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

Document Identification

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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

 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.

IMPORTANT **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 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.

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.

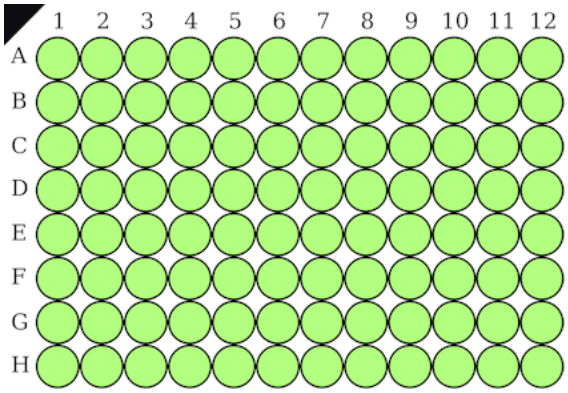

Figure 4-1: Orientation for BioSpa 8

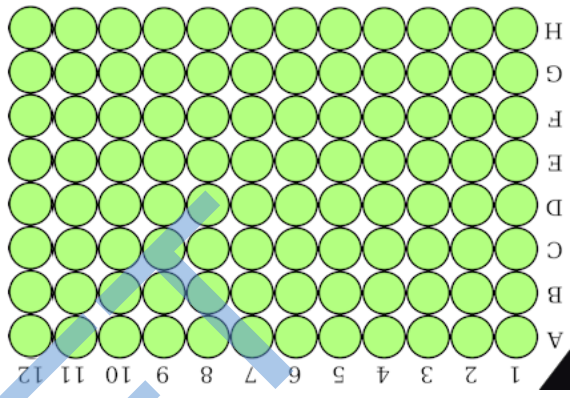

Figure 4-2: Normal orientation for Gen5 & readers

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).

Timing is Important for BioSpa Session

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

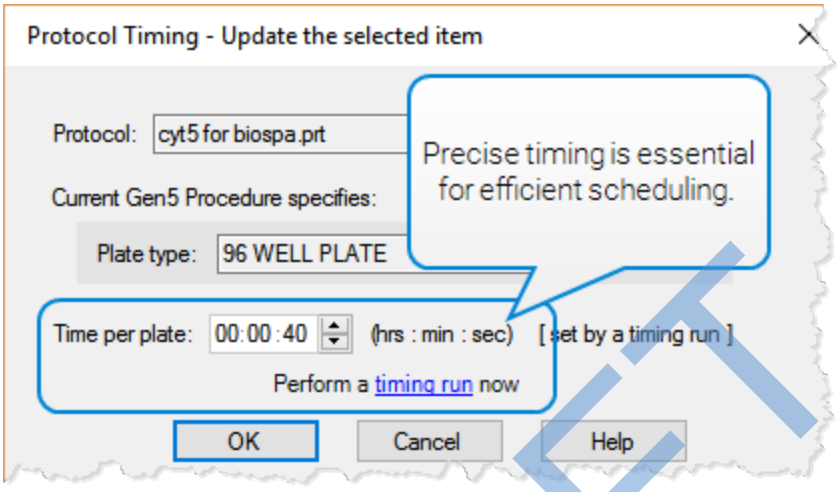

Figure 4-3: Setting an accurate time per plate.

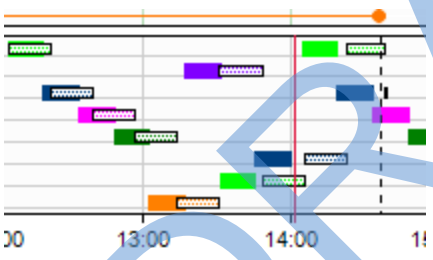

Figure 4-4: When plate processing takes longer than specified, a tight timeline soon unravels. Solid colors show the expected time, dotted bars show the actual time.

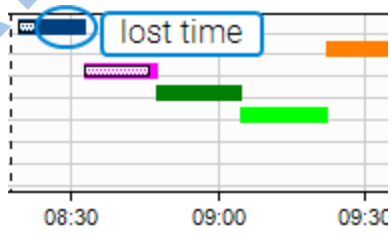

Figure 4-5: Overestimating the processing time is also a bad practice because the timeline does not automatically adjust to fill the unused time.

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:

- 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.

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**.

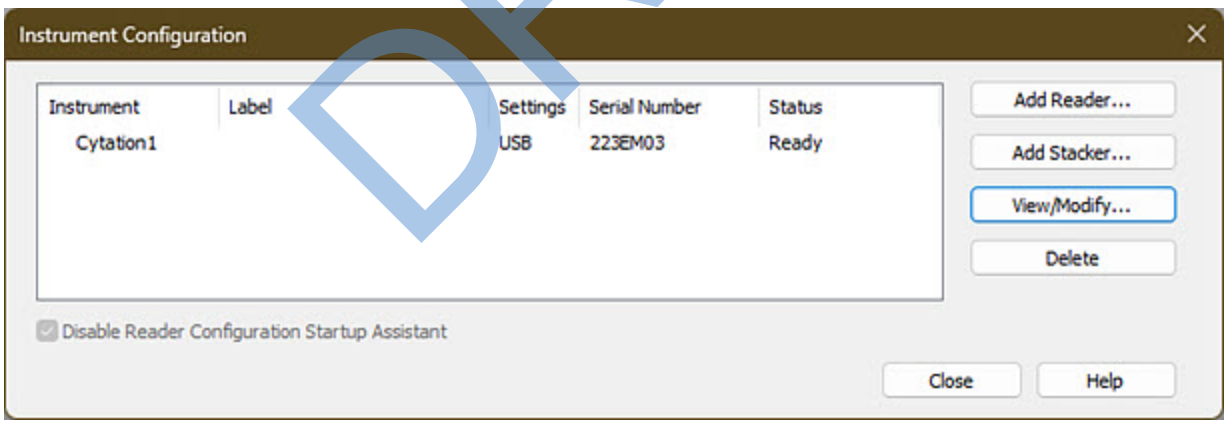

Figure 4-6: Gen5 Instrument Configuration dialog box.

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

Nice to Know

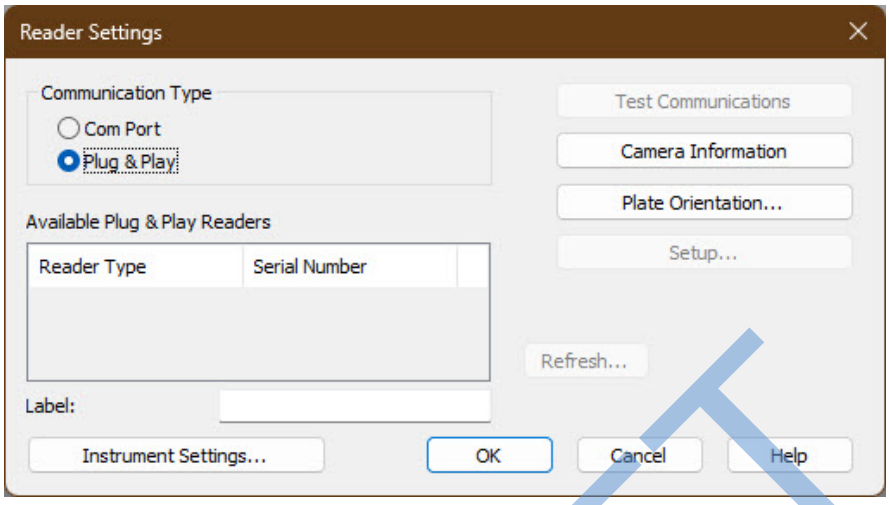

Figure 4-7: Gen5 Reader Settings dialog.

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.

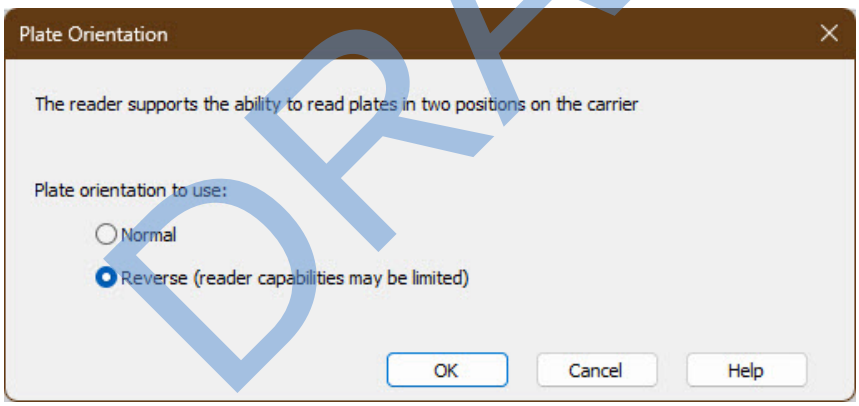

Figure 4-8: Setting the plate orientation.

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

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.

TIP

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).

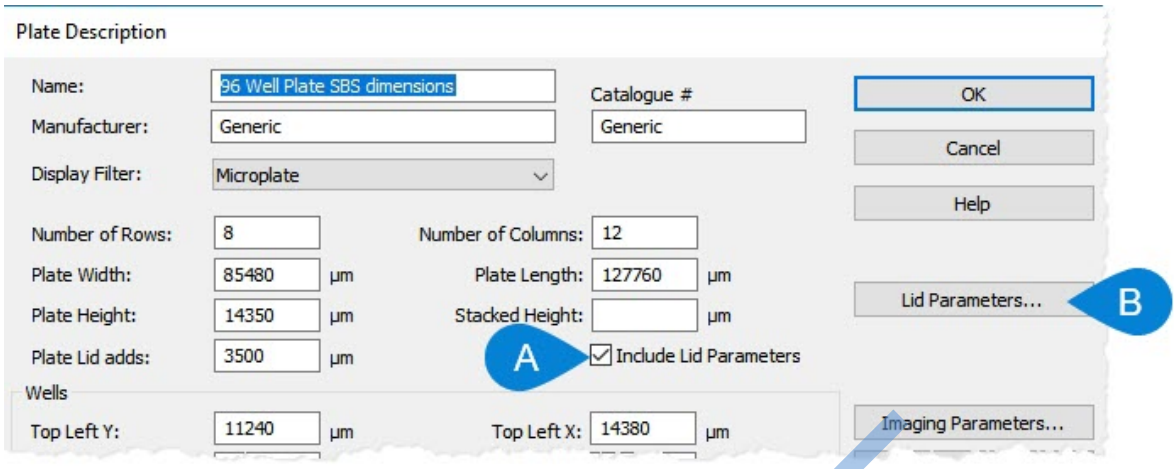

Figure 4-9: Modifying plate definition.

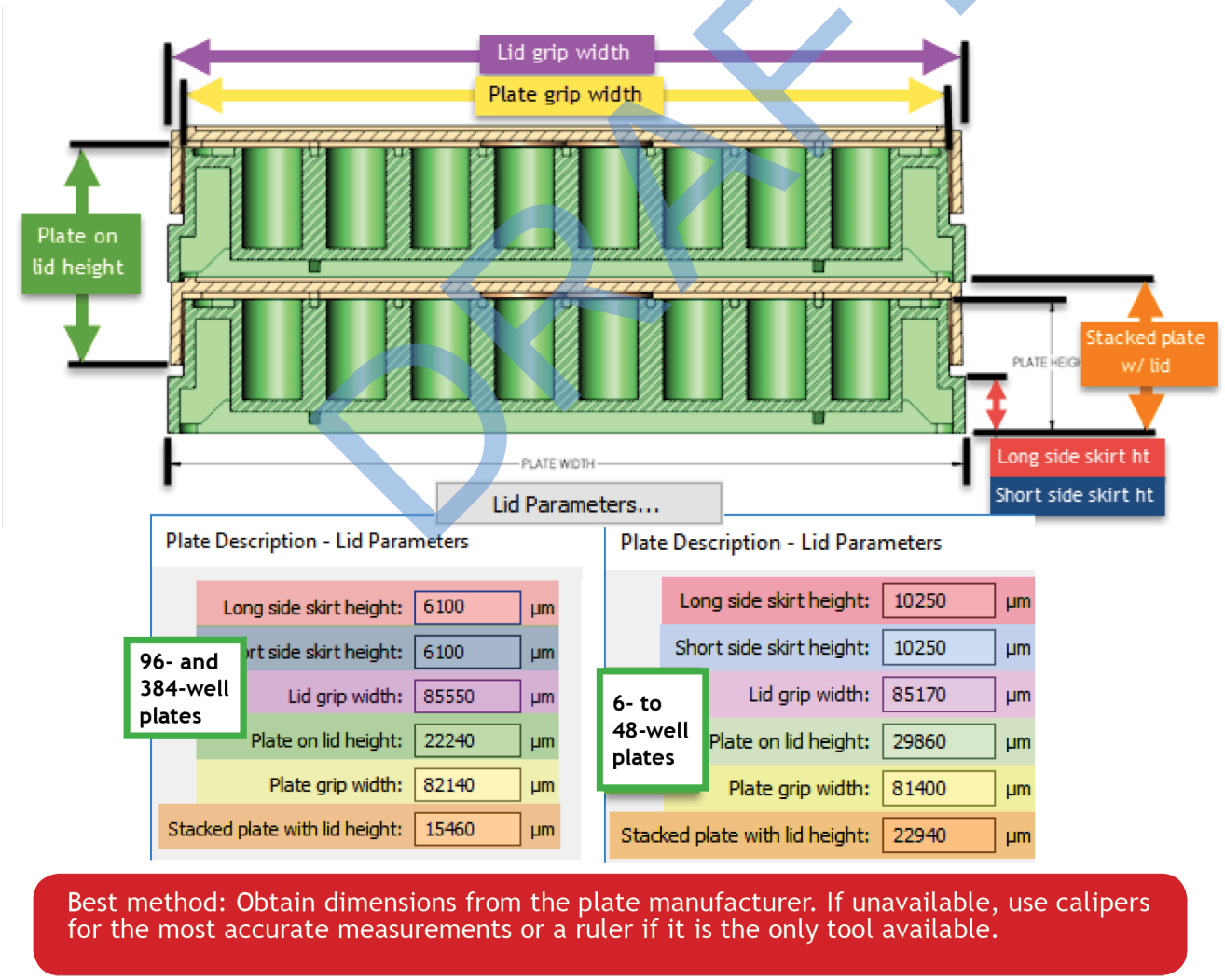

Figure 4-10: How plate and lid parameters are defined.

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.

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

- 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.

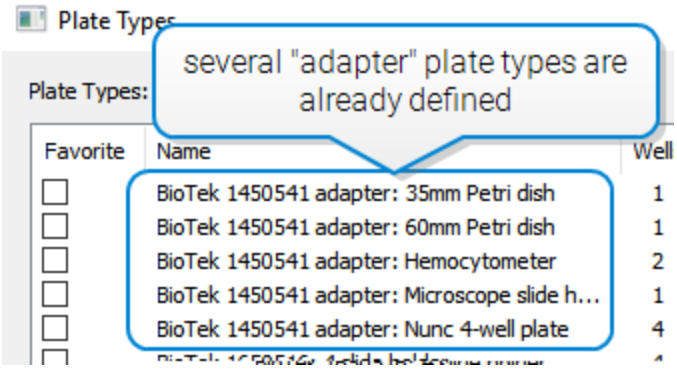

Figure 4-12: Adapter plate definitions.

Appendix A: Troubleshooting & Error Codes

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

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.

Error Code	Message and Resolution
6040 6041 6042 6043	**Invalid baud rate** **Invalid data bits selection** **Invalid stop bits selection** **Invalid parity selection** Service Only. Contact Technical Support. These codes indicate an unexpected software error that cannot be fixed without Agilent support.
6044 6045 6046	**Serial port error** **Serial write error** **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 6050 6051 6052 6053 6054 6055	**Excess data, or not enough data received.** **Invalid message header** **Invalid message object** **Invalid message body size** **Serial message timeout** **Port handle error** **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 6057 6058 6059 6060 6061	**Unauthorized to open the port.** **Out-of-range parameter for the open port function.** **Unable to open the port.** **Unable to clear the transmission buffer.** **Unable to close the port.** **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

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.