preparation is recommended. The Direct Detect® spectrometer is compatible with most solvents used in lipid research.

### 8.2 Materials Required but Not Supplied

- Protein standards (Bovine Serum Albumin - National Institute of Standards & Technology SRM 927d (optional))
- Protein sample(s)
- Lipid/detergent standards
- Lipid/detergent sample(s)
- Fixed-volume precision pipette capable of precisely dispensing 2 µL, and pipette tips

**NOTE:** To minimize pipetting error, we recommend the use of a fixed-volume precision pipette capable of dispensing 2 µL, for applying samples to the Assay-free card. Adjustable pipettes are not recommended.

- Water, PBS, or other buffer
- Chloroform or other lipid compatible solvent
- Printer (optional)

## 9 Direct Detect® Assay-free Card and Sample Preparation

### 9.1 Direct Detect® Assay-free Card

All concentration measurements are performed using the Assay-free card.



Figure 7. Direct Detect® Assay-free card

The card contains four hydrophilic polytetrafluoroethylene (PTFE) membrane positions, sized for easy sample application and surrounded by a hydrophobic ring that retains the sample to be analyzed within the IR beam. A 2  $\mu$ L sample volume is recommended in order to assure proper sample application, drying, and measurement.

By design, the positions on the card are numbered from 1 to 4, starting from the bottom. Position 1, by software default, is reserved for the "blank" measurement. The "blank" is the buffer/solvent used to resuspend the sample, but contains no protein or lipid/detergent. One or more of the remaining card positions are used for the protein and/or lipid/detergent samples.

The Assay-free card can be inserted into the system and analyzed immediately after preparation. The Direct Detect® spectrometer has a built-in drying system that automatically dries the sample to the point where it gives an accurate reading. The first sample position typically takes the longest period of time to dry, with subsequent positions taking less time. Including drying time, the total measurement time for a card loaded with 2  $\mu$ L samples does not exceed 5 minutes.

The Direct Detect® Assay-free card is intended for one-time protein and/or lipid/detergent spotting. However, already prepared cards can be stored and read again.

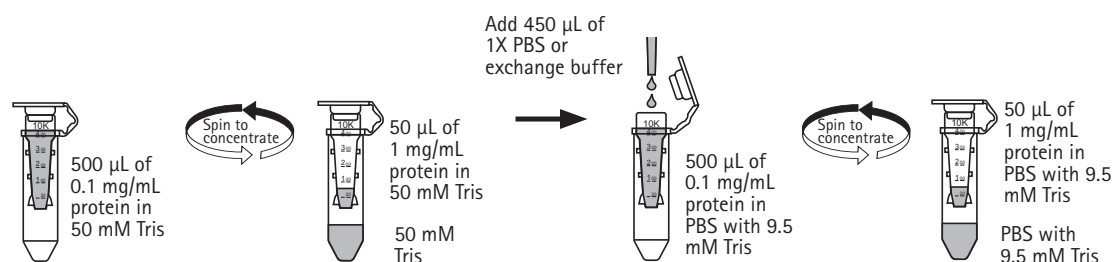
## 9.2 Sample Preparation

In general, analysis of protein samples within the concentration range of 0.25–5 mg/mL can be performed directly from the buffered solution. Buffers containing more than 5% glycerol, SDS, and/or Tween® 20 surfactant should be avoided due to the excessive time required to achieve dryness.

For analysis of samples with protein content above 5 mg/mL, single step dilution is recommended.

Concentration determination of samples below 0.25 mg/mL can be achieved after a simple spin concentration step using a centrifugal filter (e.g., Amicon® Ultra-0.5 device).

Use of centrifugal filters is also recommended for buffer exchange when the original buffer contains a high concentration of components with amide- or amine-containing functional groups. Figure 8 is an example of a diafiltration protocol that concentrates the sample and reduces the Tris buffer concentration.



**Figure 8. Buffer exchange and concentration using an Amicon® Ultra-0.5 device**

In general, lipid sample preparation involves solvent extraction. Neutral lipids may be extracted by relatively nonpolar solvents such as hexane, chloroform, or toluene while membrane-associated lipids require polar solvents like methanol or ethanol.

Lipids dissolved in organic solvents may be applied directly onto the Assay-free card. Special care in the application of highly volatile solvents (chloroform, hexane, etc.) is recommended in order to contain the samples within the hydrophobic embossing.

Analysis of detergents can be performed directly from buffer. Due to the excessive time required to achieve dryness, samples containing more than 5% of the analyzed detergent should be avoided.

The limits of lipid and detergent detection and quantification should be established experimentally for each compound analyzed.

# 10 How to Use the Direct Detect<sup>®</sup> Spectrometer

The instrument is controlled and operated by Direct Detect<sup>®</sup> Software installed on the Netbook computer. Samples are introduced to the instrument module by inserting the Direct Detect<sup>®</sup> Assay-free card into the card holder (Figure 11). An alignment notch on one corner of the card ensures correct card insertion into the card holder.

## 10.1 Accessing Direct Detect<sup>®</sup> Software

The Direct Detect<sup>®</sup> Software can be accessed through the icon on the Netbook desktop.



Figure 9. Direct Detect<sup>®</sup> Software desktop icon

Each instrument user can be assigned a unique name and optional password (Figure 10).

**NOTE:** If 21CFR11 mode is enabled, a password is mandatory. Refer to section 1.3 in the Direct Detect<sup>®</sup> Software User Guide.

The measurements performed by each user are stored and recalled based upon the user name.

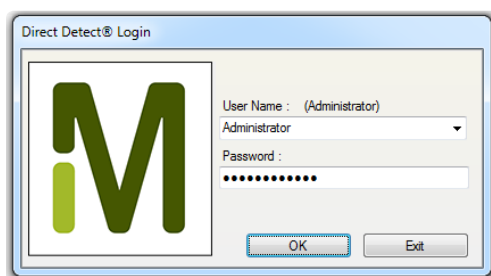


Figure 10. Direct Detect<sup>®</sup> login screen

For detailed instructions on starting up the software, refer to section 1.2 of the Direct Detect<sup>®</sup> Software User Guide.

**NOTE:** A seven minute warm-up cycle is required after initial power-up and software activation. The software will wait for seven minutes before allowing a sample measurement to begin.

## 10.2 Loading and Installing the Card

1. Place the Assay-free card(s) on the spotting tray or other clean surface. Select an appropriate buffer/solvent blank, preferably the same solution in which the protein or lipid/detergent has been prepared. If that buffer/solvent is not fully characterized, select the closest buffer/solvent possible. Pipette 2  $\mu\text{L}$  of the buffer/solvent solution to the default blank position on the card (position 1).
2. Pipette 2  $\mu\text{L}$  of sample to sample positions 2 through 4 as needed. Unused positions can be loaded and read at a later time as long as the buffer/solvent blank is appropriate for them. Card positions can be labeled if desired.

**NOTE:** Cards spotted with samples in flammable solvents must be dried at ambient conditions prior to inserting the Assay-free card into the instrument. However, for nonflammable samples, the Direct Detect<sup>®</sup> spectrometer has a built-in drying system that can automatically dry the sample to the point where it will give an accurate reading.

3. Insert the card vertically into the slot in the top of the card holder module with the card facing left and the alignment notch in the corner of the card oriented towards the center of the instrument.

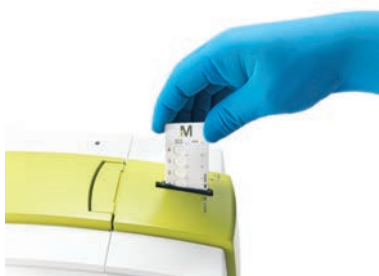


Figure 11. Inserting sample card

The arrow on the bottom right side of the card should be aligned with the arrow on the Direct Detect<sup>®</sup> card holder module (5D in Figure 5). Gently release the card and the instrument will accept it for analysis.

When the card is inserted properly the instrument will respond by jogging the card holder up and down briefly. The bottom green LED on Direct Detect<sup>®</sup> card holder module will light and the system will produce a brief tone confirming correct placement of the Assay-free card.

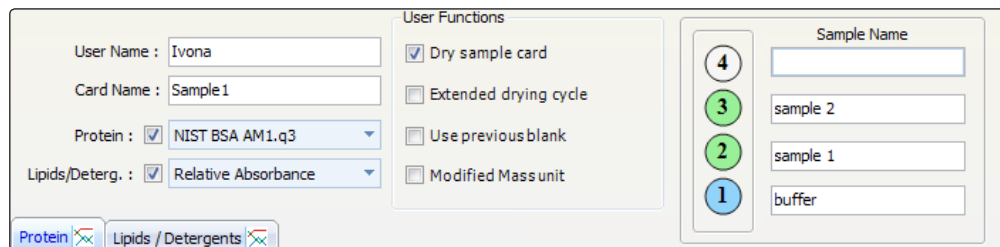
**NOTE:** The software will not allow sample measurement if the card is not inserted properly.

## 10.3 Measurement

For detailed instructions on setting up a sample measurement, refer to section 2.2 of the Direct Detect® Software User Guide.

### 10.3.1 General Measurement Procedure

1. Complete the sample measurement setup (Figure 12). Check the **Dry sample card** box to allow the instrument to dry the sample.



The screenshot shows a software interface for sample measurement setup. It is divided into several sections:

- User Information:** Fields for "User Name" (Ivona), "Card Name" (Sample1), "Protein" (NIST BSA AM1.q3), and "Lipids/Deterg." (Relative Absorbance).
- User Functions:** A list of checkboxes: "Dry sample card" (checked), "Extended drying cycle" (unchecked), "Use previous blank" (unchecked), and "Modified Mass unit" (unchecked).
- Sample Name List:** A vertical list of sample names with numbered circular icons to their left: "4" (buffer), "3" (sample 2), "2" (sample 1), and "1" (buffer).
- Buttons:** "Protein" and "Lipids / Detergents" buttons with small icons.

Figure 12. Sample information

**NOTE:** The Direct Detect® system comes with three preloaded protein calibration methods. These pre-existing methods may be used for all future measurements, however, for accurate quantification, we recommend preparation of a fresh calibration method for each buffer used in sample preparation (refer to section 4.2 in the Direct Detect® Software User Guide). Lipids and detergents can be analyzed using a **Relative Absorbance** mode.

2. Click on the Measure Card button (Figure 13). The sample measurement takes about a minute and the measurement progress is shown in the lower status bar.

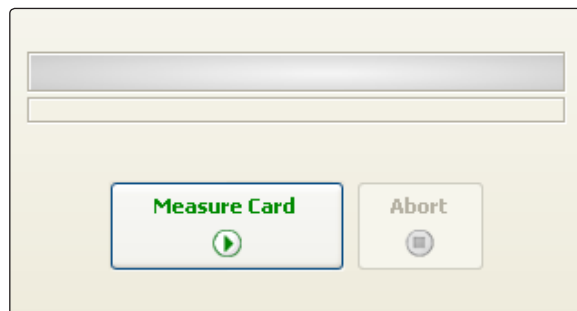


Figure 13. Measure sample

## 10.3 Measurement, continued

The result from each measured sample position is displayed in the Last Sample Result tab (Figure 14) of the main Direct Detect® screen.

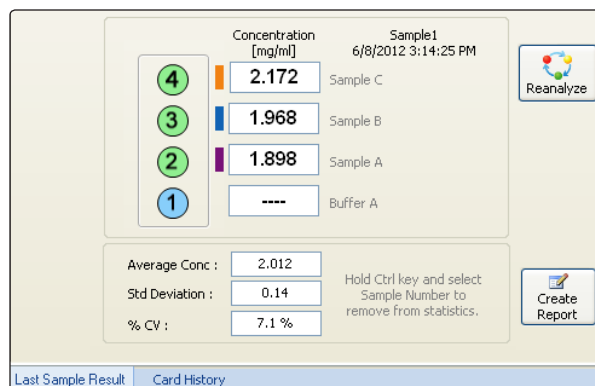


Figure 14. Last sample results screen

- For reporting options, refer to sections 2.4–2.7 of the Direct Detect® Software User Guide.

**NOTE:** Periodically the software will initiate a reference measurement which must be performed without a sample card inserted in the card holder. If a card is found in the card holder, the software will prompt you to remove it.

### 10.3.2 Calibration

The Calibration Method Wizard (Options menu, Generate Calib. Curve) is used to create or modify calibration method files that will be used to perform the analysis of the Assay-free cards.

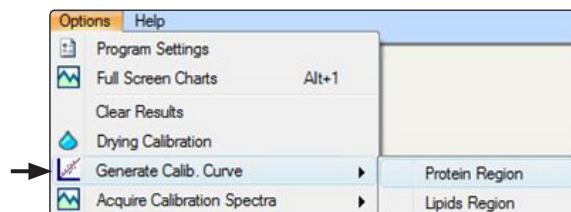


Figure 15. Calibration mode

To create a calibration method you need previously collected spectra from a series of known protein or lipid/detergent concentrations. To compile these files, select **Acquire Calibration Spectra**; **Protein Region** or **Lipids Region** from the **Options** menu and follow the instructions for collecting calibration spectra in section 4.2 of the Direct Detect® Software User Guide.

## 10.3 Measurement, continued

Section 4.2 of the Direct Detect® Software User Guide details how to use the Calibration Method Wizard to create or modify a calibration method file.

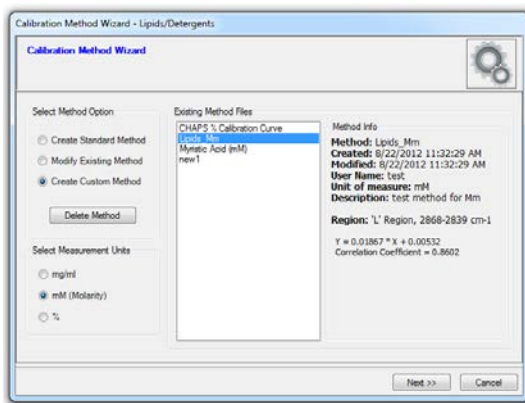


Figure 16. Calibration Method start page

## 10.4 Removing the Card

After all 4 positions on the card have been read, the instrument will sound a tone. The card will rise to the initial insertion position and can be removed.

**CAUTION:** Do not remove card during the reading. To stop a reading in progress, click on the **Abort** button.

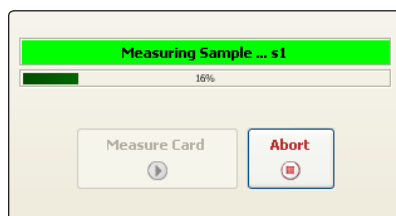


Figure 17. Abort button

When a measurement has been aborted, the progress bar will display **Measurement Aborted**. After a short period of time, the instrument will return to **Measure Card** status.

# 11 Switching from Direct Connection to Intranet Connection (Networking)

During initial setup, the Direct Detect® instrument is connected directly to the computer running the Direct Detect® software using the crossover 10Base-T cable with RJ45 connectors provided with the instrument (direct connection). After verification of instrument and software functionality, the instrument can be configured to run directly from your company's intranet (intranet connection), if desired.

## 11.1 Reconfiguration Requirements

1. Confirm that the system initializes and run the PQ test (refer to section 13.5). If a full Installation, Operation, and Process Qualification Protocol is required, perform all tests prior to changing the connections (refer to section 14).
2. The **IP Address Change Utility** is located in C:\directdetect\IPAddressChanger. This utility assists in the transition between direct connection to the computer and connection via the intranet and vice versa. Two **standard** RJ45 Cat5e Ethernet cables (not provided) are required to switch to an intranet connection.

When the **IP Address Change Utility** starts, the screen lists the requirements necessary to perform the change in operating mode (Figure 18):

- The Direct Detect® software is not running on the computer.
- The instrument is powered on and connected, either directly to the instrument or to the intranet.
- The software is able to locate the Direct Detect® installation folder and files required to reconfigure the instrument.

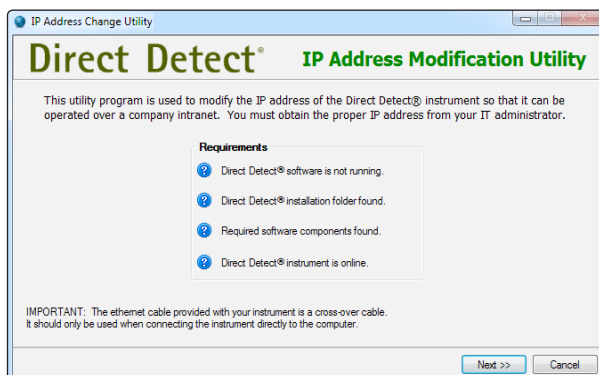


Figure 18. IP address change utility requirements

3. Select the **Next** button and the software will check the validity of each requirement.

## 11.1 Reconfiguration Requirements, continued

4. If a requirement is found to be valid, a ✓ appears next to the requirement in the list.

If a requirement is not valid then an ✗ will appear next to the requirement. A message indicating the cause of the failure will appear.

You can continue to the next step in the utility only if all requirements are valid.

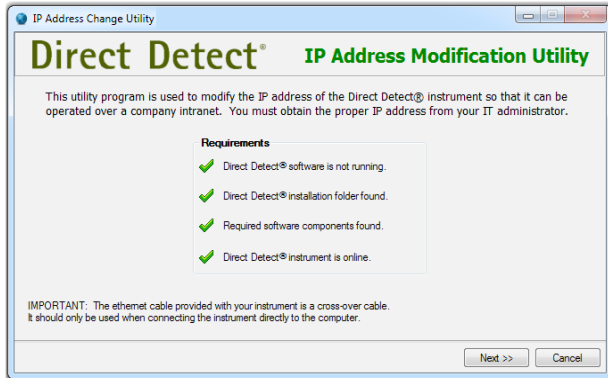


Figure 19. Status of requirements

## 11.2 Changing from Direct Connection to Intranet Connection

**IMPORTANT:** This section assumes that the Direct Detect® instrument is currently connected directly to the computer using the supplied crossover 10Base-T cable with RJ45 connectors.

1. If the requirements are valid (all check marks are green) select the Next button to proceed to the IP Settings selection screen of the utility.
2. Select the second option, I want to connect the instrument to the network using IP Address.

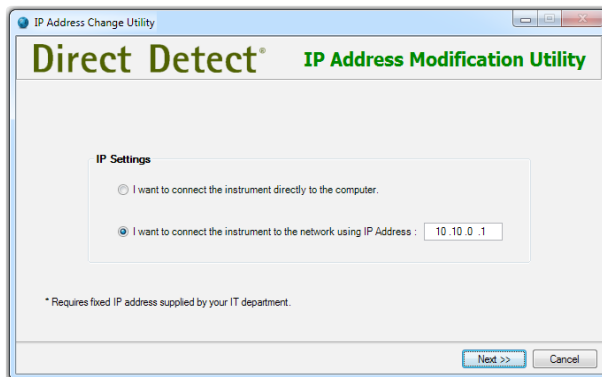


Figure 20. Selecting network IP address

3. An IP address URL field will become active to the right of this option. Enter the fixed IP address issued by your IT department. The URL must be fixed for this instrument.
4. Select the Next button to proceed.

## 11.2 Changing from Direct Connection to Intranet Connection, continued

5. The software will now make the necessary modifications to the Direct Detect® software and instrument, as well as the host computer. If the modifications are successful you will be informed that your instrument settings have been updated.

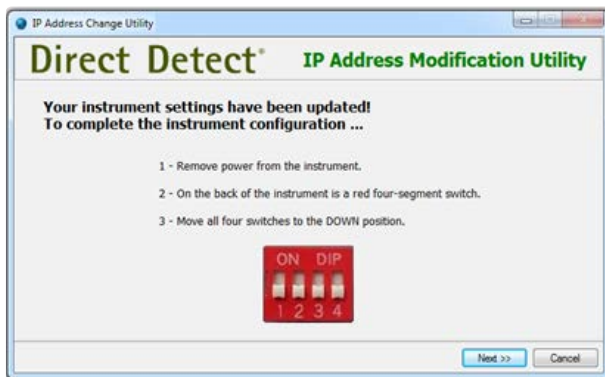


Figure 21. Successful reconfiguration to intranet connection

6. Unplug the instrument from the power source, then change the switches in the red 4-position DIP switch (6H in Figure 6) on the rear of the instrument so that all four are in the **DOWN** position (refer to Figure 21).
7. Select the **Next** button to proceed.
8. Run one of the **standard** RJ45 Cat5e Ethernet cables between the ETH/LAN port on the rear of the instrument (6B in Figure 6), and your intranet hub. Run the other **standard** RJ45 Cat5e Ethernet cable between the PC network/LAN port (3B in figure 3) of the Netbook computer and your intranet hub. The supplied **crossover** 10Base-T cable with RJ45 connectors is not required for this configuration.
9. Reconnect power to the instrument.
10. Wait for the instrument to initialize and then select the **Test Connection** button on the utility screen.

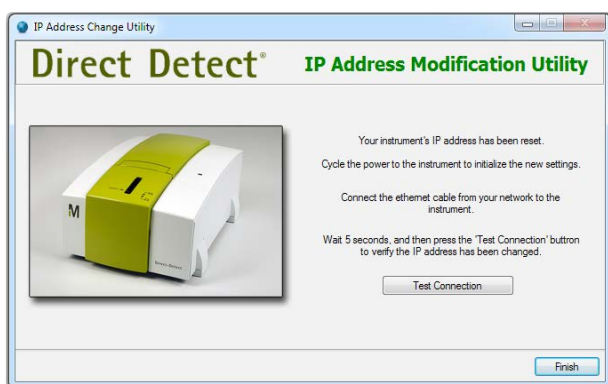


Figure 22. Testing the connection

## 11.2 Changing from Direct Connection to Intranet Connection, continued

11. If the connection was successful, the instrument will produce a tone and a message box will appear to inform you that the connection was successful. If an error message appears, check with your IT department or contact Technical Service (section 17).
12. Select the Finish button to close the software. You should now be able to perform measurements using the Direct Detect® instrument connected to your intranet.

## 11.3 Changing from Intranet Connection to Direct Connection

**IMPORTANT:** This section assumes that the Direct Detect® instrument and Netbook computer are currently connected to your intranet using two standard RJ45 Cat5e Ethernet cables.

1. Run the IP Address Change Utility (section 11.1) and confirm that the requirements are all valid (green check marks).
2. Select the Next button to proceed to the IP Settings screen of the utility and select the first option, I want to connect the instrument directly to the computer.

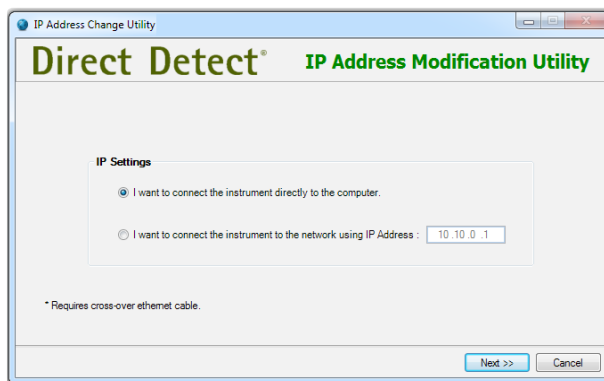


Figure 23. Selecting direct connection

3. Select the Next button to proceed.
4. The software will now make the necessary modifications to the Direct Detect® software and instrument, as well as the host computer. If the modifications are successful you will be informed that your instrument settings have been updated (Figure 24).

## 11.3 Changing from Intranet Connection to Direct Connection, continued

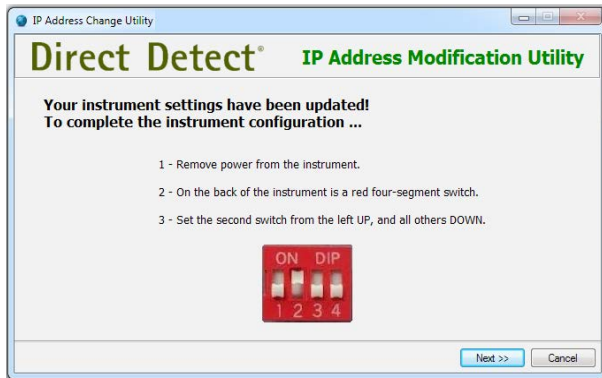


Figure 24. Successful reconfiguration to direct connection

5. Unplug the instrument from the power source, then change the switches in the red 4-position DIP switch (6H in Figure 6) on the rear of the instrument so that the second switch from the left is in the UP position, and all other switches are in the DOWN position (refer to Figure 24).
6. Select the **Next** button to proceed.
7. Run the **crossover 10Base-T** cable with RJ45 connectors supplied with your instrument between the ETH/LAN port on the rear of the instrument (6B in Figure 6) and the network/LAN port on the computer (3B in Figure 3).
8. Reconnect power to the instrument.
9. Wait for the instrument to initialize and then select the **Test Connection** button on the utility screen (Figure 25).



Figure 25. Testing the connection

10. If the connection was successful, the instrument will produce a tone and a message box will appear to inform you that the connection was successful. If an error message appears, check with your IT department or contact Technical Service (section 17).
11. Select the **Finish** button to close the software. You should now be able to perform measurements using the Direct Detect® instrument connected directly to the computer.

## 11.4 Changing Computer Network Settings Manually

Typically the IP Address Changer software makes the appropriate modifications automatically, however, in some cases you may need to do it manually.

1. To access the settings in the Windows® 7 operating system, enter TCPIP in the search field. Select the **View network connections** option in the control panel section.

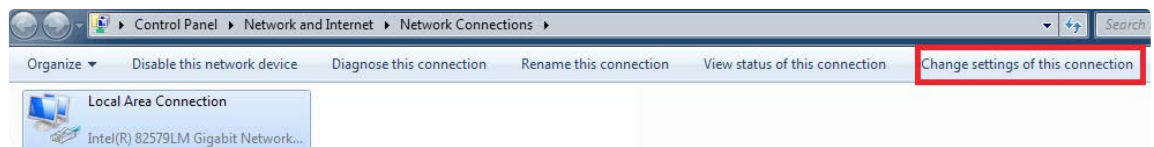
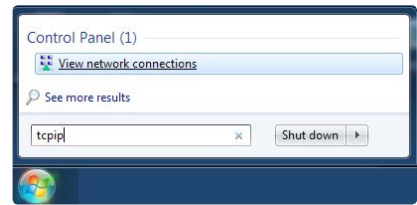


Figure 26. Accessing network connections in the Windows® 7 operating system

2. Select your network connection, and then select option **Change settings of this connection**.
3. This opens the **Local Area Connection Properties** dialog. Select the connection **Internet Protocol Version 4(TCP/IPv4)** and then select the **Properties** button to view this connection's properties.

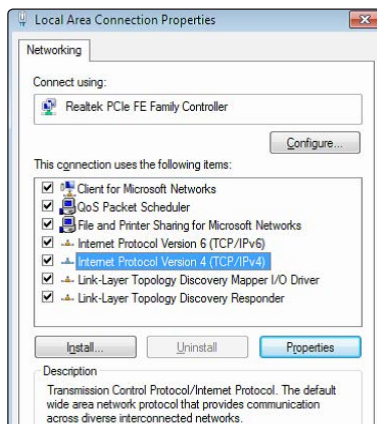


Figure 27. Accessing the connection's properties

## 11.4 Changing Computer Network Settings Manually, continued

- Depending on the desired configuration, choose one of the following options:

**For a direct connection between the Direct Detect® instrument and the computer,** enter the custom IP address 10.10.0.100 and subnet mask setting 255.255.255.0 (Figure 28).

**IMPORTANT:** Do not change the settings on your computer until after you have run the IP Address Change Utility to change the IP address on the instrument and the Direct Detect® software (section 11.2).

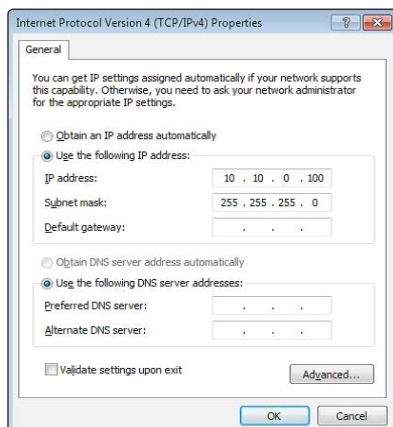


Figure 28. IP address and subnet mask settings for direct connection

OR

**For a connection between the Direct Detect® instrument and the computer via your intranet,** consult with your IT department. Typically the settings are set to obtain the IP address automatically. However, some IT departments may issue fixed addresses.

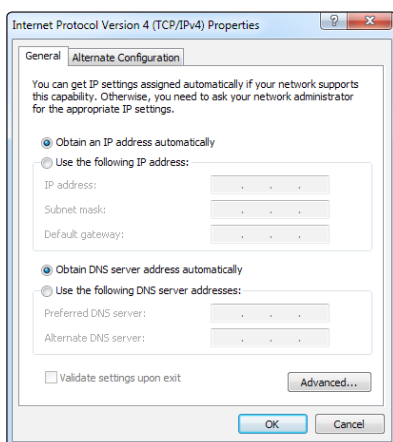


Figure 29. IP address and subnet mask settings

**IMPORTANT:** Do not change the settings on your computer until after you have run the IP Address Change Utility to change the IP address on the instrument and the Direct Detect® software (section 11.3).

# 12 Troubleshooting

## 12.1 General Information

The status of the Direct Detect® spectrometer is indicated by both the instrument status light (located on the top right side of the instrument under the Netbook tray (5E in Figure 5)) and the hardware diagnostics icon (located in the upper right corner of the Direct Detect® Software user interface (Figure 30)). The status light and hardware diagnostics icon colors are typically the same at any given time, and indicate the following:

- Green Instrument is in proper operating condition (flashing green on the instrument indicates stand-by mode)
- Yellow Instrument is initializing or indicating a warning (e.g., IR source has not yet reached its operating temperature, the validity period of the instrument has expired, the air humidity inside the spectrometer is too high)
- Red Instrument has malfunctioned, or has a defective component, or an OQ/PQ test has expired or failed

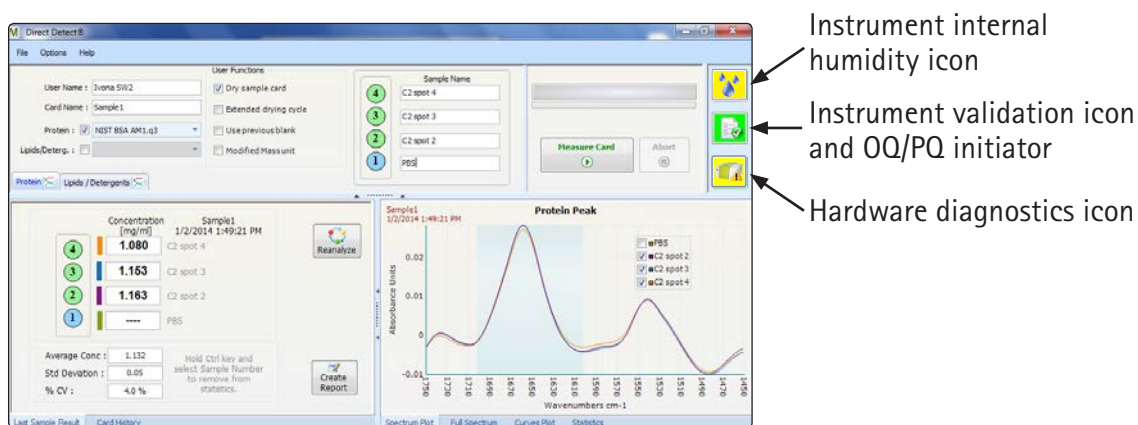


Figure 30. Direct Detect® Software user interface

## 12.1 General Information, continued

Click on the hardware diagnostics icon and the hardware diagnostics dialog opens.

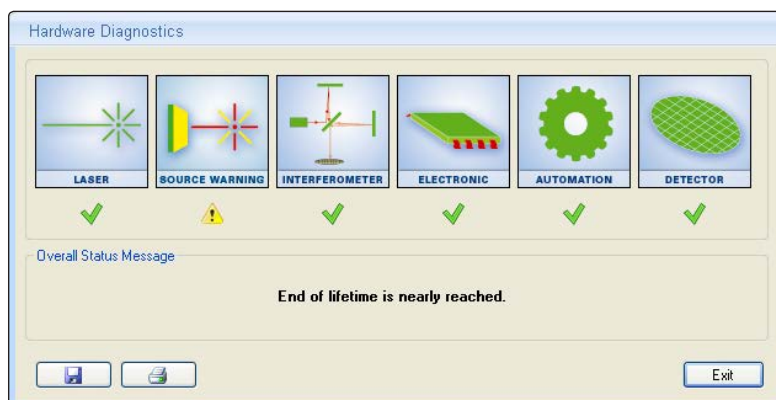




Figure 31. Hardware diagnostics dialog – Instrument Status

More information on hardware diagnostics is available in section 5.1 of the Direct Detect® Software User Guide. If an issue cannot be resolved with the help of this diagnostics section and/or the following troubleshooting tables, refer to section 17 for information on how to contact Technical Service.

## 12.2 Problems Indicated by Hardware Diagnostics Icon

Symptom	Cause	Corrective Action
Hardware diagnostics icon is yellow 	Spectrometer is still in the initialization phase.	Wait until spectrometer initialization is completed (several minutes).
	IR source has not yet reached its operating temperature.	The warm-up takes about 7 minutes. As soon as the IR source has reached its operating temperature, the hardware diagnostics icon will automatically turn green. Wait until the warm-up is completed.
	IR source is nearing end of operational lifetime.	To confirm IR source issue, click on the hardware diagnostics icon (Figure 30) to open the hardware diagnostics dialog (Figure 31). If IR source is indicated, refer to section 13.1 for instructions on how to replace it.
	Instrument internal humidity is above tolerance (also indicated by the yellow internal humidity icon, see section 12.5).	Wait 30 minutes to 24 hours for desiccant to remove the humidity. If after 24 hours the icon is still yellow, refer to section 13.2, Replacing the Desiccant.

## 12.2 Problems Indicated by Hardware Diagnostics Icon, continued



Symptom	Cause	Corrective Action
Hardware diagnostics icon is red 	Instrument test has failed.	To determine which instrument component is causing the test failure, click on the hardware diagnostics icon (Figure 30) to open the hardware diagnostics dialog (Figure 31). If the laser, interferometer, electronics, automation, or detector is defective, contact Technical Service. If the IR source is defective, refer to section 13.1 for instructions on how to replace it.
	A spectrometer component is defective.	To find out which component is defective, click on the hardware diagnostics icon (Figure 30) to open the hardware diagnostics dialog (Figure 31). If the laser, interferometer, electronics, automation, or detector is defective, contact Technical Service. If the IR source is defective, refer to section 13.1 for instructions on how to replace it.

## 12.3 Problems Indicated by Instrument Status Light (top of instrument)


Symptom	Cause	Corrective Action
Spectrometer status light does not light	Spectrometer is not connected properly to the power supply.	Check the power cord connection at the power outlet and the power port on the back of the spectrometer (Figure 3).
	No voltage is applied.	Confirm proper voltage at the power outlet.
	Short circuit in the adapter or power cable.	A short circuit is accompanied by a "ticking" sound in the power adapter. Unplug the power cord immediately and contact Technical Service.
Spectrometer status light is yellow	Spectrometer is still in the initialization phase.	Wait until spectrometer initialization is completed (several minutes).
	IR source has not yet reached its operating temperature.	The warm-up takes about 7 minutes. As soon as the IR source has reached its operating temperature the status light will automatically turn green. Wait until the warm-up is completed.
Spectrometer status light is red	Instrument test has failed.	To determine which instrument component is causing the test failure, click on the hardware diagnostics icon (Figure 30) to open the hardware diagnostics dialog (Figure 31). If the laser, interferometer, electronics, automation, or detector is defective, contact Technical Service. If the IR source is defective, refer to section 13.1 for instructions on how to replace it.
	A spectrometer component is defective.	To find out which component is defective, click on the hardware diagnostics icon (Figure 30) to open the hardware diagnostics dialog (Figure 31). If the laser, interferometer, electronics, automation, or detector is defective, contact Technical Service. If the IR source is defective, refer to section 13.1 for instructions on how to replace it.

## 12.4 Problems Indicated by Instrument Validation Icon

Instrument validation problems are indicated by the Instrument Validation Icon (Figure 30).

Symptom	Cause	Corrective Action
Instrument validation icon is yellow 	An instrument validation test has expired.	To find out which instrument validation test has expired, click on the instrument validation icon to open the instrument validation dialog. Refer to section 5.2 of the Direct Detect® Software User Guide for details on instrument validation.
Instrument validation icon is red 	An instrument validation test has failed.	To find out which instrument validation test has failed, click on the instrument validation icon to open the instrument validation dialog. Refer to section 5.2 of the Direct Detect® Software User Guide for details on instrument validation.

## 12.5 Problem Indicated by Instrument Internal Humidity Icon

Symptom	Cause	Corrective Action
Internal humidity icon is yellow 	Instrument internal humidity is above tolerance.	Click on the internal humidity icon to see what the current humidity level is. If the instrument has been on for more than 60 minutes, and the humidity warning is still displayed, replace the desiccant packets in the rear compartment of the instrument. Refer to section 13.2.

# 13 Maintenance

The Direct Detect® spectrometer is a low-maintenance instrument and the operator can replace components with a limited service life. Replacing the IR source and desiccant are described in this section.

## WARNING

Perform only the maintenance procedures described in this manual and observe the relevant safety precautions. **Failure to do so may cause property damage or personal injury.**

Maintenance or repair procedures not described in this manual should be performed only by an EMD Millipore Corporation service engineer. Refer to the Technical Assistance section for contact information.

## 13.1 Replacing the IR Source

### 13.1.1 IR Source Status Indicator

The Direct Detect® spectrometer is equipped with a highly-efficient, low-power, air-cooled infrared source with an average life span of 44,000 hours. As the end of this life span is approached, the hardware diagnostics icon turns yellow. When you click on the hardware diagnostics icon (Figure 30), the hardware diagnostics dialog appears and displays the message **End of lifetime is nearly reached** (Figure 31). Samples can still be measured, but performance and reliability may be compromised and the IR source (cat. no. DDSP00010-LA) should be replaced as soon as possible.

If the source is defective, the hardware diagnostics icon turns red. When you click on this icon, the hardware diagnostics dialog appears and displays the message **No source is connected**. Samples can no longer be measured, and the IR source must be replaced.

### 13.1.2 Replacing the IR Source



**WARNING:** During spectrometer operation the light source becomes very hot. To avoid burns, wait until the light source has cooled down before replacing it.

1. Unplug the instrument power plug.
2. On the back of the instrument, loosen the 4 cover plate Torx® screws with the Torx® TX20 screw driver supplied (Figure 32).

## 13.1 Replacing the IR Source, continued

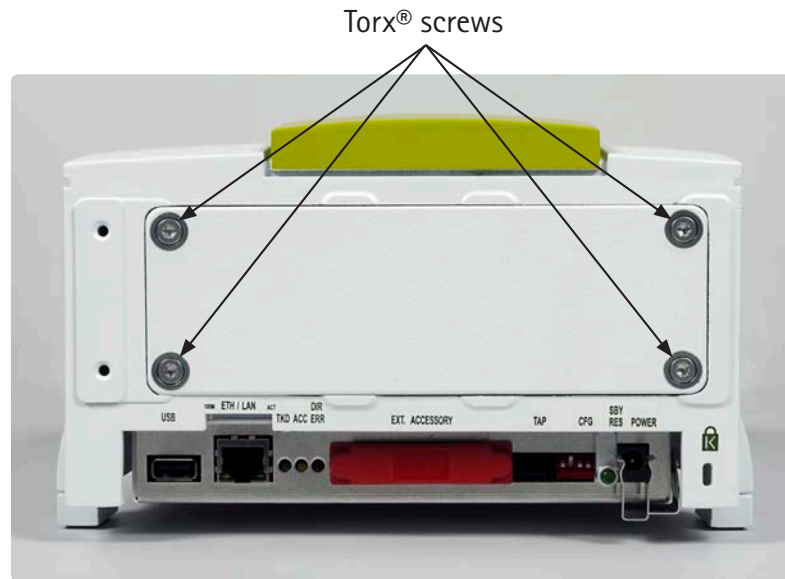


Figure 32. Removing the cover plate

3. Take off the cover plate.

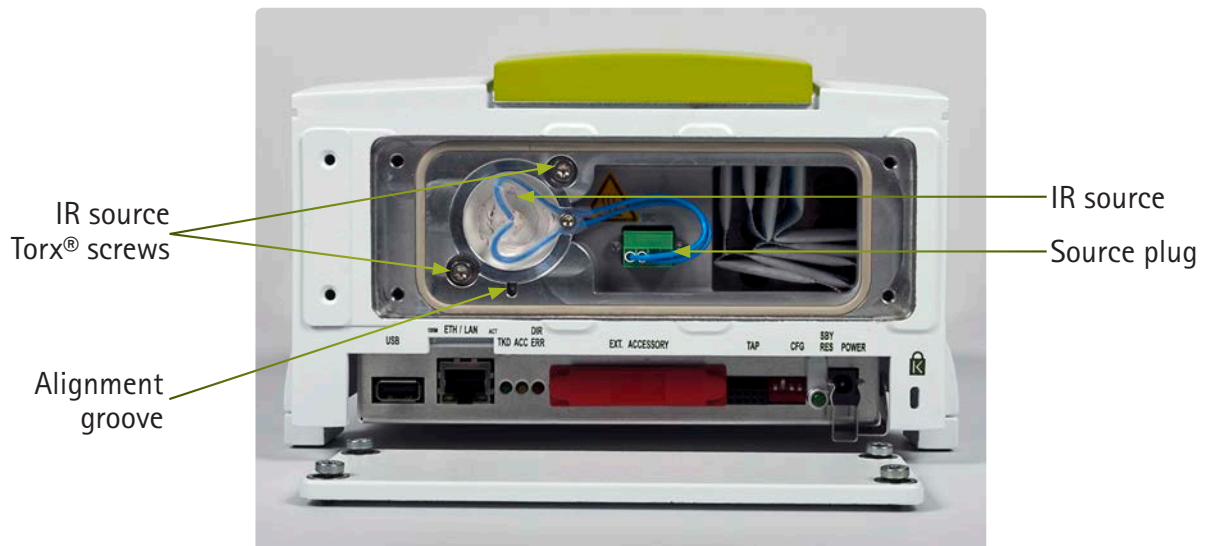


Figure 33. Removing the IR source

4. Loosen the two mounting screws on the IR source (Figure 33) with the Torx® screw driver.
5. Disconnect the green source plug (Figure 33).
6. Pull the source out from the spectrometer housing.

## 13.1 Replacing the IR Source, continued

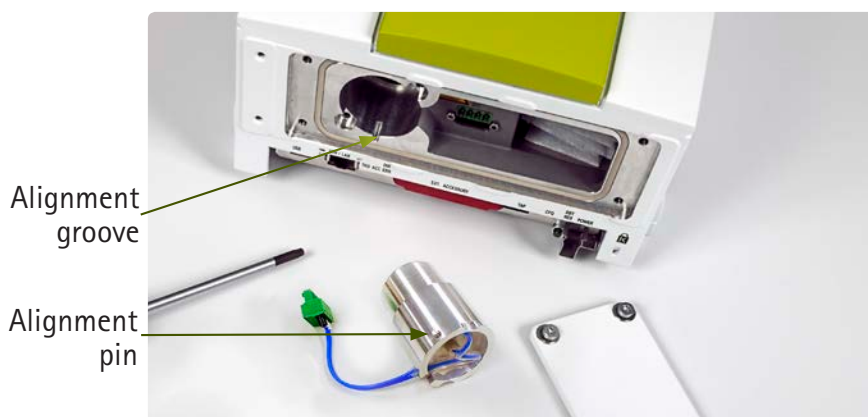


Figure 34. IR source removed from instrument

7. Insert the new IR source into the spectrometer housing. Make sure that the alignment pin fits into the alignment groove (Figure 34).
8. Secure the source by fastening the two Torx® screws (Figure 35) .
9. Plug the green source plug into the SRC female connector.

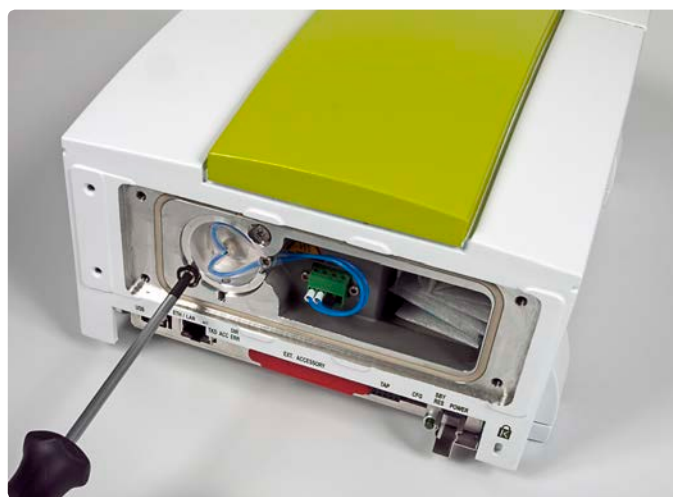



Figure 35. Replacing IR source Torx® screws

10. Attach the cover plate and fasten the 4 Torx® screws (Figure 32). Plug the power plug into the power outlet again.

## 13.1 Replacing the IR Source, continued

After replacing the IR source, perform the Performance Qualification and Operational Qualification instrument tests as follows:

11. Click on the instrument validation button .
12. When the instrument validation dialog appears, click on the **Performance Qualification Run Test** button (1 in Figure 36). The test takes about 5 minutes.

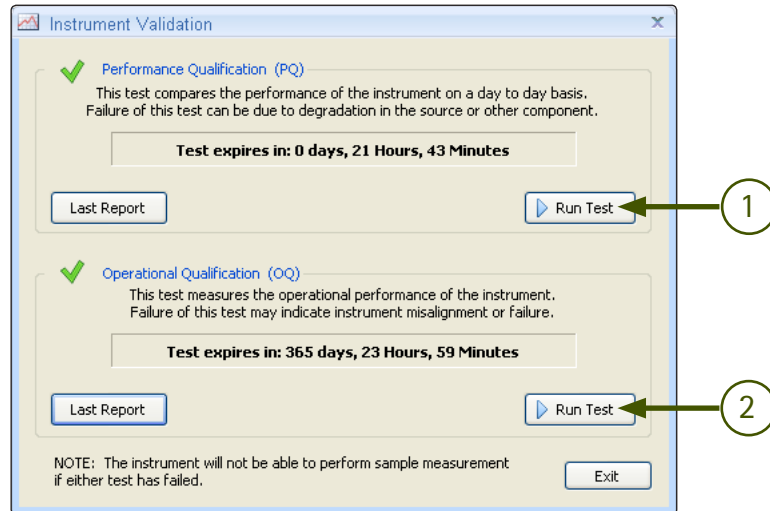


Figure 36. Instrument validation dialog

13. After the test has finished, run the Operational Qualification test by clicking on the **Operational Qualification Run Test** button (2).
14. If the instrument passes the test, the hardware diagnostics icon turns green. If the instrument fails, refer to section 5.1 in the Direct Detect® Software User Guide.

## 13.2 Replacing the Desiccant

Excessive humidity inside the spectrometer may cause H<sub>2</sub>O bands in the spectrum. To reduce H<sub>2</sub>O bands to a minimum, the air inside the Direct Detect® instrument module is convectively dried with a desiccant enclosed in a small, removable, multichambered pack.

If the desiccant is saturated and can no longer absorb humidity, it must be replaced. Both the internal humidity icon (Figure 37) and instrument status light (on top of the instrument, 5E in Figure 5) will turn yellow. When you click on the internal humidity icon, a warning message with the current humidity level is displayed (Figure 37).

## 13.2 Replacing the Desiccant, continued

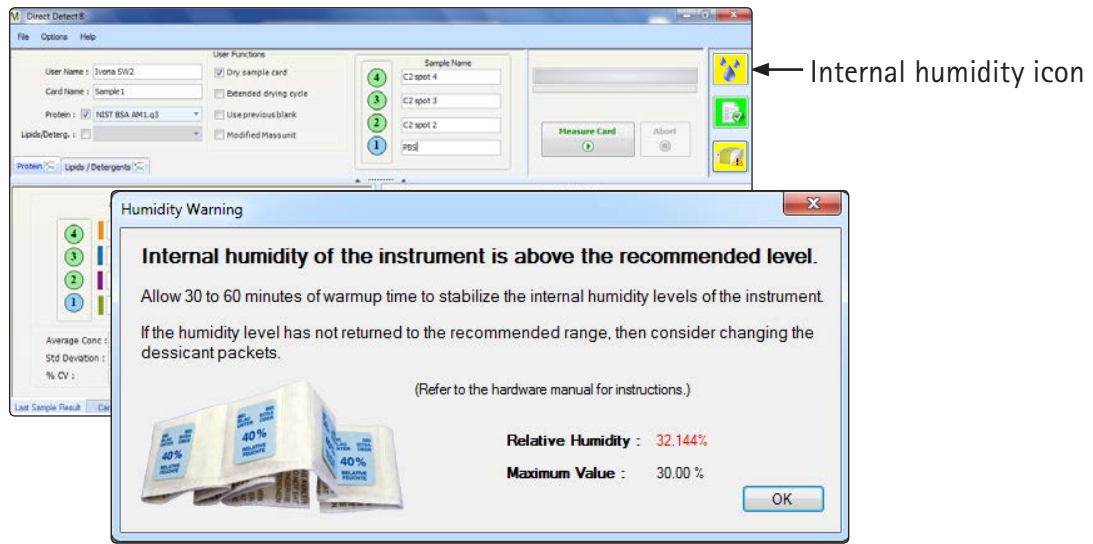


Figure 37. Instrument internal humidity warning

Measuring is still possible if the status light is yellow, but the desiccant should be replaced as soon as possible.

Replace the desiccant as follows:

1. Unplug the power plug.
2. The desiccant is accessible from the rear side of the spectrometer. Loosen the 4 Torx<sup>®</sup> screws (Figure 32) with the Torx<sup>®</sup> screw driver supplied and take off the cover plate.




Figure 38. Replacing the desiccant

3. Pull out the used desiccant pack. A pair of tweezers may be helpful.

## 13.2 Replacing the Desiccant, continued


4. Remove the external packaging from the replacement desiccant pack.
5. Insert the desiccant pack properly into the spectrometer as shown in Figure 38.

 **CAUTION:** Do not cut or tear the pack into single pieces or ingest the desiccant.

6. Attach the cover plate and fasten the 4 Torx® screws again (Figure 32).
7. Plug the power plug into the power outlet again. After the new desiccant removes the moisture from the chamber (approximately 30 minutes), the instrument status light and hardware diagnostics icon will turn back to green.

## 13.3 Cleaning

The outer spectrometer surface can be cleaned with a damp or dry cloth. Do not use detergents with organic solvents, acids, or bases!

 **CAUTION:** Do not try to clean the interior of the spectrometer as this may lead to serious spectrometer damage.

## 13.4 Description of Instrument Validation Tests

### 13.4.1 Performance Qualification Test (PQ)

The Performance Qualification test evaluates the spectroscopic performance and confirms proper function of the IR spectrometer. To ensure that the instrument is working properly, it is recommended that the PQ test be run on a weekly basis.

The factory default interval for the PQ test is one week. One week from the last PQ test, the software will alert you to run the instrument PQ test again. You can use the instrument with an expired PQ test, but the software will query you each time a measurement is initiated, asking if you wish to proceed under the expired PQ condition.

The PQ test protocol consists of the following three tests:

- **Signal-to-noise test:** The signal-to-noise ratio determines the sensitivity of the spectrometer by calculating the average signal-to-noise ratio of ten 100% spectra. A 100% spectrum is the ratio of two successively acquired single-channel spectra with no sample in the sample compartment. The ratio of these two single-channel spectra is used to generate a transmission spectrum.
- **100% Line Test:** This test verifies that the 100% line does not shift over time. It measures the maximum deviation of a 100% line within a larger frequency range. The average of ten measurements must not exceed the predefined limit.
- **Wavenumber Accuracy Test:** - This test ensures that the frequency calibration of the instrument is within the defined limits using an internal polystyrene standard sample.

## 13.4 Description of Instrument Validation Tests, continued

Figure 39 shows a successful PQ test report.

OVP - PQ Test Protocol			
Company:	MILLIPORE		
Operator:	Default		
Instrument Type:	Alpha Sample Compartment RT-DLaTGS ZnSe		
Optics Configuration:	Fibre 1 with: MIR, KBr, RT-DLaTGS [Internal Pos. 1]		
Accessory:	Millipore Direct-Detect		
Instrument Serial Number:	M3		
Instrument Firmware Version:	1.320_MDD_01 Oct 27 2011		
OPUS/DB Version:	OPUS 7.0 Build: 7, 0, 122 / DB: 7.0.105.1106 for OPUS 7.0		
Overall Test Result	PASSED		
Test expires:	12/25/2011, 3:12:41 PM (GMT-5)		
Test Date/Time:	12/15/2011, 3:12:41 PM (GMT-5)		
Test Spectra Path:	C:\Direct Detect\Validation\Data\20111215\151241		
Date of last PQ Reference Measurement			
Comment:			
Signal to Noise Test <span style="float: right;">✓</span>			
Minimum Signal to Noise Limit(area 1):	1000	Signal to Noise measured:	3458
100% Line Test <span style="float: right;">✓</span>			
Maximum 100% Line Deviation:	2.0	Measured 100% Line Deviation:	0.29
Wavenumber Accuracy Test - Polystyrene (35.5 Deg. C, Peak is T. corr.) <span style="float: right;">✓</span>			
Sample Material:	Polystyrene		
Specified Peak:	1601.45 cm <sup>-1</sup>	Maximum Deviation:	0.50 cm <sup>-1</sup>
Measured Peak:	1601.13 cm <sup>-1</sup>		
Corrected Peak:	1601.25 cm <sup>-1</sup>	Measured Deviation:	0.20 cm <sup>-1</sup>
Overall Test Result = PASSED			✓

Figure 39. PQ test report

### 13.4.2 Operational Qualification Test (OQ)

The Operational Qualification test checks instrument performance and compares this performance to the instrument specifications. The OQ test is performed during final testing before an instrument is delivered to the customer. It should also be performed after each major repair, after replacement of optical components which may influence the instrument performance, and after maintenance has taken place.

The factory default interval for the OQ test is 12 months. Twelve months from the last OQ test, the software will alert you to run the instrument OQ test again. You can use the instrument with an expired OQ test, but the software will query you each time a measurement is initiated, asking if you would like to proceed under the expired OQ condition.

The OQ test protocol consists of the following four tests:

- **Wavenumber Resolution Test:** This test verifies the achievable spectrometer resolution by measuring a single-channel (single-beam) spectrum of the water vapor available inside the spectrometer, using the maximum available resolution.

## 13.4 Description of Instrument Validation Tests, continued


- **Signal Sensitivity Test:** This test is the same as the PQ signal-to-noise test, except that the spectra are measured in a very small region (2,200–2,100 cm<sup>-1</sup>).
- **Energy Distribution Test:** This test verifies the achievable frequency range and the energy at the maximum position of the single-channel spectrum. Absolute values of energy are measured at different wavenumber positions. These values are divided by the maximum value achieved, and compared to acceptance values indicated in percent. Optical components (e.g., detector, source, and beam splitter) as well as the card holder have an effect on the limits of the frequency range.
- **Wavenumber Accuracy Test:** The wavenumber accuracy test verifies that the frequency calibration of the spectrometer is within defined limits. Water vapor is used for this test because it has extremely narrow bands, and the position of these bands can be measured with a very high degree of accuracy.

Figure 40 shows a successful OQ test report.

OVP - OQ Test Protocol			
Company:	MILLIPORE		
Operator:	Default		
Instrument Type:	Alpha Sample Compartment RT-DLaTGS ZnSe		
Optics Configuration:	Fibre 1 with: MIR, KBr, RT-DLaTGS [Internal Pos. 1]		
Accessory:	Millipore Direct-Detect		
Instrument Serial Number:	M3		
Instrument Firmware Version:	1.320_MDD_01 Oct 27 2011		
OPUS/DB Version:	OPUS 7.0 Build: 7, 0, 122 / DB: 7.0.105.1106		
Overall Test Result	PASSED		
Test expires:	12/16/2012, 9:32:23 AM (GMT-5)		
Test Date/Time:	12/16/2011, 9:32:23 AM (GMT-5)		
Test Spectra Path:	C:\Direct Detect\Validation\Data\20111216\093223		
Comment:			
Resolution Test			
Water Vapor Band:	1554.35 cm <sup>-1</sup>	Measured Resolution:	1.63 cm <sup>-1</sup>
Maximum Resolution:	2.00 cm <sup>-1</sup>		
Sensitivity Test			
Measurement Region, Start:	2200.00 cm <sup>-1</sup>	Measurement Region, End:	2100.00 cm <sup>-1</sup>
Minimum S/N:	2000	Measured S/N:	3446.07
Energy Distribution Test			
Minimum Energy Value:	5.00%	Energy at 5000.00 cm <sup>-1</sup>	9.14%
Minimum Energy Value:	0.20%	Energy at 500.00 cm <sup>-1</sup>	4.67%
Energy at 7500.00 cm <sup>-1</sup>	0.01%		
Wavenumber Accuracy Test Water Vapor			
Expected Band:	1576.130 cm <sup>-1</sup>	Measured Band:	1576.129 cm <sup>-1</sup>
Maximum Deviation:	0.050 cm <sup>-1</sup>	Measured Deviation:	0.001 cm <sup>-1</sup>

Figure 40. OQ test report

## 13.5 Running the Instrument Validation OQ and PQ Tests

1. Make sure card holder is empty.
2. To run OQ and/or PQ tests, click on the instrument validation icon .
3. When the instrument validation dialog appears, click on the Performance Qualification (PQ) Run Test button (1 in Figure 41). The test takes about 5 minutes.

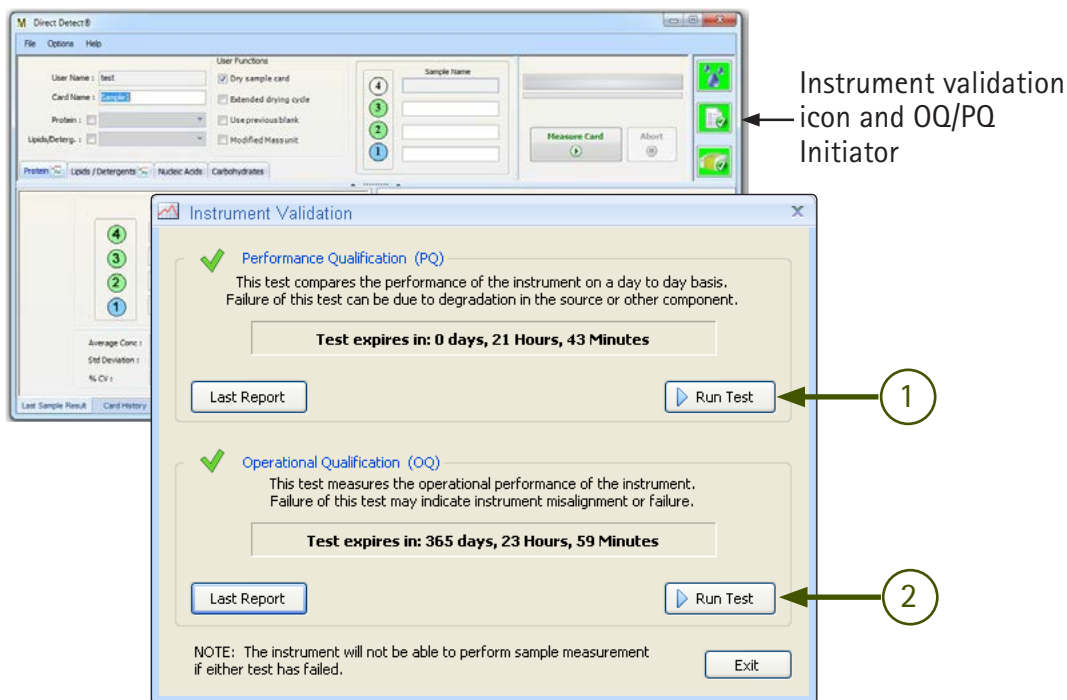


Figure 41. Running OQ/PQ tests

4. To run the Operational Qualification (OQ) test click on the Operational Qualification Run Test button (2). The test takes about a minute to run.

For information on interpreting test failures, refer to section 12.4.

## 13.6 Storage

Store the Direct Detect® spectrometer and Assay-free cards in a clean, dry environment at 15–30 °C.

# 14 Installation, Operation, and Process Qualification Protocol (Optional)

**NOTE:** A 2 mg/mL protein standard (e.g., Thermo Scientific Pierce® or Bio-Rad® Bovine Serum Albumin Standard ) is required for the protein verification test (section 14.5).

## 14.1 Installation Qualification Protocol (IQ)

**NOTE:** The installation qualification should be performed prior to making any local modifications to the PC software.

1. Set up the system as instructed in section 6.5.1.
2. Verify that the Direct Detect® system starts the initialization sequence and that successful completion is indicated by the status indicator light (5E in Figure 5) turning green. Document results on the IQ/OQ/PQ Report Summary page found in section 14.6.

## 14.2 Instrument Hardware Diagnostic Protocol

1. Access the Direct Detect® software by double-clicking on the icon on the Netbook desktop.
2. Log into the instrument using the "Administrator" user name and password "directdetect". Once in the **User Management** screen, create a user name with administrator privileges to run the OQ/PQ tests below. Refer to section 1.2.4, User Management, in the Direct Detect® Software User Guide.
3. Click on the hardware diagnostics icon to open the hardware diagnostics dialog (Figure 42).



## 14.2 Instrument Hardware Diagnostic Protocol, continued

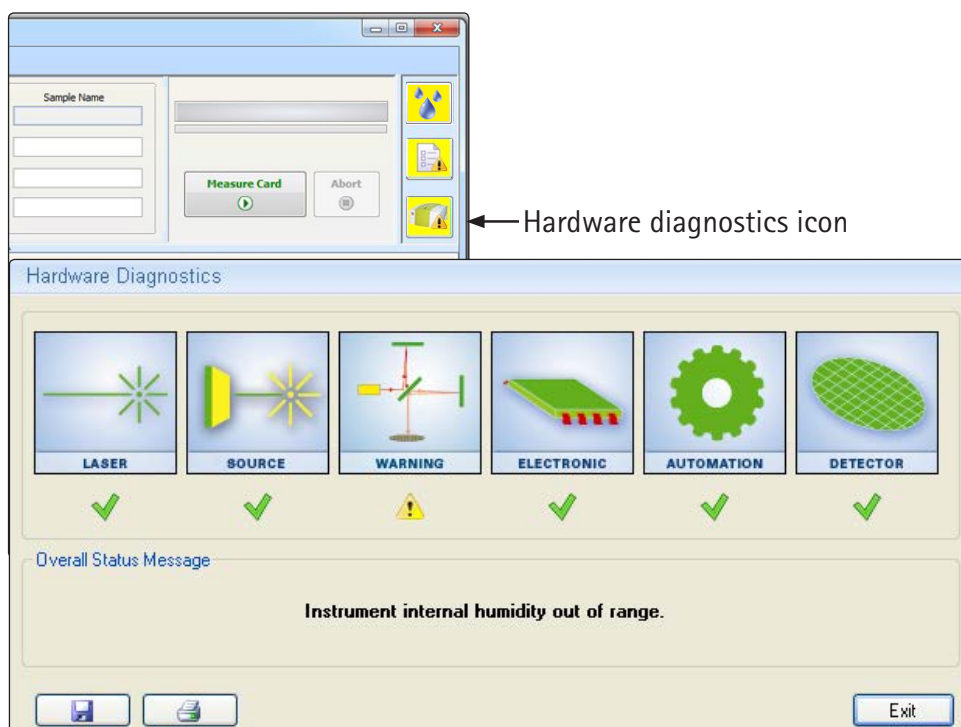



Figure 42. Instrument status

4. A green check mark indicates that each of the systems is functioning correctly. If any systems do not have a green check mark, refer to section 5, Diagnostics, in the Direct Detect® Software User Guide. Correct the problem, then click on the hardware diagnostics icon to verify that all of the check marks are green.

Select the save button  to save the hardware diagnostics report file to the hard drive (C:\DirectDetect\Validation\Reports).

Select the print button  to print the report to an installed printer.

5. Document results on the IQ/OQ/PQ Report Summary.

## 14.3 Operation Qualification Protocol (OQ)

1. Click on the instrument validation icon to open the instrument validation dialog box.

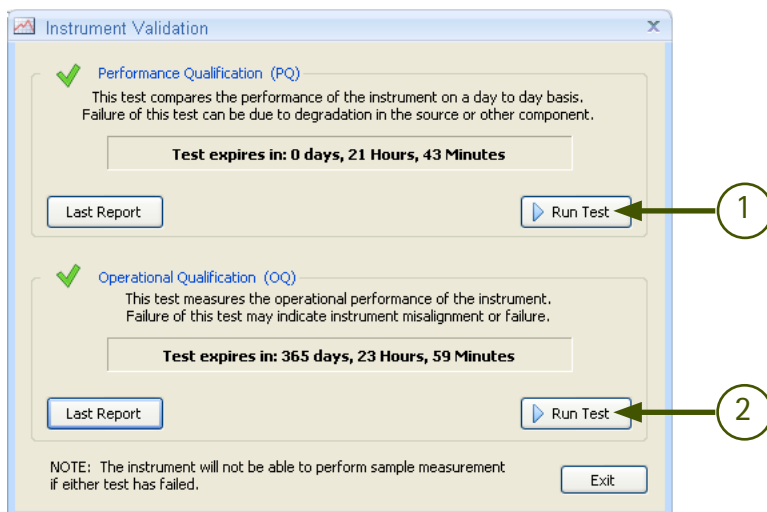


Figure 43. Instrument validation dialog

2. Click on the **Operational Qualification (OQ) Run Test** button (2). This starts a system check that takes several minutes to complete. Run this test regardless of the test expiration date. A report displaying the OQ test results will be generated and stored in C:\DirectDetect\Validation\Reports. The report can be viewed and printed by clicking on the **Last Report** button. Document results on the IQ/OQ/PQ Report Summary.

## 14.4 Performance Qualification Protocol (PQ)

1. After the OQ test has been successfully completed, run the PQ test by clicking on the **Performance Qualification (PQ) Run Test** button (1). Run this test regardless of the test expiration date. A report displaying the PQ results will be generated and stored in C:\DirectDetect\Validation\Reports. The report can be viewed and printed by clicking on the **Last Report** button. Document results on the IQ/OQ/PQ Report Summary.

## 14.5 Protein Measurement Verification

1. After successful completion of the OQ and PQ tests, the system is ready to verify that a protein spotted on the card is equivalent to a standard.
2. Open a new vial of BSA standard.
3. Place the Assay-free card(s) on the spotting tray or other clean surface. Select an appropriate buffer blank, preferably the same solution in which the BSA standard has been prepared. The majority of commercially available BSA standards use NaCl (with  $\text{NaN}_3$ ) as a buffer; check manufacturer specifications. Pipette exactly 2  $\mu\text{L}$  of the buffer solution to the default blank position on the card (position 1).

## 14.5 Protein Measurement Verification, continued


- Pipette exactly 2  $\mu\text{L}$  of sample to sample positions 2 through 4. Card positions can be labeled if desired.
- In the Direct Detect<sup>®</sup> Software main screen, complete the measurement setup as indicated in Figure 44. Choose **NIST BSA AM1** from the protein drop down menu and check the **Dry sample card** box. Sample location 1 should be blue, indicating that the buffer solution is in this location. Sample locations 2–4 should be green, indicating that there is a sample to be measured.

The screenshot shows the software interface for sample information configuration. It is divided into three main sections:

- User Information:** Includes fields for 'User Name' (Ivona), 'Card Name' (BSA Standard), 'Protein' (NIST BSA AM1.q3), and 'Lipids/Deterg.' (empty).
- User Functions:** A group of checkboxes including 'Dry sample card' (checked), 'Extended drying cycle' (unchecked), 'Use previous blank' (unchecked), and 'Modified Mass unit' (unchecked).
- Sample Name:** A vertical list of four sample positions, each with a colored circle and a text input field:
  - Position 4 (green circle): BSA STD spot 4
  - Position 3 (green circle): BSA STD spot 3
  - Position 2 (green circle): BSA STD spot 2
  - Position 1 (blue circle): Buffer

At the bottom, there are two tabs: 'Protein' (selected) and 'Lipids / Detergents'.

Figure 44. Sample information

- Follow the instructions in section 10.2 for inserting the sample card into the instrument, then click on the  button.
- The sample measurement results are displayed on the **Last Sample Result** tab of the main screen.
- Measure the BSA standard concentration using a UV reader at 280 nm.  
**DO NOT** use standard curves built into the UV reader. Use the sequence-based extinction coefficient  $42,925 \text{ M}^{-1}\text{cm}^{-1}$  or  $\text{ABS } 1 \text{ mg/mL} = 0.646$ . Zero the UV instrument with the correct buffer and read absorbance at 280 nm.
- Manually calculate the correct concentration using the sequence-based numbers. The system passes the protein verification test if the results from the UV reader and the Direct Detect<sup>®</sup> system are within 10% of one another. Document results on the IQ/OQ/PQ Report Summary.

**NOTE:** The Direct Detect<sup>®</sup> system comes pre-loaded with standard curves that were generated from phosphate-buffered saline (PBS). Analysis from buffers like NaCl with  $\text{NaN}_3$  delivers accurate quantification if the measurement is performed in a room with humidity level below 35%. Humidity levels above 35% will affect sample drying, resulting in artificially increased concentration estimates.

## 14.6 Direct Detect® System IQ/OQ/PQ Report Summary

Reference Section	Checklist	Result	
		Pass	Fail
14.1	Verify power and cable connections <ul style="list-style-type: none"> <li>● Power adapters are connected to Netbook computer and Direct Detect® instrument</li> <li>● Verify connection between Netbook computer and Direct Detect® instrument (cross over cables)</li> </ul>		
	Verify Software communication <ul style="list-style-type: none"> <li>● Direct Detect® Software launches successfully</li> <li>● Login successful</li> <li>● Instrument detected</li> </ul>		
14.2	Instrument Hardware Diagnostic <ul style="list-style-type: none"> <li>● All functions have green check mark</li> </ul>		
14.3	System OQ <ul style="list-style-type: none"> <li>● OQ test report indicates <b>Overall Test Result = PASSED</b></li> </ul>		
14.4	System PQ <ul style="list-style-type: none"> <li>● PQ test report indicates <b>Overall Test Result = PASSED</b></li> </ul>		
14.5	Verification of Protein Measurement <ul style="list-style-type: none"> <li>● Direct Detect® measurement and UV reader measurement are within 10% of one another for a 2 mg/mL BSA standard.</li> </ul>		
Testing completed by:			
Date:			

# 15 Specifications

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## General

Dimensions	22 cm wide x 30 cm deep x 15.2 cm high (including tray) (8.7 x 11.8 x 6.0 in.) NOTE: Overall height with Netbook computer opened will vary, but is typically less than 40.6 cm (16 in.).
Weight (approximate)	7 kg (15.4 lb)
Power consumption	Maximal: 100–240 VAC, 50–60 Hz (max 2 A)
Electronics	Class 1 laser product (contains 850 nm laser diode)
Environmental conditions	Operational temperature range: 18–35 °C (64–95 °F) Temperature variation: max. 1 °C/h and max. 2 °C/day Humidity (non-condensing): less than 35% (relative humidity) Humidity variation: less than 3%/hr Installation site: in a closed room, max. 2,000 m above sea level Installation overvoltage category: II Pollution degree: 2
Safety regulations	Complies with EN/IEC 61010-1/08.2002 (Safety requirements for electrical equipment for measurement, control and laboratory use)
EMI regulations	Complies with EN/IEC 61326/05.2004 (Electrical equipment for measurement, control and laboratory use - EMC requirements)

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## Performance

Spectral range	6,000–500 $\text{cm}^{-1}$
Resolution	Better than 2 $\text{cm}^{-1}$
Wave number accuracy	Better than 0.05 $\text{cm}^{-1}$
Wave number reproducibility	Better than 0.01 $\text{cm}^{-1}$
Photometric accuracy	0.1% T

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## Optics

Design	Sealed optics housing, convective desiccating
IR Source	Low voltage, air cooled, can be replaced (lifetime 44,000 operating hours)

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## Computer Specifications

Operating System	Microsoft® Windows® 7
Software Framework	Microsoft®.NET Framework 2.0
Screen Resolution	1,024 x 600 or greater
Ethernet Cable	Crossover, 10Base-T (10 or 10/100 MBit) with RJ45 connectors
Processor	Intel® Pentium® III, 800 MHz or greater
Random Access Memory (RAM)	2 GB
Graphics Card	XVGA, 256 Mb RAM, 32-bit color depth, Open GL, GDI+

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## 16 Ordering Information

This section lists catalogue numbers for the Direct Detect® spectrometer and replacement parts. See Technical Assistance section for contact information. You can purchase these products on-line at [www.millipore.com/products](http://www.millipore.com/products).

Product Description	Cat. No.	Qty/Pk
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### Direct Detect® Spectrometer

Direct Detect® Spectrometer (includes instrument with power adapter*, Dell® Netbook computer with power adapter*, Ethernet crossover cable, Netbook computer stand, 50-pack of Assay-free cards, spotting tray, spare desiccant bag, Torx® screw driver, Quick-Start Guide)	DDHW00010-WW	1
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\* Power adapters are configured for multiple countries

### Direct Detect® Spectrometer Replacement Parts/Accessories

Direct Detect® Assay-free Cards	Multiple colors	DDAC00010-4P	50 x 4
	Multiple colors	DDAC00010-8P	50 x 8
	Green	DDAC00010-GR	50
Spotting Tray		DDMISC0010-ST	5
Netbook Stand		DDMISC0010-NS	1
Direct Detect® IR Source**		DDSP00010-LA	1
Direct Detect® Desiccant Bag**		DDSP00010-DE	1

\*\* Contact Technical Service to order (see Technical Assistance section)

## 17 Technical Assistance

For more information, contact the office nearest you. In the U.S., call 1-800-MILLIPORE (1-800-645-5476). Outside the U.S., go to our web site at [www.millipore.com/offices](http://www.millipore.com/offices) for up-to-date worldwide contact information. You can also visit the tech service page on our web site at [www.millipore.com/techservice](http://www.millipore.com/techservice).

## 18 EC Representative

Millipore (UK) Ltd.  
Fleming Road, Kirkton Campus  
Livingston EH54 7BN  
UK

## 19 Standard Warranty

The applicable warranty for the products listed in this publication may be found at [www.millipore.com/terms](http://www.millipore.com/terms) (within the "Terms and Conditions of Sale" applicable to your purchase transaction).