iontorrent

Ion Personal Genome Machine™ (PGM™) System

REFERENCE GUIDE

Catalog Number 4462921

Publication Number MAN0009783

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

Thermo Fisher
SCIENTIFIC

Contents

1 Product information

About this guide

⚠ **CAUTION! ABBREVIATED SAFETY ALERTS.** Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This guide provides reference information for using the Ion PGM™ System.

Note: For information on conducting sequencing experiments, consult the user guide for your Ion PGM™ sequencing kit.

Instruments and server

Components	Cat. No.
Ion PGM™ System and accessories	4462921
Ion PGM™ Torrent Server	4483643
Ion Chip™ Minifuge:	
120 VAC	4479672
230 VAC	4479673

Kits compatible with the Ion PGM™ System

Item	Cat. No.	LIterature
Chips		
Ion 314™ Chip v2	4482261	See your sequencing user guide for instructions.
Ion 316™ Chip v2	4488145	
Ion 318™ Chip v2	4488146	
Ion template preparation kits		
Ion PGM™ Template IA 500 Kit[1]	A24622	*Ion PGM™ Template IA 500 Kit User Guide*
Ion PGM™ Hi-Q™ OT2 Kit[2]	A27739	*Ion PGM™ Hi-Q™ OT2 Kit User Guide*
Ion PGM™ Hi-Q™ View OT2 Kit[2]	A29900	*Ion PGM™ Hi-Q™ View OT2 Kit User Guide*
Ion PGM™ Hi-Q™ Chef Kit [2]	A25948	*Ion PGM™ Hi-Q™ Chef Kit User Guide*
Ion PGM™ Hi-Q™ View Chef Kit[2]	A29902	*Ion PGM™ Hi-Q™ View Chef Kits User Guide*
Ion PGM™ Hi-Q™ View Chef 400 Kit[2]	A30798	
Ion PGM™ Template OT2 200 Kit[3]	4480974	*Ion PGM™ Template OT2 200 Kit User Guide*
Sequencing kits		
Ion PGM™ Hi-Q™ Sequencing Kit	A25592	*Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (MAN0009816)
Ion PGM™ Hi-Q™ View Sequencing Kit	A30044	*Ion PGM™ Hi-Q™ View Sequencing Kit User Guide* (MAN0014583)
Ion PGM™ Sequencing 200 Kit v2	4482006	*Ion PGM™ Sequencing 200 Kit v2 User Guide* (MAN0007273)
Ion PGM™ Hi-Q™ Chef Kit	A25948	*Ion PGM™ Hi-Q™ Chef Kit User Guide* (MAN0010919)
Ion PGM™ Hi-Q™ View Chef Kit	A29902	*Ion PGM™ Hi-Q™ View Chef Kits User Guide* (MAN0014571)

[1] 500-base-read libraries
[2] 100- to 400-base-read libraries
[3] 200-base-read libraries

2 Before you begin

For additional safety information, see "Safety" on page 45.

Site requirements

For site requirements, see the *Ion PGM™ System Site Preparation Guide* (Pub. No. MAN0007516).

Update the software

IMPORTANT! Before proceeding, make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to version 5.0 or later. See "Update the Ion PGM™ System software" on page 13.

Nucleic acid contamination

IMPORTANT! A primary source of contamination is DNA fragments from previously processed samples. Do not introduce amplified DNA into the library preparation laboratory or work area.

IMPORTANT! Possible contamination can occur during the transfer of dNTPs into Reagent Tubes. Be careful to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Change gloves after handling concentrated dNTP stocks.

Instrument vibration and clearances

IMPORTANT! Significant vibration during sequencing may add noise and reduce the quality of the measurements. The Ion PGM™ System must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench (freezers, pumps, and other similar equipment).

IMPORTANT! Position the Ion PGM™ System so that the front bezel is a minimum of 12 in. (30.5 cm) and the Reagent Tubes containing dNTPs are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. Place the instrument at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

Static electricity

IMPORTANT! To avoid possible damage to the chip from static electricity, prior to handling chips, you must ground yourself on the grounding plate (located next to the chip clamp) by touching the grounding plate with your bare hand.

Do not place chips on non-grounded surfaces such as a bench.
Always use the grounding plate to hold chips that are not in the package inserted in the chip clamp or the Ion Chip™ Minifuge bucket.

CO$_2$ contamination

IMPORTANT! Dry ice (solid CO$_2$) must be kept away from areas where buffers, wash solutions, or sources of molecular biology grade water for the Ion PGM™ System are used. High air concentrations of subliming CO$_2$ may change the pH of such buffers during or after their preparation. The stability of the pH of these buffers is a critical factor in the performance of the Ion PGM™ System.

Ventilation requirements

⚠ **WARNING!** Instrumentation must be installed and operated in a well-ventilated environment, defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

Instrument should only be moved by trained personnel

IMPORTANT! The Ion PGM™ System is installed by trained Life Technologies service personnel and should not be moved.

System components

Ion PGM™ Sequencer ports and power switch
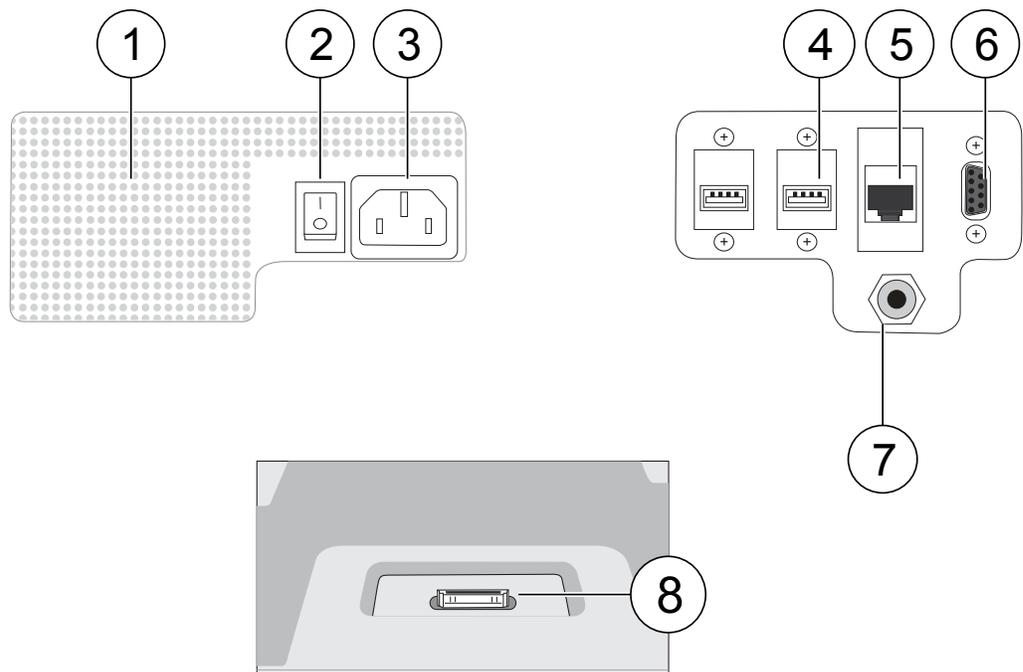
Label	Component	Description
1	Instrument fan cover	IMPORTANT! The fan cover must be unobstructed to ensure adequate cooling and proper functioning of the Ion PGM™ Sequencer.
2	On/off switch	Power switch, where the states are on (	) or off (O).
3	Power port	100–240VAC port that provides power to the instrument.
4	USB ports	Connects the barcode reader to the instrument.

Label	Component	Description
5	Ethernet port	An RJ45 port that provides Ethernet (Gigabit) communication with the Ion PGM™ Sequencer.
6	RS232 port	An instrument diagnostic port
7	Gas inlet	For nitrogen gas.
8	iPod™ port	A port for docking your iPod™ portable media player

Ion PGM™ System with Reagent and Wash Bottles attached
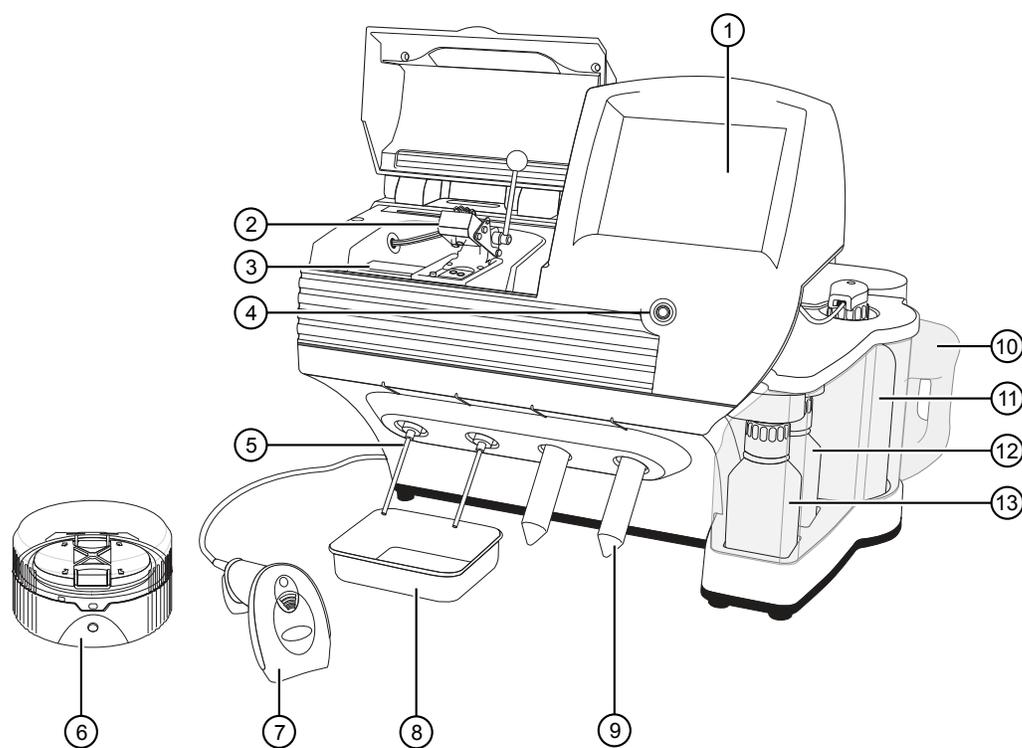
1 Touchscreen
2 Chip clamp
3 Grounding plate
4 Power button
5 Reagent Tube Sipper
6 Ion Chip™ Minifuge
7 Barcode scanner

8 Collection tray
9 Reagent Tube
10 Waste Bottle
11 Wash 2 Bottle (W2 position)
12 Wash 3 Bottle (W3 position)
13 Wash 1 Bottle (W1 position)

3

System layout

We support the layout in which the Ion PGM™ Torrent Server is directly connected to the Ion PGM™ Sequencer, rather than through the local area network from a remote location such as a server room. Data are most robustly transferred from the Ion PGM™ Sequencer to the Ion PGM™ Torrent Server when they are directly connected by a standard Category 6 Ethernet cable provided with the installation materials.

IMPORTANT! The Ion PGM™ Sequencer must be connected to the Ion PGM™ Torrent Server by a standard Category 6 Ethernet cable. We do not troubleshoot data transfer issues associated with an indirect connection between the Ion PGM™ and the Ion PGM™ Torrent Server.

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① Ion PGM™ Torrent Server   ④ Internet
② Ion PGM™ Sequencer   ⑤ Torrent Browser
③ Local area network

4
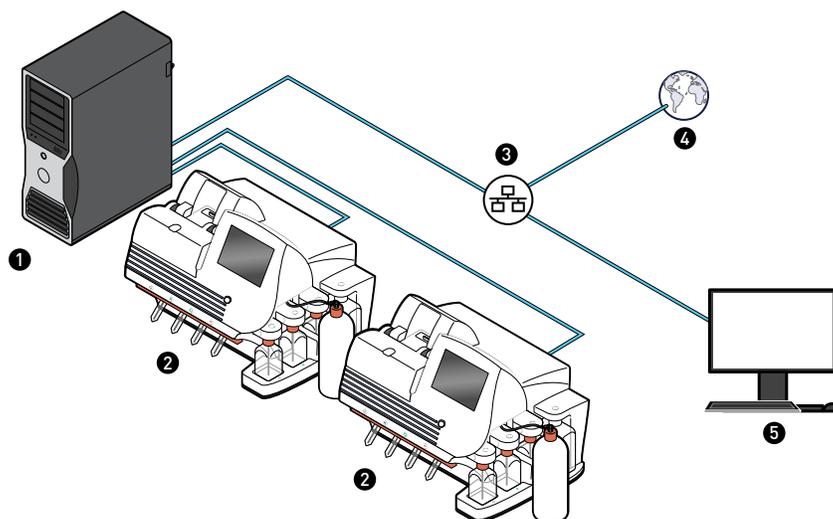
Instrument operation

Power the Ion PGM™ Sequencer on or off

Power on

Note: If the Ion PGM™ Sequencer is powered on, and the touchscreen is blank, touch the screen to "wake" the touchscreen.

1. Find the power switch on the back of the instrument and turn to the on (|) position.

2. Press the power button on the front of the instrument. The switch should illuminate. When the instrument touchscreen Main Menu appears, the instrument is ready for use.

3. See the user guide for your sequencing kit for instructions on how to perform an 18-MΩ water or chlorite solution cleaning after powering on.

Power off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument will not be used for more than 3 days, power off the instrument as follows:

1. In the Main Menu, select **Tools > Shut Down**.

2. If you have not already cleaned the instrument, select 18 MΩ water cleaning, then press **Next** to start the cleaning process.

3. When cleaning is complete, press **Shut Down**.

4. After you exit the main touchscreen, press the **Halt** button, then **OK** when prompted. The instrument will power down.

Update the Ion PGM™ System software

Note: An internet connection is required for the Ion PGM™ System to receive alerts that software updates are available.

If an update to the Ion PGM™ Sequencer software is available, the red "Alarms and Events" pop-up appears in the touchscreen Main Menu to alert you. Press the red pop-up to see the detailed messages. If a message states New Software Available, update the software as follows:

1. In the Main Menu, select **Options ▸ Updates**.

2. Select the **Released Updates** checkbox, then press **Check**.

3. When the message Press Update to begin update process appears, press **Update**.

   Note: If the message All Software Current appears, press **Back** to return to the Main Menu.

4. When the message Installing Completed displays, follow the onscreen prompts to restart the instrument.

   Note: In some cases, the instrument restarts automatically after software installation.

Clean and initialize

See your sequencing guide for instructions on cleaning and initializing the instrument.

Touchscreen reference

Clean, Initialize, and Run
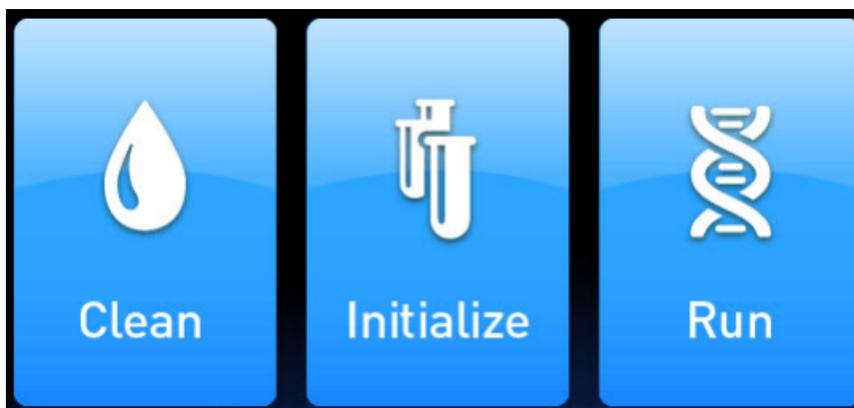
Within the Home screen the **Clean**, **Initialize**, and **Run** programs lead you through the necessary steps to prepare the instrument for sequencing and to start a sequencing run.

- Cleaning must be performed before each initialization to ensure that the reagents from the previous run are cleared from the fluid lines. The **Clean** program is normally performed automatically at the completion of the previous sequencing run. Perform a Clean if for any reason the sequencing run was not properly completed. Follow the instructions provided on the touchscreen.

- The **Initialize** program must be performed before each run to load and prepare the run reagents. The Initialize program walks you through:
  - Emptying the waste reservoir.
  - Loading the reagent cartridge, wash solution, and cleaning solution. (After this step, the instrument performs a reagent check.)

Simple easy to follow instructions are provided on the touchscreen.

- The **Run** program walks you through steps leading up to and through sequencing, including:
  – Placing a loaded chip on the instrument.
  – Selecting a planned run created in the Torrent Server Software.
  – Performing sequencing.

Options and Tools
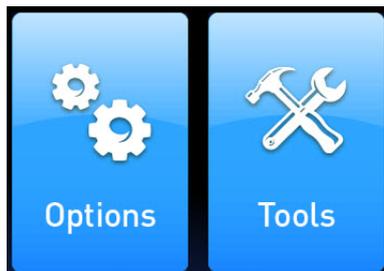
- The **Options** menu gives you access to software updates.
- The **Tools** menu gives you access to troubleshooting tools and to the instrument Shut Down and Reboot commands. See the following table for details.

Options

The Options menu gives you access to software updates and configure instrument settings.

Options menu

Item	Description	When/How to use
Updates	Checks for and installs updates	If alerted by the red "Alarms and Events" pop-up in the touchscreen Main Menu (see "Update the Ion PGM™ System software" on page 13 for details): 1. Press **Check** to determine if any software updates are available. 2. Press **Update** to install the updates.

Item	Description	When/How to use
Set Time	Synchronizes the time on the instrument with that on the Torrent Server	Use this option to configure the time on the instrument, either automatically or manually: 1. Select either the Auto or Manual tab then check the box in the tab. 2. Press **Update Time** to confirm and set the time.
Set IP	Allows you to change the instrument name, confirm the FTP, Torrent Server IP, and user information	For troubleshooting if directed to do so by Technical Support: 1. Touch the screen on the line you want to edit to activate the field. A virtual keyboard will appear. 2. Enter the new information, then press **Accept**.
Advanced	Allows you to set and/or change your Internet Parameters (DHCP or Static IP)	1. Select either the DHCP or Static IP tab. 2. Check the box to enable the internet parameter of your choice and apply the option.

Touchscreen tools
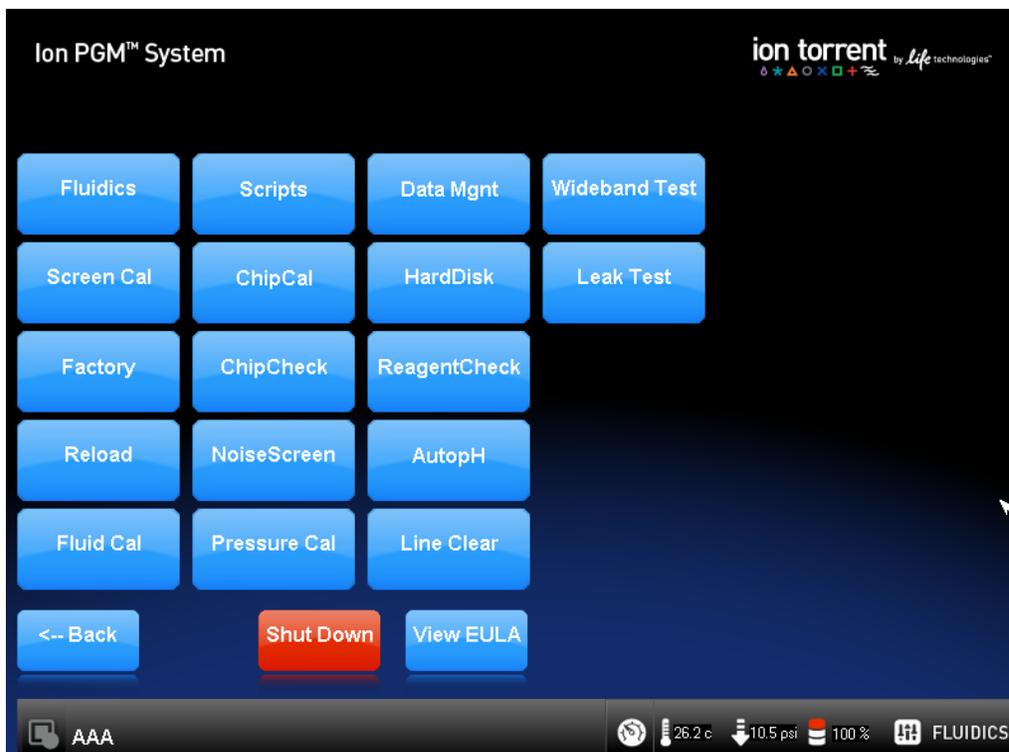
Tools menu options

Item	Description	When to use
AutopH	Adjusts the pH of the Wash 2 Bottle solution and checks the pH. This task is normally performed by the instrument as part of the Initialize program.	If directed to do so by Technical Support as part of a troubleshooting procedure.
ChipCal	Runs the chip calibration portion of the Run program. Chip calibration is performed by the instrument as part of the Run program, both before and after sample is loaded on a chip.	If necessary to calibrate chips without using the Run program.
ChipCheck	The instrument performs a QC check of a new chip.	Should be performed before each sequencing run. For details consult the appropriate sequencing guide.
Data Mgnt	Allows you to manually delete run data or transfer the data the server. Under normal conditions, run data is automatically transferred to the Torrent Server, then deleted from the instrument hard drive.	To troubleshoot data management issues. See "Error message: Not enough disk space for the necessary number of flows" on page 36.
Factory	Should be used by service engineers or under their direction only.	N/A

Item	Description	When to use
Fluidics	Should be used by service engineers or under their direction only.	N/A
Fluid Cal	Should be used by service engineers or under their direction only.	N/A
HardDisk	Should be used by service engineers or under their direction only.	N/A
Leak Test	Should be used by service engineers or under their direction only.	N/A
Line Clear	Used to identify and clear blockages in the waste lines.	See Line Clear procedure in this guide.
NoiseScreen	Provides real-time measurement of electrical noise readings on the chip.	For troubleshooting if directed to do so by Technical Support.
Pressure Cal	Should be used by service engineers or under their direction only	N/A
Reagent Check	Measures the pH of all reagents on the instrument. This task is normally performed by the instrument as part of the Initialize program.	If directed to do so by Technical Support as part of a troubleshooting procedure.
Reload	Should be used by service engineers or under their direction only.	N/A
Screen Cal	Calibrates the touchscreen.	• Follow the onscreen prompts. • If the touchscreen continues to malfunction after Screen Cal, contact Technical Support.
Scripts	Runs various procedures on the instrument.	If directed to do so by Technical Support as part of a troubleshooting procedure.
Shut Down	Access to "Shut Down" and "Reboot" commands. **Note:** It is not necessary/recommended to power off the instrument overnight or over the weekend. If necessary to power off the instrument, see "Power off" on page 13.	If directed to do so as part of a troubleshooting procedure.

Item	Description	When to use
View EULA	View End User Licence Agreement.	Want to check End User Licence Agreement.
Wideband Test	Should be used by service engineers or under their direction only.	N/A

Line clear procedure

1. Click on **Line Clear** (Home screen >**Tools**>**Line Clear**)

2. Click on **Flow Check**.

3. Click on **Line Clear**.

4. Follow the instructions on the screen.

Touchscreen gauges

Press the Gauges icon in the lower right corner of the touchscreen to show or hide the instrument gauges.
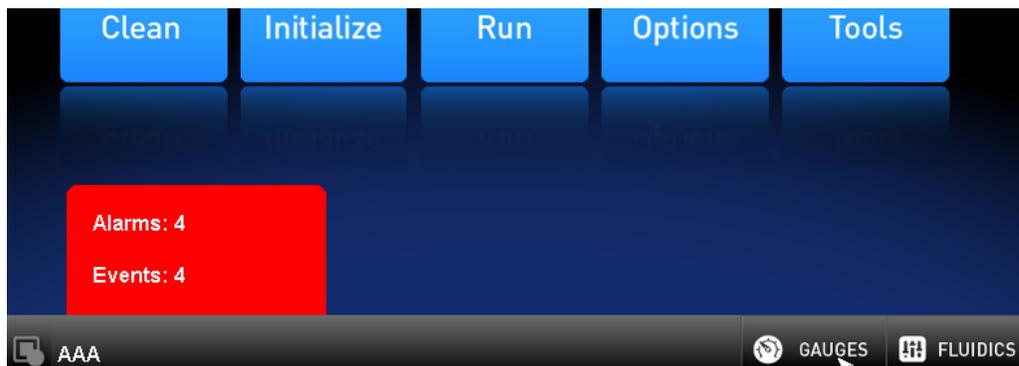
Gauges icon
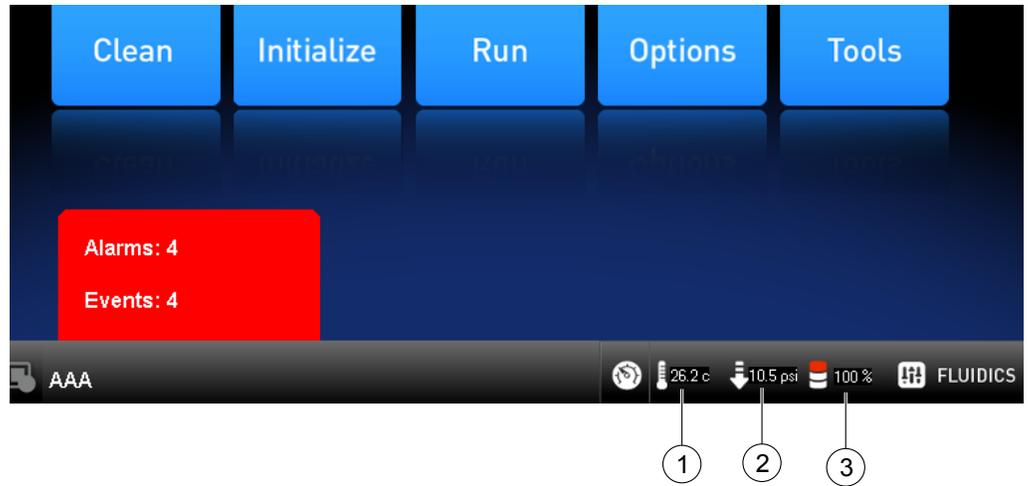
Label	Icon	Description
1		Chip compartment temperature. The expected value when the lid is closed is 35.00 C. If the icon is red, see "Temperature icon indicates chip compartment temperature is out of range" on page 36.
2		Instrument gas pressure. The expected value is 10.50 psi during cleaning and initialization, and 8.0 during a sequencing run. If the icon is red, see "Error message: Confirm instrument has gas pressure" on page 37.
3		Percent instrument hard drive and SSD in use. If the icon is red, see "Error message: Not enough disk space for the necessary number of flows" on page 36.

Ion Chip™ Minifuge and barcode scanner

Ion Chip™ Minifuge

The Ion Chip™ Minifuge is supplied with one custom rotor and two buckets. The buckets are designed to hold two chips: one in each bucket. The rotor and bucket design enables effective and efficient reagent loading of chips.

Safety precautions

⚠ **CAUTION!**
- Make sure your supply voltage matches the voltage label on the minifuge, i.e., never plug a 120V minifuge into an 220–240 VAC outlet. Operating the minifuge with a supply voltage outside the specified range may cause a fire or electric shock.
- Do not run the minifuge for more than 30 seconds.
- Never operate the minifuge without a rotor properly attached to the shaft.
- Never operate with only one chip in place. A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.
- Never put hands in the rotor area unless the rotor is completely stopped.
- Never move the minifuge while the rotor is spinning.
- Do not leave the minifuge running when not in use.

Voltage selection

Two different minifuges are available, depending on your supply voltage: 120 VAC and 220–240 VAC. Make sure that the voltage specification on the label of your minifuge matches the supply voltage. If they do not match, change your supply voltage or contact Customer Support to request the appropriate minifuge.

⚠ **CAUTION!** Never plug a 120V minifuge into an 220–240 VAC outlet, or vice versa. Operating the minifuge with a supply voltage outside of the range specified on the label may cause a fire or electric shock.

Voltage, RPM, and RCF

The following tables list the revolutions per minute (RPM) and relative centrifugal force (RCF) at different voltages.

120/50 VAC, 60 Hz	RPM	RCF
90	4100	836
100	4550	1030
110	4960	1224
120	5330	1424
130	5710	1628

230/50 VAC, 60 Hz	RPM	RCF
210	5070	1279
220	5310	1403
230	5515	1513
240	5705	1619
250	5900	1732

Operation

1. Place the Ion Chip™ Minifuge on a level, clean surface near an accessible power outlet so that the cord and outlet are within easy reach of the operator.

2. Make sure the power switch on the minifuge is in the "off" position.

3. Load a chip into each bucket.

   IMPORTANT! A chip must be present in each bucket to balance the rotor. If necessary, you can balance a loaded chip with a used chip of any type.

4. Turn the power switch on.

5. To begin centrifugation, close the lid of the minifuge. (The centrifugation time will vary depending on the step in the chip-loading protocol.)

6. To stop centrifugation, press down on the lid release tab on the front of the minifuge.

   ⚠ **CAUTION!** Do not attempt to open the lid or remove the chips until the unit has come to a complete stop.

7. After the rotor has stopped, open the lid by grabbing it with the thumb on the front and fingers on the back, then lifting the lid back on the hinge.

Cleaning

To clean the minifuge, use a damp cloth and a mild, noncorrosive detergent (pH <8). After cleaning, ensure that all parts are dried thoroughly before attempting to operate the unit. Do not immerse the centrifuge in liquid or pour liquids over it.

Note: Use only the cleaning protocol described above.

Barcode scanner

The barcode scanner provided with the Ion PGM™ System uses a low-power, visible-light diode.

⚠ **CAUTION!** As with any very bright light source, you should avoid staring directly into the light beam. Momentary exposure to a Class 2 laser is not known to be harmful.

⚠ **CAUTION!** Use of controls, adjustments, or performance of procedures other than those specified in this guide may result in hazardous laser light exposure.

The barcode scanner specifications are listed below.

Wavelength	Rated Power
630–680 nm	1 mW

6 Sharing Planned Runs between Torrent Servers

Enable Planned Run sharing

Starting in Torrent Suite™ Software v4.4, Planned Runs created on one Torrent Server can be transferred to another Torrent Server. This is useful if a sequencer connected to a particular server is offline or busy.

For example, the figure below illustrates a scenario where three Torrent Servers are on the same subnet, and a Planned Run created on TS 1 (the "origin" server) is transferred to TS 3 (the "destination" server) for use on the sequencer connected to TS 3.
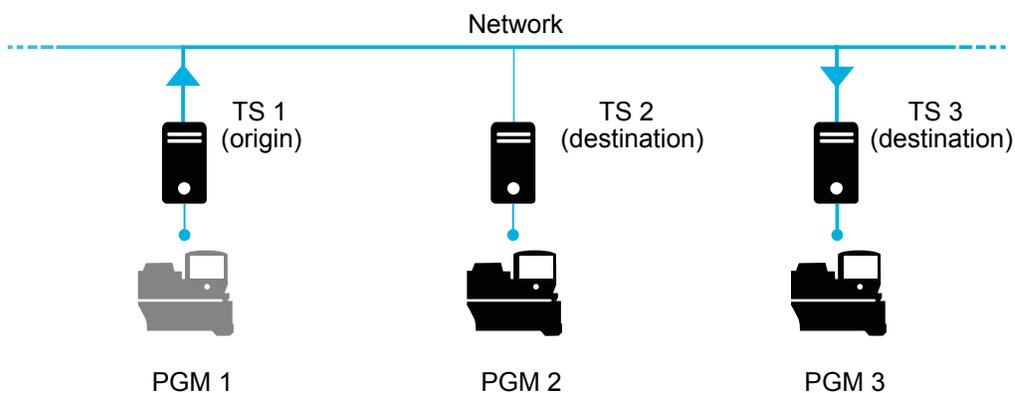
Requirements include:

*   All Torrent Servers must be on the same subnet.
*   All Torrent Servers must be running the same software version.
*   All Torrent Servers must have the same genomic reference, barcode set, BED files, Variant Caller config files, etc.

Set up Server Network (admin action)

1. On the *origin* server (e.g., TS1) Site administration page, scroll down and select Shared servers. The Select shared server to change window appears.

2. If you are adding your *destination* server for the first time, click **Add shared server**.

3. Define your *destination* server.

   a. Enter the name, address (can be IP address), user name and password for the *destination* server.

   b. Click **Active** if you want this server enabled for sharing.

   c. *(Optional)* Add a comment.

   d. Click one of the **Save** options.

4. *(Optional)* If you want to configure the origin Torrent Server to also be a destination server, you must go to another server and repeat these steps to set the origin server as a destination server. Once the Torrent Servers are configured, you or a user can now transfer Planned Runs between Torrent Servers.

Transfer a Planned Run

1. Using the Torrent Browser on the *origin* Torrent Server, go to **Plan ▸ Planned Run List**.

2. Open the Gear ⚙ menu of the Planned Run you want to transfer, and select **Transfer**. Then select the *destination* Torrent Server.
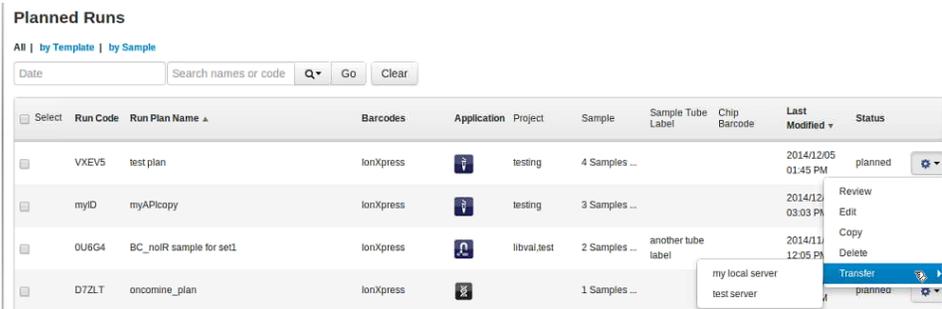
3. A confirmation window appears. Check the information, then click **Transfer**.

   Note: You can no longer access this Planned Run on the origin server after it has transferred.
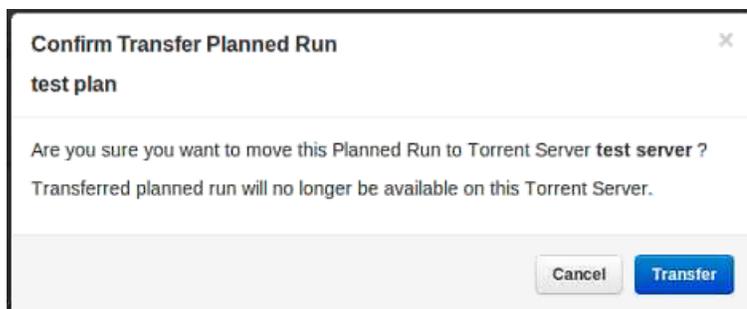
4. A status window displays the results of the transfer:
   - The green box lists the samples successfully processed and the required target BED files found on the destination server.
   - The red box lists any required BED files or plugins that are not present on the destination server. To successfully perform the run, you will need to edit the transferred Planned Run on the destination server and manually add the missing BED files or plugins.

test plan

Successfully created test plan on Torrent Server **test server**

....processed Samples: Sample 2, Sample 3, Sample 1

....found BED files: target.bed

....found IR account IonEast IR (Version: 4.2 | User: Ion User | Org: IR Org)

Planned run data is incomplete, please **Edit test plan** to fix the following errors

Unable to find bedfile: HSMv12.1_hotspots.bed for reference: hg19

Unable to find bedfile: atarget.bed for reference: hg19

Close

5. To edit the transferred Planned Run and add missing files:
   a. Download required files using the References tab of the Torrent browser of the destination server
   b. Go to the Edit Plan wizard of the transferred Planned Run by selecting Edit on the gear pull-down menu to the right of the Planned Run.
   c. Select the files or plugins as needed, then click **Update Plan**.

   Note: You can also navigate to the Edit Plan wizard by clicking the **Edit test plan** link in the status page above.

Undo a Planned Run transfer (administrator)

1. On the *destination* server, delete the transferred Planned Run from either the Planned Run page or the admin page.

2. On the *origin* server, locate the plan on the /admin/rundb/plannedexperiment/page, uncheck **PlanExecuted** and change **PlanStatus** to **Planned**.

Change planned experiment

PlanName:	test_plan
PlanGUID:	97ef4510-b7c0-4b09-8a91-6fc8008d9aae
PlanShortID:	VXEV5
☐ PlanExecuted	
PlanStatus:	Planned ▾
Username:	ionadmin
PlanPGM:	
Date:	Date: 2014-12-05 Today	🗓
	Time: 14:07:07 Now	⊘
PlanExecutedDate:	Date: Today	🗓
	Time: Now	⊘
MetaData:	{"destination":"test server","location":"http://6wvvpq1.ite/rundb/api/v1/plannedexperiment/92/"}
ChipBarcode:	
SeqKitBarcode:	
ExpName:	
☑ UsePreBeadfind	
☑ UsePostBeadfind	
Cycles:	
AutoName:	

Undo a Planned Run transfer (user)

If you transferred a Planned Run in error, you can transfer it back to the origin server or to another server.

1. On the destination Torrent Server, navigate to **Plan ▸ Planned Run List** and locate the transferred Planned Run.

2. From the Gear ⚙ menu of the Planned Run, select **Transfer**, then select the Torrent Server to which you wish to transfer the run.

Manually adjust W2 pH

Materials and equipment needed

- Orion Star™ A111 pH Benchtop Meter Kit (Fisher Scientific, Cat. No. 13-645-503), or equivalent
- Nitrogen gas tank, tube, and flow meter
- 100 mM NaOH (prepared fresh daily)
- Pipette tips and pipette
- Magnetic stirrer and stir bar
- 100 mM HCl

Procedure

If an error message during the automatic pH process indicates that there is a problem adjusting the pH of the W2 Solution, use the following procedure to adjust the pH of the W2 Solution in the Wash 2 Bottle manually.

1. Before proceeding, rinse an empty Wash 2 Bottle and have it ready next to the instrument. Also have an extra Wash 2 Bottle cap ready.

   Note: Gas will be flowing out of the Wash 2 cap, so perform the next steps as quickly as possible (flowing gas will not harm the W2 Solution, and is not a hazard).

2. Remove the Wash 2 Bottle attached to the instrument, then cap the bottle.

3. Secure the empty Wash 2 Bottle (from step 1) to the instrument—do not remove the sipper. This bottle contains the gas flowing out of the instrument while adjust the pH of the W2 Solution, and protects the sipper from contamination.

4. Move the Wash 2 Bottle containing the W2 Solution to the stir plate near the nitrogen gas tube.

5. Secure the gas tube so that it extends inside the mouth of the Wash 2 Bottle but not below the surface of the W2 Solution.

6. Set the gas flow to 0.5 lpm. Start mixing the W2 Solution fast sufficient for a small whirlpool to form.

7. Calibrate the pH meter using a three-point calibration. Rinse any buffering solution from the pH probe before preparing solutions.

8. Adjust the pH of the W2 Solution to 7.55 ± 0.1 by adding a small amount of freshly prepared 100 mM NaOH to the solution, then measuring the pH using the pH meter. Add small aliquots, then allow the pH to equilibrate before adding more.

   Note: If the pH rises above 7.75, use 100 mM hydrochloric acid (HCl) to readjust the pH to 7.55 ± 0.1.

9. When the pH is stable, turn off the gas, remove the gas line, then cap the Wash 2 Bottle.

10. Move the bottle to the instrument, remove the empty Wash 2 Bottle from the instrument, then place the sipper inside the Wash 2 Bottle whose pH adjusted.

11. Secure the cap firmly. Press **Next** to exit the automated pH check, then continue with instrument initialization.

Sequencing run times

Number of flows	Average read length [1]	Average run time by chip type: 314/316/318	Single read runs/kit[2]
850	400 bp	4.8 / 6.3 / 9.4 hours	4
500	200 bp	2.4 / 3.1 / 4.5 hours	8
260	100 bp	1.3 / 1.7 / 2.4hours	12

[1] Read length may vary based on library size.

[2] Only 4 runs are supported for any read length. For best results, run should be started within 1 hour after initialization.

A

Troubleshooting

Alarms/events pop-up

If the red Alarms/Events pop-up appears, press the pop-up to see detailed messages.

Alarm/Event message	Recommended action
Pressure too low	1. Verify that the gas cylinder has at least 500 PSI and 30 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and the Ion PGM™ Sequencer are open. 2. Once you confirm gas pressure leading to the instrument, press **Yes** to retry verification of gas pressure. If the test continues to fail, contact Technical Support.
Pressure too high	Ensure input pressure to the Ion PGM™ Sequencer is correct.

Alarm/Event message	Recommended action
Failed to set digital pressure regulator	Check input pressure.
Temperature too low/high	Verify that lab temperature meets the recommendation. If temperature is normal and alarm persists, contact Technical Support.
Unable to read internal temperature	Contact Instrument Hardware Services.
Unable to read chip temperature	
Results drive not accessible	
Bad boot drive detected	Contact Field Services.
Valve board not accessible	
Valve board downstream errors	
Fan current usage low	
Heater current usage low	
FPGA memory test failed	
Bad results data drive detected	Contact Technical Support.
Unexpected image file size	
Hard drive write slow possible due to bad hard drive	
U-boots don't match	Contact Technical Support.
Kernels don't match	Contact Technical Support.
Potentially losing connection to Torrent Server	1. Disconnect, then re-connect the ethernet cable. 2. Confirm the router is operational. 3. Verify that the network is up and running.
No connectivity to Torrent Server	
No connectivity to FTP server	

Alarm/Event message	Recommended action

Cannot connect to Torrent Server due to
 	
• invalid user ID or password
• invalid IP address
	1. Check that your user id and/or password are correct.
	2. If alarm persists:
	   a. Power off the instrument: In the Main menu, select **Tools ▸ Shut Down ▸ Shut Down**.
	   b. Wait 30 seconds, then press the button on the front of the instrument to power on the instrument.
	3. If the message still appears in the main menu, contact Technical Support.

Newer software available	Update your software. See "Update the Ion PGM™ System software" on page 13.

Observation	Possible cause	Recommended action
Error message: Not enough disk space for the necessary number of flows (The sequencer hard drive does not contain enough space for the Planned Run) and/or Hard drive icon indicates hard drive is almost full ()	Data normally transfer automatically from the hard drive to the Torrent Server, however this may not happen in the case of: • Data transfer manually aborted by user • Issue with connectivity or network • Incorrect configuration of the Torrent Server	1. Check for connectivity or network issues, for example, unplug and replug the ethernet cable, confirm that the router is operational, and verify that the network is up and running. 2. If in "Select Planned Run", select **Data Management** in the touch screen, otherwise select **Tools ▸ Data Management** from the Main Menu. 3. In the Data Management screen, select **All**, then review the runs. If there are runs that do not need to be transferred to the Torrent Server (for example test or aborted runs), select the checkbox next to the run names, then press **Delete Sel**. 4. If there are runs that you do want to transfer, you may need to wait until connectivity is restored for the run to transfer and then autodelete.
Temperature icon indicates chip compartment temperature is out of range 	Thermistor in chip compartment is damaged	Contact Technical Support. **Note:** Do not perform sequencing runs until this problem is corrected; non-optimal temperatures in the chip compartment may affect sequencing.

Initialization—General errors

Observation	Possible cause	Recommended action
Error message: Confirm instrument has gas pressure	Gas cylinder may be turned off or empty.	1. Verify that the cylinder has at least 500 PSI and 30 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and the Ion PGM™ Sequencer are open. 2. Once you confirm gas pressure leading into the instrument, press **Yes** to retry verification of gas pressure. If the test continues to fail, contact Technical Support.
Bottle leak check fails	• Bottle seal is not tight. • Bottle may be damaged / defective.	1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.

Initialization—Auto pH errors

Observation	Possible cause	Recommended action

Error message: Please insert a chip and press **Start**	Instrument cannot detect the chip in chip socket.	1. Open the chip clamp and remove the chip.
		
		2. Check for debris under the chip or in the chip socket. Remove any debris by rinsing with 18-MΩ water and gently dabbing the socket with a lab wipe tissue.
		
		   IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.
		
		3. Look for liquid outside the flow cell of the chip:
		
		4. If you see liquid, replace the chip with a new (unused) one. Wash the new chip once with 100% isopropanol and twice with SEQ Sample Buffer before using.
		
		   Note: The new chip can be used for sequencing after initialization completes.
		
		5. Close the clamp, then press **Start** to restart the process.
		
		6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support.

Error message: Chip calibration failed	• Chip is not seated in socket correctly. • Chip is damaged. • Sipper is loose.	Follow the procedure for "Error message: Please insert a chip and press Start." Follow the procedure for "Error message: Wash 2 average not stable."

Observation	Possible cause	Recommended action
Error message: The system did not reach the target W2 pH and/or has a clog	The waste lines can be clogged.	1. Press the **Troubleshoot** button. **Note:** You can skip the **Troubleshoot** button and change the chip to restart the Auto-pH routine. 2. Remove the waste bottle. 3. Place lab wipes under the waste arm. 4. Gently wipe the waste arm with a lab wipe to clear liquid near the waste line. 5. Press **Next** to start buffer flow. Observe flow rates from both waste lines. One line should drip slightly faster than the other. Following the flow rate check, one of 3 results is possible: a. If flow rate appears normal, press **Cancel** and test another chip. If Auto pH failure persists, contact Technical Support. b. If flow is blocked, press **Line Clear** to run the standard Line Clear procedure. If the line is unable to clear, contact Technical Support. c. If the result of the flow rate check is uncertain, press **Re-flow** to re-flow the buffer and re-test the flow.
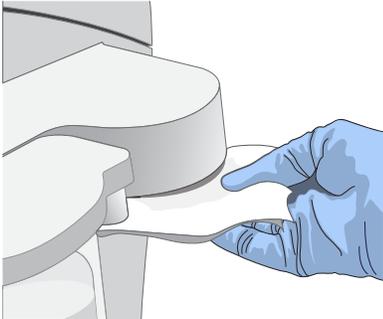
Observation	Possible cause	Recommended action
Error message: The system did not reach the target W2 pH (*continued*)	Wash 1 or Wash 2 sipper may be loose.	1. Loosen the Wash 1 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.) 2. Loosen the Wash 2 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 3. Press **Start** to re-start the auto-pH process.
	Forgot to add NaOH to the Wash 1 Bottle.	1. If there is no NaOH in the Wash 1 Bottle, loosen the cap and add 350 µL of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.) 2. Recap the bottle and shake gently to mix. 3. Press **Start** to restart auto-pH.
	Chip is damaged.	1. Replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. **Note:** The new chip can be used for sequencing after initialization completes. 2. If the error persists, there could be a problem with the chip clamp. Contact Technical Support.

Observation	Possible cause	Recommended action
Error message: W2 average not stable. Try reseating/replacing chip	Reading for W2 solution is not stabilizing quickly enough.	1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe. 2. Check for leaks and reseat the chip (see troubleshooting for "Chip Check" and "Chip calibration" above). Replace the chip with a new (unused) one if needed. **Note:** The new chip can be used for sequencing after initialization completes. 3. Loosen the cap in the W2 position and re-tighten the sipper. Because the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful and not a hazard.) 4. After performing one or more above steps, press **Start** to re-start auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support and manually adjust the pH of the Wash 2 Bottle as described in "Manually adjust W2 pH" in the *Ion PGM™ System Reference Guide* (Pub. No. MAN0009783).
Error message: W2 out of range	• Chip measurements are very unstable. • Chip is damaged.	See troubleshooting tips for "W2 average not stable" above.
Error message: Chip reading inconsistent. Please replace chip and try again	• pH response of the chip is not uniform or reliable. • Ran out of W3 Solution or volume too low.	1. Verify that there is enough W3 Solution (>25 mL) in the Wash 3 Bottle and that the sipper is secure. 2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the W3 Solution and is not a hazard.) 3. If there is enough W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. **Note:** The new chip can be used for sequencing after initialization completes.

Observation	Possible cause	Recommended action
Error message: Added too much W1 to W2	• Water quality is poor. • 18 MΩ water was exposed to air for too long. • Incorrect solution was added to the Wash 2 Bottle. • Too little NaOH was added to Wash 1 Bottle. • Chip is damaged.	1. Check whether the water meets the 18 MΩ specification and 100 mM NaOH and W2 Solution were added correctly. 2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use high-quality water. Abort the initialization and restart using correct solutions/water. 3. If solutions are correct and water meets specifications, abort the initialization, return to the main menu, and proceed to the next steps. 4. Leave the Wash 2 Bottle on the instrument. 5. Remove the Wash 1 Bottle, leaving the sipper on the W1 port. Empty the bottle, and rinse the bottle twice with 18 MΩ water. 6. Add 350 µL of 100 mM NaOH to the Wash 1 Bottle and reinstall on the instrument. 7. Press **Initialize**, select the kit type, and keep pressing the **Next** button to skip all bottle prep steps until the instrument begins purging air from the bottle. Then proceed through the touchscreens as normal to complete the initialization. 8. The next time you initialize the instrument, add 140 µL of 100 mM NaOH to the Wash 2 Bottle instead of 70 µL. Continue to use this larger volume for subsequent initializations until you receive an "Overshot Target" error message at the first auto-pH iteration, at which point follow the troubleshooting steps in "Error message: The system overshot the target W2 pH." on page 43, then return to adding 70 µL of 100 mM NaOH. 9. If you still receive the same initialization error ("Added too much W1 to W2"), contact Technical Support.
Error message: UNDERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH couldn't add enough Wash 1 to the Wash 2 before the maximum iterations, 10, occurred.	1. A blockage may have occurred. Follow the procedure for "Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again." 2. Press **Start** to re-start auto-pH. If you still get the "Undershot target pH" error, try replacing the chip with a new (unused) chip and restarting auto-pH. **Note:** The new chip can be used for sequencing after initialization completes.

Observation	Possible cause	Recommended action
Error message: The system overshot the target W2 pH.	Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed, and reports the pH value.	1. Press the **Overshoot** button to proceed with W2 pH adjustment. 2. Unscrew the cap of the Wash 2 Bottle. Without removing the sipper from the bottle, lift the cap high enough to pipette 15 µL of 100 mM HCl into the Wash 2 Bottle, close and tighten cap. 3. Press **Next** to re-pressurize the Wash 2 Bottle and mix the W2 solution. 4. Press **Start** to retry auto-pH.

Initialization—Reagent pH verification

Observation	Possible cause	Recommended action
Red failure screen, reagent pH is displayed	One or more reagents are not within the target pH.	1. Press **Start** to repeat the pH measurements to confirm the measurement. 2. If any reagents still fail, try replacing the chip with a new (unused) chip and repeating. **Note:** The new chip can be used for sequencing after initialization completes. 3. If any reagents still fail, clean and re-initialize the instrument with fresh reagents and a new chip.
	A possible line clog exists which persisted through the Auto pH process.	1. From the **Tools** menu, perform a W1 line clear. 2. Press **Start** to repeat the pH check.

Observation	Possible cause	Recommended action
Red failure screen, reagent pH is *not* displayed	Chip did not calibrate.	1. Replace the chip with a new (unused) one. **Note:** The new chip can be used for sequencing after initialization completes. 2. Press **Start** to restart the pH measurement. 3. If the second test fails, contact Technical Support.

Safety

⚠ **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
⚠	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
⏚	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)

Symbol	English	Français
⌧	Do not dispose of this product in unsorted municipal waste ⚠ **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. ⚠ **CAUTION!** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	French translation
⚠ **CAUTION! Hazardous chemicals.** Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
⚠ **CAUTION! Hazardous waste.** Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination	⚠ **CAUTION! Cleaning and Decontamination.** Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Laser	☀ **CAUTION! LASER HAZARD, Bar Code Scanner.** The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 15	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment

Chemical safety

⚠️ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Documentation and support

Obtaining information from the Help system

The Ion PGM™ Instrument has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the window.
- Select **Help ▸ Contents and Index**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

You can also access PDF versions of all documents in the document set from the Help system.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
    - Product FAQs
    - Software, patches, and updates
    - Training for many applications and instruments
- Order and web support
- Product documentation, including:
    - User guides, manuals, and protocols
    - Certificates of Analysis
    - Safety Data Sheets (SDSs; also known as MSDSs)

        Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/ terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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