



Agilent BioTek BioSpa 8 Automated Incubator

User Manual



ERRATA NOTICE: This document contains references to BioTek. Please note that BioTek is now Agilent. For more information, go to www.agilent.com/lifesciences/biotek.

Notices

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CAUTION

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Contents

Notices	i
Contents	ii
1 General Information	1
Contact Information	2
Worldwide Sales and Support	2
Technical Support and Service	2
Customer Care	2
Intended Use Statement	3
Quality Control	3
Safety Notices	4
Warnings and Precautions	4
Chemical/Environmental	5
Safety Symbols	8
Electromagnetic Compatibility (EMC) Information	12
Emission	12
Immunity	14
Disposal	14
2 Introduction	15
Product Description	16
Instrument Models	16
Compatible Agilent BioTek Instruments	16
Typical Applications	17
Introducing the BioSpa	18
Physical Specifications	18
Environmental Conditions	19
Labware	19
Plate Dimensions	20
Performance Specifications	20
Temperature Control	20
CO ₂ Control	21
O ₂ Control	21
Humidity	21
Power Usage	21
Package Contents	22
Optional Accessories	22
Integration Kits	22

Contents

Instrument Qualification Tools	23
Gas Control Accessories	23
3 Installation	24
Select an Appropriate Location	25
Unpack and Inspect	26
Remove the Shipping Hardware	26
Remove the Shipping Platform	28
Install a Cytation with an Isolation Table	28
Installing a Synergy Neo2 or Cytation (without an isolation table) on the Left Side	29
Install Feet to Support Right-Side Rotating Base Installation	31
Remove the Shipping Brackets	31
Install Internal Components	32
Insert the Baffles	34
Install the HEPA Filter	35
Install Plate Holders	36
Install the Water Pan	37
Close Lid and Install Cover	38
Install External Components	38
Install the Deck Spacers	39
Install the Gripper and Robot Covers	40
Install the Platform/Cover and Guard Rail	40
Install the Rotating Base	44
Install Companion Instruments	50
Integration Kits	51
Cytation 1/5/7 Right-Side Installation	52
Cytation 1/5/7 Left-Side Installation	53
Cytation 1/5/7 with Isolation Table Installation	54
Cytation C10 with Isolation Table Installation	56
Epoch 2 Installation	60
Synergy H1 Installation	61
Synergy Neo2 Right-Side Installation	62
Synergy Neo2 Left-Side Installation	63
405 TS-LS Installation	64
EL406 Installation	65
MultiFlo FX Installation	66
Install BioSpa Software	67
Minimum System Requirements	67
Disable Sleep Mode	68
Turn Off Automated Updates	68

Contents

Launch the BioSpa Session Software	68
Connect Required Components	69
Connect the Power Supply	69
Connect the Gas	70
Gas Connection Recommendation	72
Initial Setup Steps	72
Configure LHC Software for the BioSpa (for BioSpa Session only)	74
Align Instruments	74
Select Your Favorite Plate Types	77
Set Up Assay Protocols for the BioSpa	78
Set up Email and Text Messaging	81
Fill the Water Pan	82
Do a Test Run	83
Run a Test Using BioSpa Session	83
Repackage the Instrument for Shipping	85
Prepare Instrument for Shipping	86
Repacking Step 1	87
Repacking Step 2	88
Repacking Step 3	89
4 Operation	90
BioSpa Rules of Operation	91
One Session at a Time	91
OnDemand Runs Continuously	91
During BioSpa 8 Operation	91
Timing is Important for BioSpa Session	93
Keeping it Clean	93
Gen5 Limitations	94
Nice to Know	95
Gen5 Plate Orientation	95
Using Lidded Plates	96
Condensation on Plate Lids	96
Lidded vs. Non-Lidded Plates	97
BioSpa Session vs OnDemand	102
Important Information for Users	103
Guidelines for Imaging with the BioSpa 8	103
Exploring the BioSpa 8 Session Workspace	104
LHC and Gen5 Software Programs	104
BioSpa Session Workspace Overview	104
Session Workspace	105

Contents

Define the Environment and Assay Steps	105
Gen5 Setup	107
LHC Limitations	108
Priming Options	108
Running a BioSpa Session	110
BioSpa Session Steps	126
Exploring the BioSpa 8 OnDemand Workspace	130
Gen5 Software	130
BioSpa OnDemand Workspace Overview	130
Control panel	130
Protocol and Plate Information	131
Rules for OnDemand	134
Assign Plate IDs - Barcode in OnDemand	136
Gen5 Protocols List	137
Scheduling Options	138
Users	142
Processing Options	142
Gen5 Imaging Workflow for BioSpa OnDemand	143
Gen5 Protocols for OnDemand	149
Power Up Sequence	150
Shut-Down Procedure	151
Reset Button	152
Status LED	152
Define your Preferences	153
Preferences for OnDemand	153
Colors	153
File Locations	153
Session Options	153
Sounds	154
Startup Options	154
Notification Options	154
Send an Email or Text	155
Email and Text Message Settings	156
Email Directory	156
Notify Me	156
Critical Notice	157
Controlling the Environment	158
BioSpa Utilities	159
BioSpa Utilities: Advanced	159

Contents

To Open Drawers	159
Water Level Controls Humidity	160
5 Maintenance	162
Warnings and Precautions	163
Recommended Maintenance Schedule	163
Daily Maintenance	163
Daily Safety Checks	164
Weekly Maintenance	164
Required Materials	164
Clean the Water Pan	165
Replace the HEPA Filter	166
Clean the Internal Chamber and Plate Holders	166
Replace the Gas Line Tubing and Filter	168
Clean the Fan Inlet Filter	169
Decontamination	169
Tools and Supplies	169
Decontaminate Exterior Surfaces	170
6 Qualification	171
Recommended Qualification Schedule	172
Test Plate Transfers	172
Calibrate Gas Sensors	172
Calibrate Zero	175
Calibrate Gain	175
Calibrate Temperature Sensor	176
Gas Sensors - Theory of Operation	178
Appendix A: Troubleshooting & Error Codes	181
Error Codes	182
BioSpa 8 Software Error Codes	182
System Error Codes	184
Motor Error Messages	187
Gas Error Messages	188
Temperature Error Messages	189
USB Communication Errors	190
Plate Transfer Test	192
Active X Registration Problem	192
Remove the Gas Sensor	192
OLE Automation Registration Problem	193
Technical Support	194

Contents

Appendix B: BioSpa 8 Integration Space Requirements	195
405TS 405LS - BioSpa 8	196
405T LS - Cytation 1-5-7	197
405T LS - Epoch 2	198
405T LS - Synergy Neo 2	199
405T LS - Synergy H1	200
Cytation 1-5-7 - Iso Table Install	201
Cytation 1-5-7 - Right-side Install	202
Cytation 1-5-7 - Left-side Install	203
Cytation 1-5-7 - on Rotating Base at Full Rotation	204
Cytation 1-5-7 - on Rotating Base at Home	205
Cytation 1-5-7 - on Rotating Base at Mid Rotation	206
Cytation 1-5-7 - on Rotating Base	207
Cytation C10 Iso Table Install	208
EL406 - BIOSPA 8	209
EL406 - Cytation 1-5-7	210
EL406 - Epoch 2	211
EL406 - Synergy Neo 2	212
EL406 - Synergy H1	213
Epoch 2 - BioSpa 8	214
MultiFlo FX - Cytation 1-5-7	215
MultiFlo FX - Epoch 2	216
MultiFlo FX - Synergy Neo 2	217
MultiFlo FX - Synergy H1	218
Synergy H1 - BIOSPA 8	219
Synergy Neo 2 - Left-side Install	220
Synergy Neo 2 - Right-side Install	221
Synergy Neo2 - on Rotating Base	222
Appendix C: Safety Information	223
Safety Notices	224
Warnings and Precautions	226
Electrical Hazards	226
Chemical/Environmental	228
Components	233
Intended Product Use	236
In This Book	239

1 General Information

Contact Information

Contact Information



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4 Operation

This chapter describes the [BioSpa Rules of Operation](#) and instructions for controlling the BioSpa 8 using BioSpa Session or BioSpa OnDemand.

BioSpa Rules of Operation	91
BioSpa Session vs OnDemand	102
Exploring the BioSpa 8 Session Workspace	104
Exploring the BioSpa 8 OnDemand Workspace	130
Power Up Sequence	150
Shut-Down Procedure	151
Define your Preferences	153
Notification Options	154
Controlling the Environment	158

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4 Operation

BioSpa Rules of Operation

BioSpa Rules of Operation



Most of these rules apply to both BioSpa 8 Session and BioSpa 8 OnDemand processing. Session and OnDemand are separate applications using the same BioSpa hardware. See [BioSpa Session vs OnDemand on page 102](#) to learn the differences. Understanding these general operating rules will improve your experience using BioSpa 8 software.



One Session at a Time

BioSpa 8 can only run one session at a time. And, once a session is started, it cannot be stopped and restarted. A session can be paused and restarted, providing an opportunity during an idle time, for example, when plates are incubating, to use the companion instruments offline. Use Pause to load plates after a session has started, if necessary. See [Using Instruments Outside of a Session on page 116](#).



OnDemand Runs Continuously

OnDemand is designed to run continuously. Each plate in the incubator is a distinct experiment with its own schedule. A run cannot be paused except to fill the water pan. Environment-condition warnings and processing errors are limited and always give users the ability to resume the run (rather than potentially abort another user's experiment). See [Rules for OnDemand on page 134](#).



Only one application — Session or OnDemand — can be run at a time.

Close all other Agilent BioTek software programs such as Gen5, LHC, and the open BioSpa 8

IMPORTANT application, before running either option.

During BioSpa 8 Operation

Temperature and Gas Settings: BioSpa 8 monitors and displays the current actual temperature and gas values and the **Set point** or desired settings for a session. The BioSpa 8 warns you when the actual settings do not match the set points when:

- temperature is <1.5° or >1.5° of set point,
- gas is >1% or <1% of set point.

Out-of-range readings are highlighted in the control panel. Warning messages displayed at session/run start and during a session offer the option to ignore the warning and proceed with the session or abort the session. All warnings are recorded in the Session Log.

NOTE

BioSpa 8 waits 1/2 hour for temperature and gas readings to stabilize following a door/drawer opening before displaying a warning.

4 Operation

BioSpa Rules of Operation

Water Pan: For both BioSpa 8 Session and OnDemand, the water pan must be empty when running with unlidded plates to avoid excessive humidity in the chamber. Learn more: [Water Level - Maintaining Humidity on page 160](#).

Plate Orientation for Readers on the Right: When the reader is installed on the right side of the incubator, BioSpa 8 puts microplates on the plate carrier in a 180-degree rotation from normal.

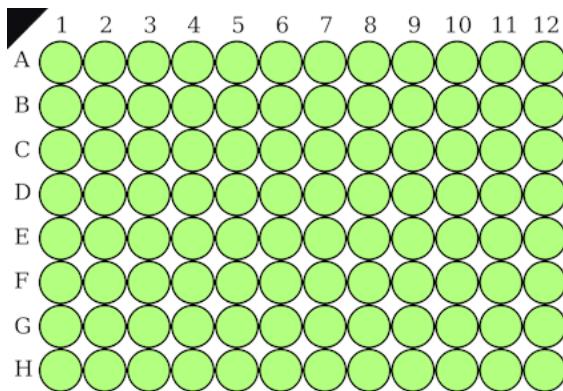


Figure 4-1: Orientation for BioSpa 8

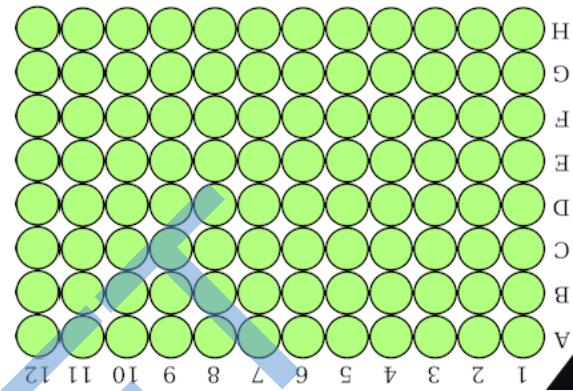


Figure 4-2: Normal orientation for Gen5 & readers

Normally, plates are placed on the reader's plate carrier with A1 closest to the reader. When the reader is positioned on its right, the BioSpa 8 puts plates on the reader's carrier with A1 furthest from the reader. Gen5 automatically corrects for this anomaly by reversing the orientation of the data for review and analysis. See [Nice to Know on page 95](#) for more information.

When performing test runs of your Gen5 protocols on the reader or when using the reader outside of a BioSpa 8 session, load plates in the normal orientation.

A liquid handler or reader positioned left of the incubator receives the plate in its normal orientation, with A1 closest to the instrument, so correction is not needed.

Readers with Dispensers/Injectors: Gen5 dispense protocols are not supported in a BioSpa 8 session or On Demand run. In a BioSpa 8 session, you can take the reader off-line: [Using Instruments Outside of a Session on page 116](#) to run a Gen5 dispense protocol, that is, inject solution before a read.



BioSpa Session only. Plate-wise Processing: BioSpa 8 Session always processes plates in a plate-wise way, that is, it builds the timeline to first perform all steps on the first plate before fitting the next plates into the timeline for processing. When an incubate step, for example, is long enough to allow it, the BioSpa 8 fits subsequent plates into the timeline. BioSpa 8 will process plates as efficiently as possible (based on the timing required for each step).

4 Operation

BioSpa Rules of Operation



Timing is Important for BioSpa Session

The BioSpa 8 creates the timeline based on the specified **Time per plate** protocol timing.

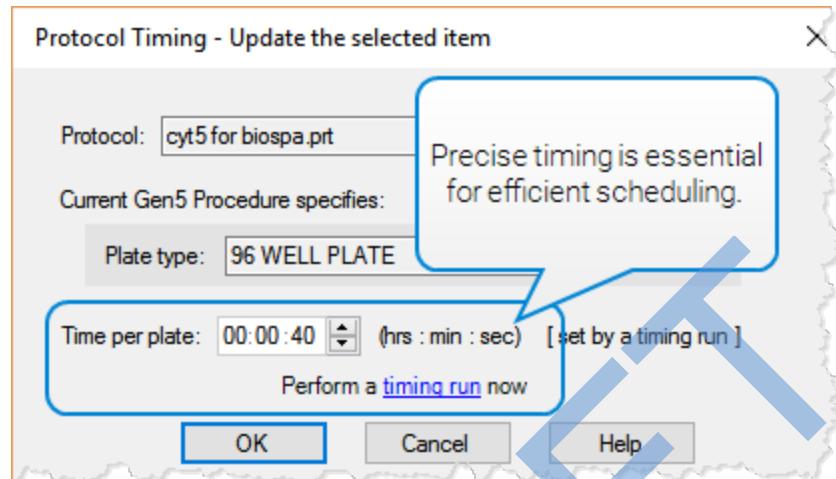


Figure 4-3: Setting an accurate time per plate.

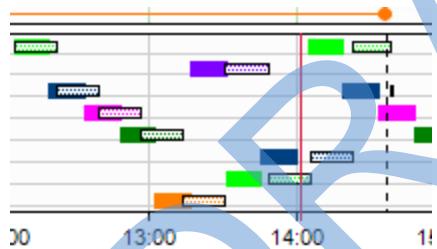


Figure 4-4: When plate processing takes longer than specified, a tight timeline soon unravels. Solid colors show the expected time, dotted bars show the actual time.

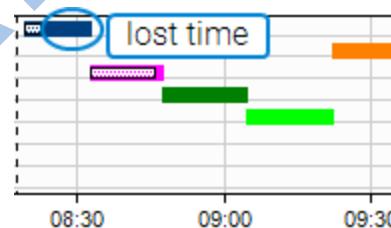


Figure 4-5: Overestimating the processing time is also a bad practice because the timeline does not automatically adjust to fill the unused time.

BioSpa 8 always allots the specified **Time per plate** in the timeline regardless of the actual time required. Redo the timing run for protocols that consistently exceed or outpace the expected processing time.

Keeping it Clean

For maximum cleanliness, keep the incubator powered up at all times. Set the temperature to ambient+4 to conserve power, if desired. If the incubator will not be used for an extended period of time, empty and dry the water pan and follow [Shut Down Procedure on page 151](#) to avoid potential condensation on the gas sensors and condensation elsewhere that may lead to bacteria growth.

CAUTION

Aggressive chemicals, especially acids and their vapors, may damage chamber surfaces and the water pan.

- Do not use hydrogen chloride (HCl).
- If you must use harsh chemicals, minimize their time in the chamber:

4 Operation

BioSpa Rules of Operation

- Optimize session scheduling.
- Use the **Open drawers at finish** option.
- Use lidded microplates.

CAUTION

Clean up spills promptly. Do not let fluid seep into the robotic components beneath the platform or internal compartments where it can damage electronics.

Gen5 Limitations

NOTE

Close the BioSpa 8 software when you want to use Gen5 software, and close the Gen5 software before launching the BioSpa 8 app.

When running Gen5 in both BioSpa 8Session or BioSpa 8 OnDemand, make sure your protocols do **not** contain these options:

- Dispensing
- Monitor Well reads
- Runtime well selection
- Stop/Resume step
- Multi-plate Assay protocol type, for example, calibrator plate protocol
- Cuvette read (and cannot be used in path length correction)
- BioCell adapter plate (and cannot be used in path length correction)

NOTE

Some non-standard vessels, like 35 mm Petri dishes in their adapters can be transferred to a companion reader/imager. However, the T25 flask (and its adapter) cannot be transferred by the robot, only stored manually in the incubator.

See also [Using Lids in Gen5 Protocols on page 101](#).



IMPORTANT

Make sure the Plate Type ([Tools > Plate / Lid Definition](#)) contains a lid definition to run plates with lids.



TIP

Remember to [Select Your Favorite Plate Types on page 77](#).

Gen5 Discontinuous Kinetic protocol setting



BioSpa 8 OnDemand: All Gen5 protocols must be defined as discontinuous kinetic. See [Defining Kinetic Assay Protocols on page 119](#).



BioSpa 8 Session: If you want to repeat a protocol multiple times in BioSpa Session, you must define it as Discontinuous Kinetic in Gen5. Learn more about the [Repeat Block on page 126](#).

4 Operation

BioSpa Rules of Operation



BioSpa 8 OnDemand requires a default setting in the Gen5 protocols to be disabled. When preparing protocols for OnDemand use:

In the **Procedure** choose **Options** and deselect **Eject plate when procedure is finished** (disable **Eject plate when procedure is finished**).

NOTE

This setting has no effect when running BioSpa Session, which takes control of the reader's plate carrier.

Nice to Know

Gen5 Plate Orientation

When the reader is installed on the right side of the incubator, BioSpa 8 tells Gen5 to reverse the read data acquired in a session to correct for the improper placement of the plate on the carrier. You do not have to tell Gen5 to change its Plate Orientation, the BioSpa 8 does this for you. However, when you want to read a plate outside of a BioSpa 8 session or OnDemand run, put the plate on the carrier in the standard orientation with well A1 closest to the reader.

Alternatively, when performing a read without the robot, you can choose the plate orientation:

- 1 Close the BioSpa 8 software.
- 2 Start Gen5.
- 3 Select **System > Instrument Configuration**.
- 4 Choose an instrument and click **View/Modify**.

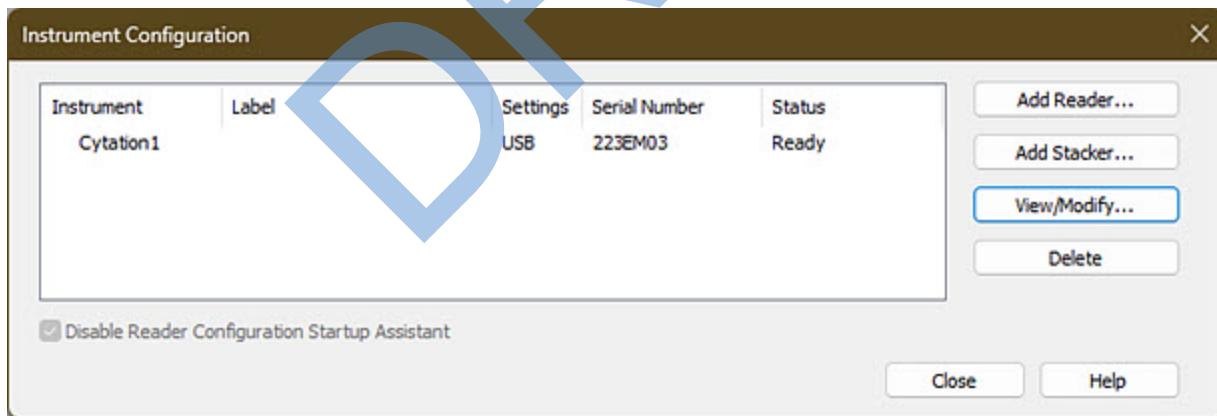


Figure 4-6: Gen5 Instrument Configuration dialog box.

- 5 In the **Reader Settings** dialog box, select the reader in the list. Click **Refresh** if you do not see the reader.

4 Operation

BioSpa Rules of Operation

Nice to Know

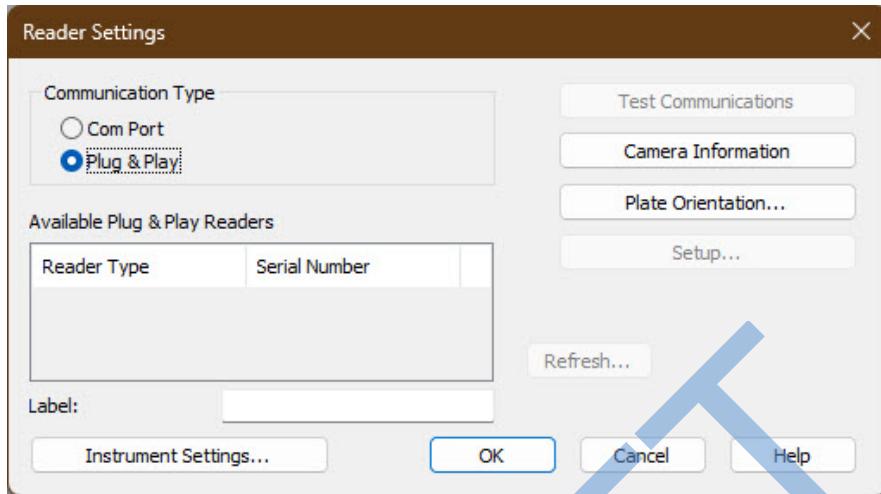


Figure 4-7: Gen5 Reader Settings dialog.

- 6 Double click your reader and then select **Plate Orientation**.
- 7 In the **Plate Orientation** dialog for a stand-alone Gen5 experiment: either **Normal** (for the reader) or **Reverse** (standard orientation for the BioSpa 8). Either setting works for BioSpa 8.

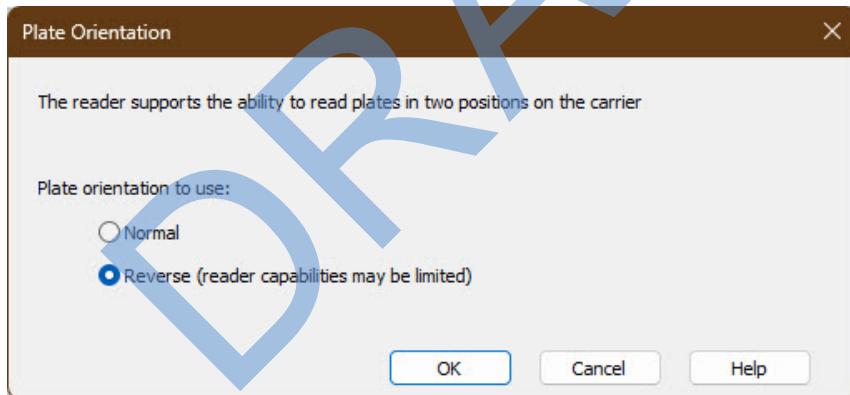


Figure 4-8: Setting the plate orientation.

- 8 Run your experiment.
- Remember to close Gen5 before starting a BioSpa 8 session.

Using Lidded Plates

If you haven't already done so, review this information about using lidded plates with the BioSpa 8: [Lidded vs. Non-Lidded Plates on page 101](#).

Condensation on Plate Lids

When moving plates with lids to and from the incubator, condensation may form on the inside of the lid. When returned to an incubator of like temperature (either the BioSpa or a reader), the

4 Operation

BioSpa Rules of Operation

condensation will slowly evaporate. Typically, this does not affect your assay. However, just as when you're using a stand-alone reader, always test with and without lids to be sure any difference in readings is not a problem for your assay. In particular, absorbance reads, top fluorescence reads, and to a lesser extent bright field imaging may be affected. In case of problems, let the BioSpa 8 remove the lid. If the lid must be used, the following guidelines may be helpful.

Keep in mind:

- **Ambient temperature versus incubator temperature:** the greater the difference in temperature, the faster the condensation will form.
- **Relative humidity:** the higher the humidity, the faster condensation will form.

To minimize the condensation effects:

- **Absorbance:** perform dual wavelength reads and background subtraction, which has been shown to effectively cancel condensation effects.
- **Set the same temperature on both the incubator and reader:** Make sure all calibrations are up to date.
- **Enable the reader's temperature gradient:** the higher the gradient the faster lid condensation will evaporate.
- **Set a delay in the Gen5 protocol:** This allows the condensation to evaporate before reading the plate. Experiment with the delay time to determine the appropriate duration for your configuration.
- **Change ambient temperature and relative humidity:** Increase laboratory ambient temperature and decrease relative humidity, if possible. Put the BioSpa in a controlled environment and then use that environment to control ambient temperature and humidity.

Lidded vs. Non-Lidded Plates

Make sure the Plate Type has a lid definition to support handling plates with lids.



Precise lid dimensions are most important when delidding is required. The default parameters work for most plate types in most setups.

View/Modify your favorite plate types to make sure their lid is defined:

This example uses a Gen5 plate type record.

- 1 Select **Include Lid Parameters** (A).
- 2 Select **Lid Parameters** (B).

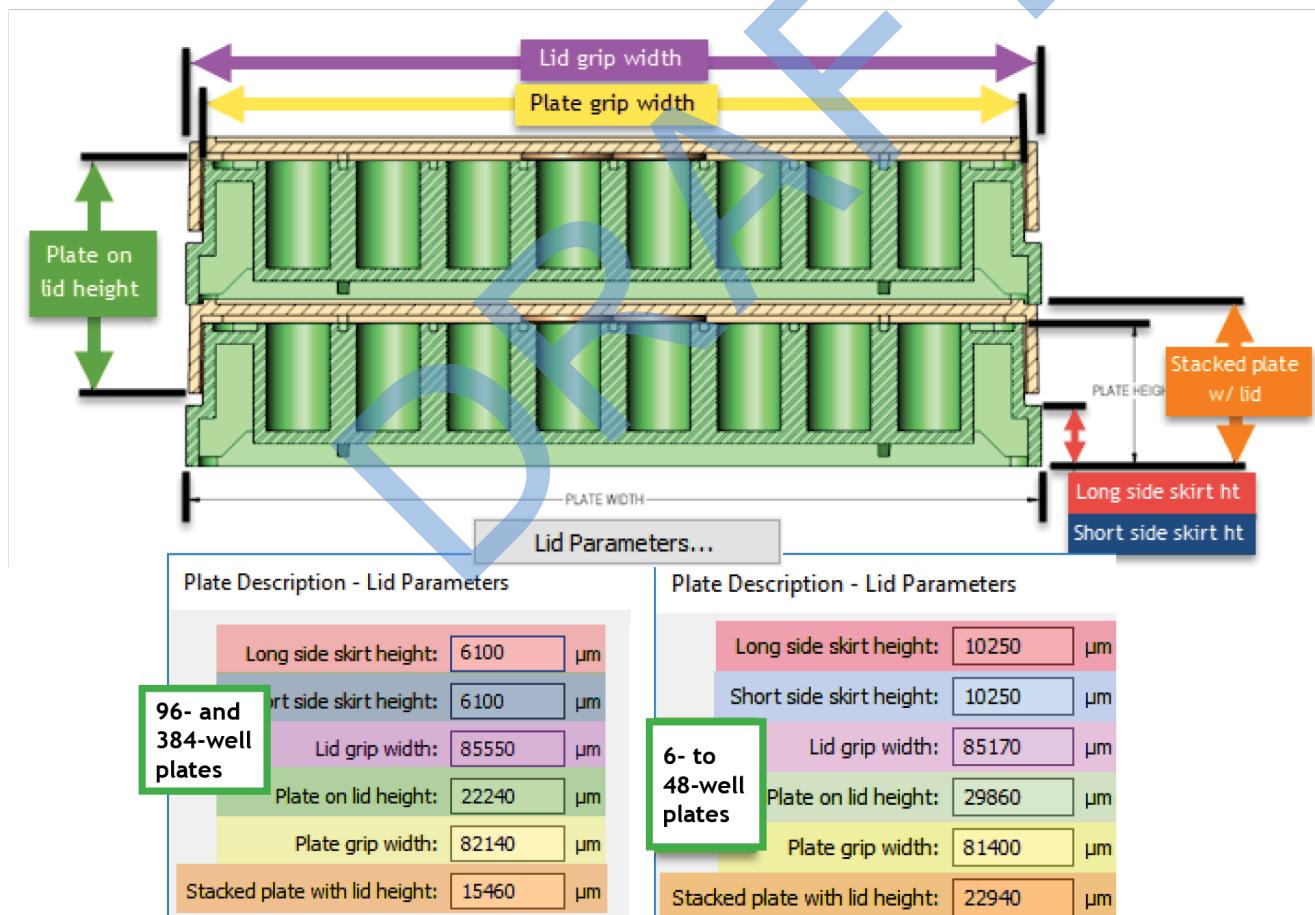
4 Operation

BioSpa Rules of Operation

Plate Description

Name:	96 Well Plate SBS dimensions	Catalogue #	OK		
Manufacturer:	Generic	Cancel			
Display Filter:	Microplate	Help			
Number of Rows:	8	Number of Columns:	12		
Plate Width:	85480	μm	Plate Length:	127760	μm
Plate Height:	14350	μm	Stacked Height:		μm
Plate Lid adds:	3500	μm	<input checked="" type="checkbox"/> Include Lid Parameters	Lid Parameters...	B
Wells					
Top Left Y:	11240	μm	Top Left X:	14380	μm
				Imaging Parameters...	

Figure 4-9: Modifying plate definition.



Best method: Obtain dimensions from the plate manufacturer. If unavailable, use calipers for the most accurate measurements or a ruler if it is the only tool available.

Figure 4-10: How plate and lid parameters are defined.

4 Operation

BioSpa Rules of Operation

Special Vessels - Plate Types

Here are some tips to help you define lid parameters for specific vessels:

- **Low-density plate** lid parameters (6-48 wells): **Copy** a Nunc plate type that best matches your vessel to create a custom low-density plate record. Most Nunc plates in the database have been assigned lid parameters.



Low-density-plate lids have different dimensions than 96-well plates.

IMPORTANT

- **High-density plate** lid parameters (96-1536 wells): Numerous records for 96- and 384-well plates are provided in the Plate Type Database. Shown below are examples with lid parameters already defined. Copying one that matches your vessel is a shortcut to creating your own precise plate type.

Name	96-well	384-well (low profile)	1536-well (low profile)
	Costar 96 black opaque	BRAND 384 HTS standard	Aurora 1536 Lobase 200um bottom

- **T25 Flasks** (No LHC protocol): Can only be placed in the BioSpa 8 (in its Agilent-supplied adapter) for incubation; the T25 flask cannot be moved by the robotic arm.
- **Petri Dishes** (No LHC protocol): Update the plate type record for the Petri dishes you are using. You can use default lid parameters. The robot cannot remove the Petri dish lid. Select **Tools > Plate/Lid Definitions** and select **Include Lid Parameters** to comply with the BioSpa's validation rules. Keep the default lid values and always define the Gen5 protocol to **Use Lids**.
- **Chamber Slides in Multi-Vessel Adapter** (No LHC protocol): Similar to Petri dishes, modify the plate type record for chamber slides (in the adapter) to contain Lid Parameters. You can use default lid parameters as the robot cannot remove the lid. Define the Gen5 protocol to **Use lids**.



Figure 4-11: Chamber slide in an adapter.

Plate Types

several "adapter" plate types are already defined

Favorite	Name	Well
<input type="checkbox"/>	BioTek 1450541 adapter: 35mm Petri dish	1
<input type="checkbox"/>	BioTek 1450541 adapter: 60mm Petri dish	1
<input type="checkbox"/>	BioTek 1450541 adapter: Hemocytometer	2
<input type="checkbox"/>	BioTek 1450541 adapter: Microscope slide h...	1
<input type="checkbox"/>	BioTek 1450541 adapter: Nunc 4-well plate	4

Figure 4-12: Adapter plate definitions.

Appendix A: Troubleshooting & Error Codes

This appendix provides guidelines for error recovery and troubleshooting performance problems.

Error Codes	182
USB Communication Errors	190
Plate Transfer Test	192
Active X Registration Problem	192
Remove the Gas Sensor	192
OLE Automation Registration Problem	193
Technical Support	194

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Appendix A: Troubleshooting & Error Codes

Error Codes

Error Codes

The BioSpa 8 alerts you to an error condition in multiple ways.

When an error flag appears in the workspace, as shown above, click the **Info** link to view details.



Click **Utilities** at any time to [Run a Self-Test](#) to restore functionality.

If running the self-test does not clear an error: close the BioSpa 8 software; press the [Reset button](#); and restart the software. If errors persist, contact Technical Support.

Three of the incubator's internal components may generate an error message:

- [Motor Error Messages on page 187](#)
- [Gas Error Messages on page 188](#)
- [Temperature Error Messages on page 189](#)

BioSpa 8 software may also display an error code:

- [Software Error Codes below](#)
- [System Error Codes on page 184](#)
- See also [Active X Registration Problem on page 192](#).
- For Gen5 related errors that may be coded as -1 or 1 and report: "Unexpected file format," see [OLE Automation Registration Problem on page 193](#).

NOTE

Contact Technical Support at bio.tac@agilent.com. See also [Contact Information on page 2](#).

BioSpa 8 Software Error Codes

Generally, these errors are caused by a communication failure between an instrument and the computer.

Use only new USB cables supplied with the BioSpa 8 to connect the instruments.

Error Code	Message and Resolution
6000	General communication error during download. Select Tools > Configure Instruments . If the correct instruments are defined, run a self-test to potentially clear the errors.
6001	COM port created by USB converter no longer active. Service Only. Contact Technical Support .
6002	Invalid basecode part number; instrument is not BioSpa 8. Service Only. Contact Technical Support .
6003	Invalid Basecode Data Version; basecode needs to be updated. Contact Technical Support to obtain latest basecode.
6010	The data is invalid or out-of-range. Service Only. Contact Technical Support .

Appendix A: Troubleshooting & Error Codes

Error Codes

Error Code	Message and Resolution
6040	Invalid baud rate
6041	Invalid data bits selection
6042	Invalid stop bits selection
6043	Invalid parity selection Service Only. Contact Technical Support . These codes indicate an unexpected software error that cannot be fixed without Agilent support.
6044	Serial port error
6045	Serial write error
6046	Unable to communicate. Serial read error Select Tools > Configure Instruments . If the correct instruments are defined, run a self-test to potentially clear the errors.
6047	Checksum error Contact Technical Support .
6048	Serial NAK error Restart the companion instrument. If error reoccurs, contact Technical Support .
6049	Excess data, or not enough data received.
6050	Invalid message header
6051	Invalid message object
6052	Invalid message body size
6053	Serial message timeout
6054	Port handle error
6055	Read timeout value is invalid. Select Tools > Configure Instruments . If the correct instruments are defined, run a self-test to potentially clear the errors. If error reoccurs, contact Technical Support .
6056	Unauthorized to open the port.
6057	Out-of-range parameter for the open port function.
6058	Unable to open the port.
6059	Unable to clear the transmission buffer.
6060	Unable to close the port.
6061	Port is no longer available. Select Tools > Configure Instruments . If the correct instruments are defined, run a self-test to potentially clear the errors. If error reoccurs, contact Technical Support
6062	Unhandled exception while transmitting message. Contact Technical Support .
8001	Invalid request. Contact Technical Support .
8107	Request not supported by instrument. Contact Technical Support .
8300	Invalid password. Contact Technical Support .
8301	No matching item. Contact Technical Support
8999	Invalid request. Contact Technical Support

Appendix A: Troubleshooting & Error Codes

Error Codes

System Error Codes

Most of these error conditions require technical expertise to correct. A few errors may be caused by an obvious obstruction to a device's movement. Fix these kinds of errors and restart your instrument to give it an opportunity to clear the error code.

Code	Message and Resolution
200	Motor didn't find home opto-sensor transition Clear any obstructions to permit free movement of the robot. If error occurs again, contact Technical Support .
350	Motor interlock safety switch open Service Only. Contact Technical Support .
400	Motor failed positional verify Clear any obstructions to permit free movement of the robot. Run self-test. If error reoccurs, contact Technical Support .
550	Motor currently in use Run self-test. If error reoccurs, contact Technical Support .
600	Invalid position specified motor currently in use Service Only. Contact Technical Support .
650	Autocal Jig error Autocal jig placement error, sensor in unexpected state. Make sure jig is correctly placed and try again.
700	Interface checksum error Check USB cable connections to PC. Contact Technical Support .
750	Instrument checksum error Check USB cable connections to PC. Contact Technical Support .
800	Motor not homed Service Only. Contact Technical Support .
900	Invalid profile error. Motor profile does not exist. Reboot PC and try again. Contact Technical Support .
1000	Autocal checksum error Reboot PC and try again. Contact Technical Support .
1100	Autocal sequence error Contact Technical Support .
1300	Timeout sending or receiving serial data Check USB cable connections to PC. Reboot and try again. Contact Technical Support .