

Gen5®

Getting Started Guide



Gen5™

Getting Started Guide

Microplate Data Collection and Analysis Software

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Notices

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Revision History

| Rev | Date | Changes |
|-----|--------|---|
| I | 2/2015 | Minor text edits, updated references to Windows 8 to Windows 8.1; added .tif files to list of file types Gen5 creates |

Install Gen5

An "install wizard" guides you through the installation of Gen5—just follow the prompts. Before doing so, make sure your computer and BioTek instruments meet the system requirements outlined in this section. You may also want to review the installation options, software warranty, and related information.

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Gen5 License Agreement and Warranty

License Agreement

BioTek Instruments, Inc. (also referred to as “BioTek”) has developed Gen5 Microplate Software for Windows. The price paid for this software includes the license granting the purchaser a non-exclusive use of the Gen5 product per the guidelines given below.

Gen5: The purchase of Gen5 entitles the installation of the licensed copy on one or several computers, depending on the purchased version. If the purchased version supports multiple installations: (1) The installation of Gen5 on one computer for the purpose of controlling one or two BioTek microplate readers at a time and (2) multiple, simultaneous use of the software on multiple computers solely for the purpose of analyzing, reporting, and otherwise working with data already collected are expressly permitted.

This license agreement does not grant the user title to the software or any copyrights of proprietary rights in the software. The user may not sublicense, rent, lease, modify, translate, decompile or disassemble the software for any purpose. The license is not a sale of the original software or any copy.

Limited Warranty

BioTek warrants that for a period of ninety (90) days from the date of shipment, the software product manufactured and sold by BioTek will conform, as to all substantial operational features, to BioTek’s then current published specifications (meaning those specifications in effect as of the date of purchase), and will be free of defects which, in BioTek’s sole judgment, substantially affect system performance. This warranty is limited to the original purchaser and cannot be assigned or transferred. All claims under this Limited Warranty must be made in writing to BioTek, Attention: Service Department or to BioTek’s authorized representative or dealer. The purchaser must notify BioTek within ninety (90) days after the date of shipment of the software to the purchaser of any claim under this limited warranty. This 90-day period shall not be extended by the delivery or receipt of any subsequent modifications to the software. Modifications to the software within a version (“Updates”) are expressly excluded from the terms of this warranty. If the software is found to be defective by BioTek, BioTek’s sole obligation under this warranty is to use efforts consistent with BioTek’s regular business practices to attempt to remedy such a defect. In no event shall BioTek’s liability under this Warranty exceed the original purchase price of the software.

This warranty shall be null and void if the purchaser makes any modifications to the software. Correction of difficulties of defects traceable to Purchaser’s errors or system changes shall be billed to the purchaser at BioTek’s standard time and material charges.

System Requirements

Computer Requirements

Minimum Requirements: For Gen5 to run successfully, the computer must meet the following requirements:

- Windows 7 Professional Editions, Windows 8.1 Professional Editions
- Intel® Celeron™ Dual Core Processor T1600 (1.66 GHz, 667 MHz FSB, 1 MB L2 cache) or equivalent
- 512 MB RAM or higher/4 GB RAM or higher for Cytation 3 or Cytation 5 with imaging
- 2 GB hard drive space or higher
- Monitor resolution 1024 x 768 or higher/1280 x 800 or higher for Cytation 3 or Cytation 5 with imaging
- CD-ROM readable drive
- Keyboard/mouse
- Microsoft Internet Explorer v 5.0 or higher (for online Help)
- Microsoft Excel 2003 or later for QuickExport and Power Export (excluding Excel 2010 and Excel 2013 64-bit)
- Serial or USB port
- FireWire IEEE 1394b port for Cytation 3 and Cytation 5 with imaging (included with the Cytation 3 and Cytation 5) and open PCIe/expansion slot to support the FireWire card

Gen5 will run on a computer system that meets the minimum requirements for most applications. For very fast kinetic reactions in multiple wells, especially in higher density plates where large amounts of data are captured, BioTek recommends a 2 GHz processor speed and at least 4 GB RAM for best performance.

Change the Virtual Memory Settings

For instruments with the imaging module, it is recommended that you not allow Windows to automatically manage paging file size.

1. From the Windows Start menu, go to **Control Panel**, and select **System**.
2. In the left pane, select **Advanced system settings**.
3. In the System Properties dialog, on the Advanced tab in the Performance area, click **Settings**.
4. In the Performance Options dialog, on the Advanced tab in the Virtual memory area, click **Change**.
5. Clear **Automatically manage paging file size for all drives**, if it is selected. This is the default setting for both Windows 7 and Windows 8.1.
6. Select **Custom Size**, enter the following minimum and maximum values, and then click **OK**:
 - Initial size: 10 GB
 - Maximum size: 20 GB
7. You will need to restart your computer for the change to take effect.

Instrument Basecode Software Requirements

Verify that the basecode built into your BioTek instrument is compatible with Gen5.

If your instrument reveals a basecode with a version number lower than those provided here, please contact TAC for instructions for downloading and installing updated software.

| Instrument | Minimum Basecode Version |
|------------|--------------------------------------|
| BioStack | 2.03 |
| BioStack3 | All basecode versions are compatible |
| BioStack4 | All basecode versions are compatible |
| Cytation 3 | 1.12 |
| Cytation 5 | All basecode versions are compatible |
| ELx800 | 3.07 |
| ELx808 | 3.15 |
| Eon | All basecode versions are compatible |
| Epoch | All basecode versions are compatible |
| Epoch 2 | All basecode versions are compatible |

| Instrument | Minimum Basecode Version |
|---------------------|--------------------------------------|
| FLx800 | 1.15 |
| PowerWave | 1.21.1 |
| PowerWave XS | 1.06 (any PowerWave XS2 basecode) |
| Synergy 2 | 1.03 |
| Synergy 4 | 1.03 |
| Synergy H1 | All basecode versions are compatible |
| Synergy H4 | All basecode versions are compatible |
| Synergy HT | 2.24 |
| Synergy HTX | All basecode versions are compatible |
| Synergy Mx | All basecode versions are compatible |
| Synergy Neo | All basecode versions are compatible |
| Synergy Neo Stacker | All basecode versions are compatible |
| Synergy Neo 2 | All basecode versions are compatible |

Gen5 Software Level Comparison

BioTek offers seven levels of Gen5, from the most basic Reader Control utility to the full-featured, imaging-capable, and FDA-compliant Gen5 Image+ IVD.

Check out www.biotek.com for a detailed breakdown of the distinctions between software levels.

| | Gen5 RC | Gen5 | Gen5 Image+ | Gen5 Secure | Gen5 Secure Image+ | Gen5 IVD | Gen5 IVD Image+ | Gen5 TS |
|-------------------------------|------------|------|----------------|----------------|--------------------------|-------------|--------------------|------------|
| Control readers | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Data analysis | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Reporting/exporting data | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Save data | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| FDA 21 CFR Part 11 compliance | | | | ✓ | ✓ | ✓ | ✓ | |
| Data audit trail | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| System audit trail | | | | ✓ | ✓ | ✓ | ✓ | |
| QC Trending | | | | | | ✓ | ✓ | |

| | Gen5 RC | Gen5 | Gen5 Image+ | Gen5 Secure | Gen5 Secure Image+ | Gen5 IVD | Gen5 IVD Image+ | Gen5 TS |
|--|------------|------|----------------|----------------|--------------------------|-------------|--------------------|------------|
| Imaging: 40X and 60X objectives | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Imaging: Hit picking | | | ✓ | | ✓ | | ✓ | |
| Imaging: Cell counting only | ✓ | ✓ | | | | ✓ | | |
| Imaging: Full cellular analysis | | | ✓ | | ✓ | | ✓ | |
| Imaging: Subpopulations | | | ✓ | | ✓ | | ✓ | |
| Image processing (stitching, Z-projection, digital phase contrast) | | | ✓ | | ✓ | | ✓ | |
| Installations per license | 2 | 5 | 2 | 25 | 2 | 25 | 2 | NA |

Install the Software on a PC

Prerequisite

Gen5 requires the user who is installing the application to have Administrator privileges for the Windows operating system. If a user with restricted access attempts to install the application, errors may occur. Contact your organization's system administrator if you are uncertain about your privileges.

BioTek strongly recommends running a Windows Update to ensure the latest Windows security fixes and critical updates are installed prior to installing Gen5.

Install Gen5 (all versions on a PC)

1. Start Windows.
 - Be sure you have administrative privileges.
2. Follow the instructions from the insert, **Installing Gen5 from a USB Stick**, found on the inside of the USB stick case.

The Typical installation option is strongly recommended for most users (see below).

Gen5 asks for the serial number shown on your product packaging. Enter it and click **Continue** to save time later. If the number is unavailable you can click **Cancel** and provide this information later.

Be sure to register with BioTek for the fastest response from our support team, should the need arise.

Custom vs. Typical Installation

The Typical installation option is recommended for most users. It installs:

- Gen5 application (Gen5.exe and supporting files)
- Gen5 Diagnostic module
- Gen5 Take3 module

For Custom installations, click the arrow next to a feature to display the options menu. Select the desired option. When you opt to not install a feature, its disk icon is replaced with a red X.

Gen5 OLE Automation Toolkit

Custom installation is required during software installation to install the Gen5 OLE Automation Toolkit for programming robotic instruments to use Gen5. Instead of selecting the Install Wizard's default option for Typical installation, select **Custom**. Change the setting for OLE Automation Toolkit to install this feature.

Gen5 installs an OLE Automation folder when this option is selected. You'll find the Gen5 Automation Programmer's Guide (in PDF format), the BTIStatusCodes.h file, and a Samples folder containing several program samples in common programming languages.

To learn about security options when using OLE Automation, see **Login/Password Controls** in the Gen5 Help.

Next Steps: Register Gen5 and Initial Setup

After registering your software with BioTek, review the recommended initial setup suggestions for the level of Gen5 you're running.

Register with BioTek

The Pre-Registration screen appears when you launch Gen5 until the software is registered. A trial-version serial number can be used for the specified number of days until a licensed version is purchased. Unless the purchased version of Gen5 is a higher version than the trial version there is no need to reinstall the software; simply register the software.

As required for installing the software, you must have administrative privileges to register with BioTek. Generally, the user who logged in to Windows when installing Gen5 should be logged in when registering the software.

Windows 7 and Windows 8.1 users: If prompted for administrative privileges, engage them before registering the software: Locate and right-click the Gen5 desktop icon, and select **Run as administrator**. At the User Account Control dialog, click **Allow**.

Launch Gen5 and Register the Software

1. Open Gen5 by clicking its desktop icon or by using the Windows Start button and selecting **Programs > Gen5 > Gen5**.
2. At the Pre-Registration dialog enter the product serial number (if it wasn't entered during installation or if you've been using a trial version).
3. Click **Register** to register the software and receive a password. The Registration dialog appears with the serial number and the site key; this is information provided by your computer.

Click **Demo** to run Gen5 without registering it for the number of days displayed below the Demo button.

4. Click **Go to Internet Site**.
 - Alternatively, if your Internet browser and Gen5 are on different computers, enter the BioTek URL into your browser's address field. Make note of the serial number and site key to proceed.
 - If you do not have access to the Internet, contact BioTek. See **Getting Technical Assistance** on page 50.
 - When using the same computer, you can copy and paste (Ctrl+C and Ctrl+V) the serial number and site key into the registration form.
5. At BioTek's Software Registration website page, enter or paste the serial number and site key information into the form and click **Submit**.
 A registration form will be displayed containing any information BioTek already has about you and your organization.
6. Review and edit the information as necessary, then click **Submit Registration Form**.
7. Your password will be displayed on screen. Copy or make note of it.

8. Return to the Gen5 Registration dialog to paste or enter it into the Password field.
9. Click **Validate Password**.

The software should now be registered and you will not see the Pre-Registration screen again.

Gen5 stores the serial number and site key in the **Help > About Gen5** screen so you can log in to BioTek's product registration site at any time.

Initial Setup

The first thing all users must do after installing Gen5 on a PC is connect an instrument to the computer and tell Gen5 how to communicate with it. Other initial setup steps can be performed to improve your experience using Gen5. Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD, to meet FDA submission criteria, require establishing security conditions. You'll find instructions for performing all these tasks in this section.

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Connect an Instrument

System > Instrument Configuration

Before connecting the instrument: If you plan to connect the instrument to the computer via the USB port, you must first install a necessary USB driver. The driver and installation instructions are included on the Gen5 software USB flash drive. Connect the instrument after the driver installation is complete.

When you start Gen5 without an instrument connected to your computer, Gen5 prompts you to add one. Connect the instrument to the computer and then perform the following steps:

1. Click **Yes**.
2. Select an instrument from the list, then click **OK**.

You can also access the Instrument Configuration dialog later by clicking **System > Instrument Configuration**.

3. Click **Add** to define the Instrument Settings:
 - Select either **Plug & Play** or **Com Port** as the communication type.

Plug & Play

- Select an available instrument from the list.
- Click **OK** to save the settings.

For Com Port instruments:

- Select the Instrument Type from the list.
- Enter the number of the Com port in the Com Port field.
- If necessary, select a baud rate. **BioTek recommends retaining the default baud rate.**

Mismatched baud rate settings can cause “serial read” errors. When the baud rate is set to a non-default setting for non-keypad instruments, such as the PowerWave XS2, Synergy Mx, and Epoch, Gen5 is unable to communicate with the instruments if they are turned off and then turned on again while Gen5 is running. For keypad instruments, ensure that the instrument has the baud rate set to 9600. If the baud rate is changed, the instrument must be rebooted.

- If needed, click **Setup** to change the factory-tested and defined configuration values. This step is rarely required.
 - Click **OK** to save the settings.
4. Click **Test Comm**. Gen5 attempts to communicate with the instrument.
 5. After you receive a passing message, “The reader is communicating,” click **OK** and then **Close** at Instrument Configuration. If you receive any other message, look for a remedy in the **Troubleshooting** section of this guide.

Gen5 captures the information it needs from the instrument itself, including probe size, wavelength, bandwidth capability, filter set and mirror configuration, and any other applicable information.

Two models of PowerWave XS are listed in Gen5: PowerWave XS and PowerWave XS2. If you are connecting a PowerWave XS reader that has a USB port and an MQX200R**2** product number (take note of the 2), you must select the PowerWave XS2. Our changes to the PowerWave XS hardware to incorporate a USB/RS-232 COM port require unique reader identification in Gen5. There is no difference in the optical performance characteristics of the reader.

Set Up Gen5 or Gen5 Image+

Gen5 fulfills the instrument control and analytical needs for a wide range of laboratory settings. The degree to which you follow the recommendations provided here depends on the needs of your organization.

Recommended Tasks to Perform

1. Designate a System Administrator.
2. Install Gen5 on the Administrator’s computer.
3. Change the System Administrator’s password.

4. Determine the optimal way to store Gen5's protocol and experiment files. See **About File Storage** on page 55.
 - Organize the database or your Windows file structure.
5. Install Gen5 for other users and connect an instrument to each computer. See **Connect an Instrument** on page 18.
6. If applicable, direct each user's database configuration to point to the correct shared database.

Set Up Gen5 Secure or Gen5 Image+ Secure

1. Designate a System Administrator.
2. Complete the System Administrator's To-Do List (the Initial Setup tasks).
3. Organize the database. See **Organize Your Database Files** on page 25.
4. Review/modify Signature Reasons and other security controls (select **System > Security**).
5. Install Gen5 on each user's computer.
6. Set up each user's database configuration to point to the correct shared database. See **Move or Copy a Database to a Network** on page 26.
7. Connect an instrument to each user's computer.
8. Advise users to change their passwords.

Set Up Gen5 IVD or Gen5 Image+ IVD

1. Designate a System Administrator.
2. Complete the System Administrator's To-Do List (the Initial Setup tasks).
3. Organize the database. See **Organize Your Database Files** on page 25.
4. Review/modify Signature Reasons and other security controls (select **System > Security**).
5. Install Gen5 on each user's computer.

6. Set up each user's database configuration to point to the correct shared database. See **Move or Copy a Database to a Network** on page 26.
7. Connect an instrument to each user's computer.
8. Advise users to change their passwords.

Set Up Gen5 Reader Control

1. Install Gen5 on the computer.
2. Determine the optimal way to store Gen5's protocol and experiment files.
 - Organize the database or your Windows file structure.
3. Connect an instrument to the computer. See **Connect an Instrument** on page 18.
4. Set user preferences. See the Gen5 Help for more information.

System Administrator's To-Do List

Initial Setup Tasks: Gen5 Image+ Secure and Gen5 Secure

- | | |
|--------------------------|---|
| <input type="checkbox"/> | 1. Make sure all designated computers (PCs) and BioTek instruments meet the minimum requirements. See System Requirements on page 9. |
| <input type="checkbox"/> | 2. Install Gen5 Image+ Secure or Gen5 Secure on one computer (PC). |
| <input type="checkbox"/> | 3. Start Gen5 and log in as the System Administrator. |
| <input type="checkbox"/> | 4. Change the System Administrator's password. |
| <input type="checkbox"/> | 5. Copy the database Shared.mdb to a secure network location. |
| <input type="checkbox"/> | 6. Test database configuration of the Shared.mdb on the network. |
| <input type="checkbox"/> | 7. Create/modify user groups, as needed, and assign user permissions to the groups. |
| <input type="checkbox"/> | 8. Create new user accounts and assign the users to a group.* |
| <input type="checkbox"/> | 9. Connect instrument(s) to the PC and establish communication. |
| <input type="checkbox"/> | Repeat Steps 2, 3, 6, and 8 for the remaining PCs. |

Initial Setup Tasks: Gen5 Image+ IVD and Gen5 IVD

- | | |
|--------------------------|---|
| <input type="checkbox"/> | 1. Make sure all designated computers (PCs) and BioTek instruments meet the minimum requirements. See System Requirements on page 9. |
| <input type="checkbox"/> | 2. Install Gen5 Image+ IVD or Gen5 IVD on one computer (PC). |
| <input type="checkbox"/> | 3. Start Gen5 and log in as the System Administrator. |
| <input type="checkbox"/> | 4. Change the System Administrator's password. |
| <input type="checkbox"/> | 5. Copy the database Shared.mdb to a secure network location. |
| <input type="checkbox"/> | 6. Test database configuration of the Shared.mdb on the network. |
| <input type="checkbox"/> | 7. Copy the database QCDB.mdb to a secure network location. |
| <input type="checkbox"/> | 8. Test database configuration of the QCDB.mdb on the network. |
| <input type="checkbox"/> | 9. Create/modify user groups, as needed, and assign user permissions to the groups. |
| <input type="checkbox"/> | 10. Create new user accounts and assign the users to a group.* |
| <input type="checkbox"/> | 11. Connect instrument(s) to the PC and establish communication. |
| <input type="checkbox"/> | Repeat Steps 2, 3, 6, 8, and 10 for the remaining PCs. |

* For Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD, you can use Windows Authentication as an alternative. See **Configure Windows Authentication** on page 32.

Periodic/As Needed Tasks

- Customize the security features to accommodate your organization's needs.
- Organize your database files.
- Educate users on regulatory requirements and Gen5 best practices.
- Establish and implement a procedure and schedule for record retention and archival.
- Review records, including any training/user-qualification records.

Before modifying a user's account, make sure he/she is not logged in to the system. You can check the System Audit Trail to determine who is currently logged in.

Change the System Administrator's Password

System > Security > Users

System > User Setup > Administrator tab

BioTek recommends changing the System Administrator's password immediately following Gen5 installation to ensure a secure operating environment.

To change the password:

1. Log in as the System Administrator, if you haven't already done so.
 - Select **System > Login/Logout**.
 - Set the User to **Administrator**.
 - Enter the default password: **admin**.
2. Select **System > Security > Users**.
3. Double-click the System Administrator user (to edit the record).
4. Define and confirm the new password. The System Audit Trail will open to log the change and accept your comments.

Record and store the new password in a secure location. If you forget the password, contact BioTek TAC for assistance.

About Gen5 Databases

Gen5 installs two databases during regular installation: SharedDB and LocalDB. Only Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD are initially set up to use the Gen5 Database for experiment and protocol file storage. All other levels of Gen5 must elect to use the database to store experiment and protocol files at **System >**

Preferences > File Storage Mode.

- SharedDB can be set up on a network for sharing information among multiple users. It contains all protocol and experiment data files and their associated audit trails, the plate types, and reader-diagnostic history data. In Gen5 Secure and Gen5 IVD, SharedDB also contains security settings, user accounts, and a system audit trail for shared events. This database can be moved, renamed, and copied. So, if desired, you can create a unique database for individual projects, teams, or other classification.

Gen5 Image+ IVD and Gen5 IVD also install the QC Trending database, QCDB.mdb. It can be set up on a network for sharing among multiple users, moved, renamed, and copied. It is initially installed in a QC folder in the default database location described below.

- LocalDB contains the local setup information, including the Instrument Configuration. For Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD, this database also contains an audit trail for local events. LocalDB is stored on the computer's hard drive, and it cannot be moved or renamed.
- **Default database location:** During normal installation, Gen5 installs its databases in Windows Common Application Data Folder:
 - **Windows 7 and Windows 8.1:** C:\Program Data\BioTek\Gen5 (software level)\(version #)\SharedDB or LocalDB

You may need to change your operating system settings to view the Application Data folder.

- **Database Names after Upgrade:** The Gen5 Upgrade Utility changes the names of the databases to help distinguish them:
 - **SharedDB:** The file name of the database selected for upgrade is not changed during the process, but the upgraded version is identified by this suffix: "Upgraded_<date_time>.mdb".

The Gen5 Periodic Backup routine appends the filename with "Auto_Backup_<date_time>".

- **LocalDB:** The file name of the LocalDB selected for upgrade is changed because Gen5 requires the database stored on your hard drive to be named LocalDB. After the upgrade the older version is named with this suffix: "Before_<date>_<time>.mdb".
- **Max Size:** the maximum size of the database files is 2 gigabytes (GB). At startup, Gen5 checks the remaining size of the database. Warning messages are displayed when the database size exceeds 1536 MB. Use Gen5 maintenance and backup features to archive your database records.
- Gen5 has **built-in error recovery modes**. When your connection to the database is lost for any reason, Gen5 saves any unsaved files as Temporary Files. After a system failure, the next time you open an affected protocol or experiment file, Gen5 offers to replace the unsaved files with the Temporary Files. Select **Yes** to recover any changes made to the files before the system failure; select **No** to open the files as they were last saved, before the unsaved changes were made. Newly

created files are also saved as Temporary Files. Following a system failure, you can rename these temporary files with the proper file name extension (.xpt or .prt) using Gen5 Maintain Files controls.

- **File locking:** When a file is opened in Gen5 it is “locked” to protect it from being modified (saved or renamed) by a different user. When a second user attempts to open the file, he or she receives a message stating: “File <filename> is already in use. Do you want to open it in read-only mode?”
- Gen5 offers **automatic backup**. You can define settings for regularly and automatically backing up and optimizing databases with Gen5 Auto-Optimize feature.

Organize Your Database Files

During regular installation, Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD use the shared database to store experiment and protocol files. All other levels of Gen5 must elect to use the database at **System > Preferences > File Storage**.

All of your file management requirements can be fulfilled using Gen5 databases. You'll be most satisfied with the final structure if you spend some time planning it up front. In a multiple-user environment, you can set up a Gen5 database on a shared-network drive (LAN) so multiple users can access the same protocol and experiment files.

Backups: Performing backups on a regular schedule is highly recommended to preserve your data. And, Gen5 provides a tool to schedule backups to occur periodically. See below.

File Management Recommendations

- Put a **copy of the SharedDB on a shared-network drive** where all your Gen5 users can access it. Be sure to set each user's database configuration to point to the correct location.
- Before moving the SharedDB to a network location, make a copy of it to use as a template for future use:
 1. In the default SharedDB folder, highlight the original, right-click, and select **Copy**.
 2. Deselect the original (click elsewhere in the dialog), right-click, and select **Paste**.
 3. Highlight the copy, right-click, and select **Rename**.
 4. Give the copy a unique name, like SharedDB_original.mdb.

- Consider setting up **shared databases** for different projects or teams within your organization. You can follow the steps defined above to create multiple databases in the same folder (or directory), or you can move the unique databases to a different network location/folder. Use Database Configuration to point users' Gen5 sessions to the correct database.
- **Regularly archive and back up the database** to preserve your records. Use Gen5 Optimize and Backup Settings to backup and clean small errors in your database. BioTek recommends following your organization's existing policy for securing data, for example, putting the shared database on the network to be backed up every night.
- Consider using the Gen5 automatic Save feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 organizes all that data by date. Define this kind of file management setting in the Initial Protocol Settings (**System > Preferences**) so it will apply to all newly created protocols.
- Gen5 handles multiple, simultaneous users performing database management tasks by giving precedence to the user with the greater administrative rights.

Move or Copy a Database to a Network

System > Database Configuration

Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD install and enable the databases during regular installation. All other levels of Gen5 must elect to use the database to store protocol and experiment files at **System > Preferences > File Storage**.

In a multiple-user environment, you can set up the Gen5 database on a shared network drive so multiple users can access the same protocol and experiment files. This is a recommended step for System Administrators. You can also set up multiple databases, one for each team or project, for example. During a Gen5 session, access is provided to only one database at a time.

How To

1. Select **System > Database Management > Database Configuration**.
2. Select the **SharedDB** tab.
3. Next to the Source field, click **Browse**.

4. In the Open dialog, highlight and right-click the file **SharedDB.mdb**, and select **Copy** or **Cut**; use cut to move and copy to copy (see File Management Recommendations below).

SharedDB is the installed/original name for the shared database. Because you can change the name, it's possible it has already been changed.

5. Navigate to the desired location in the Look in field.
6. When the correct location is selected, right-click in the window and select **Paste**.
7. Click **Open** to save and close the window, and return to the Gen5 Database Configuration dialog.
8. Shut down and restart Gen5 to make the changes take effect.

If you're moving the Shared DB to a network drive and you have Windows XP, you may want to consider disabling file caching. See the Gen5 Help for more information.

About User Accounts

System > Security > Users

Prerequisite

This function is available only to the System Administrator. You must log in as the Administrator (**System Menu > LogIn/LogOut**) to access all the controls. Non-administrators are limited to changing their own password and selecting a Startup Action and Protocol Folder.

How to Create, Modify, or Delete User Accounts

Only an Administrator can add, modify, or delete users. You can designate multiple individuals to be system administrators. Any user account can be changed or deleted, except there must always be at least one System Administrator:

- Click **New** to set up a new user.
- Double-click or highlight a user, and click **Edit** to modify its name, password, or Group assignment.
- Highlight a user, and click **Delete** to remove the user account.

About User Groups

System > Security > Groups

Prerequisite

This function is available only to the System Administrator. You must log in as the Administrator (**System > LogIn/LogOut**) to access this control.

Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD use groups to manage the rights or permissions granted to users. When creating (or maintaining) a group, you define the level of access and the controls available to certain types of users and then assign actual users to the groups. Gen5 ships with three groups: Administrator, Power User, and Standard User.

The System Administrator and Power User groups are given access rights to all functions. The Administrator's rights cannot be changed and include additional rights to manage user accounts that are not extended to Power Users. When Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, or Gen5 IVD is installed, the Standard User is limited to the following permissions. The System Administrator can change these rights as needed:

- Open a protocol
- Add a new plate
- Create/Edit Sample IDs
- Edit plate information
- Edit Plate Layout
- Edit Report/Export Builders
- Define test plates
- Create folder in database

Create New Groups and Modify Existing Groups

Only a System Administrator can add, modify, or delete groups. Except for the Administrator group, any group can be changed or deleted, and any group can be renamed.

- Click **New** to set up a new group.
- Highlight a group and click **Edit** to modify its name and permissions.

- Highlight a group and click **Delete** to remove it as an option. First you must reassign any users to another group. You cannot delete a group with users assigned to it.

Create/Maintain User Accounts

For Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD only.

System > Security > Users

Prerequisite

Most options for user accounts are available only to the System Administrator. Non-administrators are limited to changing their own password and selecting a Startup Action and Protocol Folder.

User ID

Enter a unique ID using 1 to 16 alphanumeric characters. The user will enter or select this ID when logging into Gen5 and when signing files.

Full Name

Enter the user's name. This name will be associated with events logged by this user's actions and with the digital signature applied by this user.

Group

Choose a Group membership to assign access rights and permissions to the user. See **About User Groups** in the Gen5 Help for information. Users receive the rights assigned to the Group.

Status

The check box shows whether the user's account is currently locked. The System Administrator can lock or unlock the account. When a user's account is locked, the user cannot log in to Gen5 and cannot sign files. A user's account may become locked due to one of three events:

- Intentional lock by the Administrator using this dialog
- Automatic lock if the user exceeded the number of successive failed login attempts
- Automatic lock if the user's password expired

Unlocking a user's account following an automatic lock resets its counter or clock. The reset is specific to the reason for the lockout: When it is caused by password expiration, the password expiration clock is reset; when it is caused by failed logins, the user's history of "successive failed login attempts" is reset to 0.

When lockout occurs due to an expired password, unlocking the account allows the user to log in to Gen5 with the same password, providing a chance to change it. Alternatively, as system administrator, you can simply change the password yourself (which will by default unlock the account) and tell the user to log in with the password you have assigned him or her.

Startup Action

Select the preferred method for starting Gen5:

- **Display Task Manager/Last used page** is the default setting. You can also specify a specific Task Manager page, such as Read Now page or Experiment page.
- **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected **Experiments > Create New**.
- **Start at system menu** opens Gen5 showing the File, Take3, Window, System, and Help menus only. Since neither a protocol or experiment is open, the workspace is blank.

Protocol and Experiment Folders

Browse to or enter the full path and directory to define the folder in which the current user will typically store protocol and experiment files. Gen5 defaults to the most recently accessed folder.

Password

Assign a password for the user to enter the first time he or she logs in to Gen5. Instruct users to change their password after the first login using the password you've assigned. Users can change only their own password. System Administrators can change any user's password.

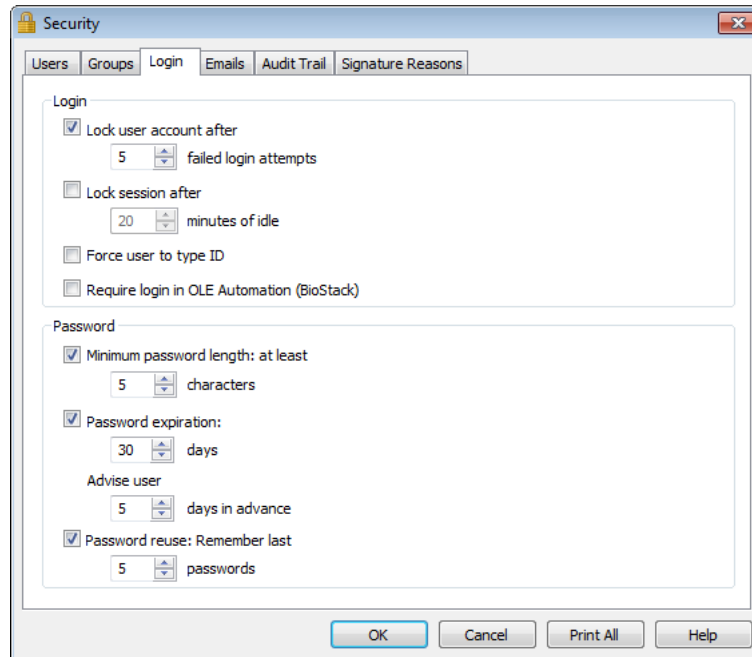
Login/Password Controls

System > Security > Login

Prerequisite

Only the System Administrator can access these controls. You must log in as the Administrator (**System > LogIn/LogOut**) to change the settings.

The default settings shipped with Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD, as shown in the screenshot below, comply with the FDA's 21 CFR Part 11 requirements for controls for identification codes/passwords.



Login

- **Lock user account after:** Specify the number of successive failed login attempts a user may make before being locked out of Gen5. This feature does not apply to System Administrator accounts, and only a System Administrator can reinstate a locked-out account. Valid entry range: 2–10. When this feature is not selected, users' login attempts are unlimited. Compliance with 21 CFR Part 11 requires setting a limit for failed login attempts.
- **Lock session after:** Specify the number of minutes that a Gen5 session can be idle before it is locked and requires successful user login to reactivate. A session is considered idle when there is no keyboard or mouse activity and Gen5 is not controlling a reader activity. Valid entry range: 1–1440 minutes. Compliance with 21 CFR Part 11 requires setting an idle-time limit.
- **Force user to type ID:** Apply this control if your security rules require users to enter their ID at login and to apply their Signature. When this feature is not selected, the last user's ID is displayed in the login and signature screens, and users can select an ID from a drop-down list of users.
- **Require login in OLE Automation (BioStack):** Select this option to ensure that Gen5 security permissions are enabled when Gen5 Image+ Secure, Gen5 Image+

IVD, Gen5 Secure, or Gen5 IVD is run as an OLE Automation server, for example, for using the BioStack. If Windows Authentication is enabled, the login process may take place automatically. If the user does not have Windows Authentication enabled, Gen5 prompts the user to enter a login ID and password. Logins performed while this option is selected are tracked in the Audit Log.

Password

- **Minimum password length:** Specify the minimum number of alphanumeric characters required for a valid password. Valid entry range: 2–10 characters.
- **Password expiration:** Specify the number of days a password can be used before users are required to change it. When users let their password expire without changing it, their accounts are locked out and only a System Administrator can reinstate a locked-out account. Valid entry range: 1–10000 days. If this feature is not selected, passwords do not expire. Compliance with 21 CFR Part 11 requires an expiration period.
- **Lock out:** When a user's password has expired, the system administrator has two choices:
 - Manually remove the Locked out flag. This resets the password expiration period allowing the user to log in using his/her current password.
 - Enter a new password for the user (which unlocks the account) and tell the user to log in with the password you have assigned him/her. Advise the user to change the password after logging in.
- **Advise user:** If password expiration is set, specify the number of days before passwords expire to alert users to change their password. Valid entry range: 1–30 days, but cannot exceed the number of days to Password Expiration.
- **Password reuse:** Specify the number of passwords Gen5 will remember for each user's account to prevent a recently used password from being reused. Valid entry range: 2–20.

Configure Windows Authentication

Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD provide an option to allow users to sign in to Gen5 using Microsoft Windows Authentication instead of user accounts defined in Gen5. Through Windows Authentication, users log in to their workstations once and have access to Gen5 without having to log in again. The Windows Authentication feature benefits system administrators as well by providing a single location for the management of user settings.

To use the Windows Authentication feature, workstations must be running Windows 7 or Windows 8.1; earlier versions of the operating system will not work properly. Servers must be running Windows 2003 or Windows 2008.

BioTek has validated this feature on Microsoft Windows Server 2003 (SP2 and SP3) and Microsoft Windows Server 2008 (SP1). BioTek does not provide support for the use of the Windows Authentication feature on a server that has not been validated.

Before Windows Authentication can be activated in Gen5, both the Gen5 Administrator and the IT administrator must perform some setup tasks.

Gen5 Administrator Tasks

Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD use groups to manage the rights or permissions granted to users. The Group Names are defined in Gen5 by the Gen5 Administrator.

When creating or maintaining a group, the Gen5 Administrator defines the level of access and the controls available to certain types of users, and then assigns actual users to the groups. Gen5 ships with three default groups: Administrator, Power User, and Standard User.

The System Administrator group is given access rights to all functions. The Administrator's rights cannot be changed. When Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, or Gen5 IVD is installed, the Standard User is limited to the following permissions (the System Administrator can change these controls as needed):

- Open Protocol
- Use Gen5 Protocols (requires Gen5 Native)
- Add a New Plate
- Create/Edit Sample IDs
- Edit Plate Information
- Edit Report/Export Builders
- Create folder in database
- Edit Paneled protocols
- View protected/read-only protocol items

IT Administrator Tasks

The IT administrator must create user groups on the server to mirror the Gen5 User Groups defined in Gen5. The user groups on the server must be named Gen5_<Gen5

Group Name>, where <Gen5 Group Name> is the name of a group in Gen5, such as Gen5_System Administrators. The IT administrator can also define permissions that apply to Windows resources, such as access to folders, printers, and so on.

Each user who has permission to run Gen5 must be defined as a member of a single Gen5 group in Windows. If a user is defined as a member of more than one Gen5 group, an error message will appear when the user attempts to log in to Gen5. If the user is not assigned to a Gen5 group, a warning message will appear, indicating that the user is not a valid Gen5 user. The user can then log in with another, valid Gen5 account.

Configure the Email Notification Feature

Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, and Gen5 IVD can be set up to send an email notification to specified recipients when a predefined event, such as a reader error or failed login, occurs. The email server settings must be configured to support this feature. The Gen5 Administrator must test these settings to verify that the Email Notification feature is functioning correctly.

To verify that the email server settings are configured correctly to support this feature, check with your IT administrator.

Defining a Custom Email Template

By default, Gen5 generates the title and body of the email notification messages, but you can define a custom template that includes specific variables.

1. On the Email tab of the Security dialog, click **Auto** in the Template column.
2. In the Email Definition dialog, select **Custom** as the mode and either **Text** or **HTML** as the format.
3. Modify the Subject and Body text as necessary. Variables associated with the defined event are listed in the Variables text box. To insert them in the Subject or Body of the email notification, place the cursor where you want the variable to occur, then double-click the variable in the Variable text box.
4. Click **OK**.

Configure the Email Server

System > Preferences > Email Server

System > Security > Emails tab > Email Server

The email server must be configured correctly to support the Gen5 Email Notification feature. Check with your IT administrator to verify your configuration settings.

In the **Send Emails from** area, you can define the email addresses used in the Email Notification feature.

- **Display name:** This field is required. By default, Gen5 displays Gen5_Notice_Do_Not_Reply@biotek.com as the address from which the notification message is sent. The user can change this address, if desired.
- **Forward errors to:** The address specified in this field will receive any mail delivery error messages generated by the server related to the Email Notification feature. If no address is entered, error messages will be sent to the address in the Display Name field.
- **Reply to:** The address specified in this field will receive messages sent as a reply to an Email Notification message. If no email address is entered in this field, the address in the Display Name field will receive any reply emails.

Getting Started

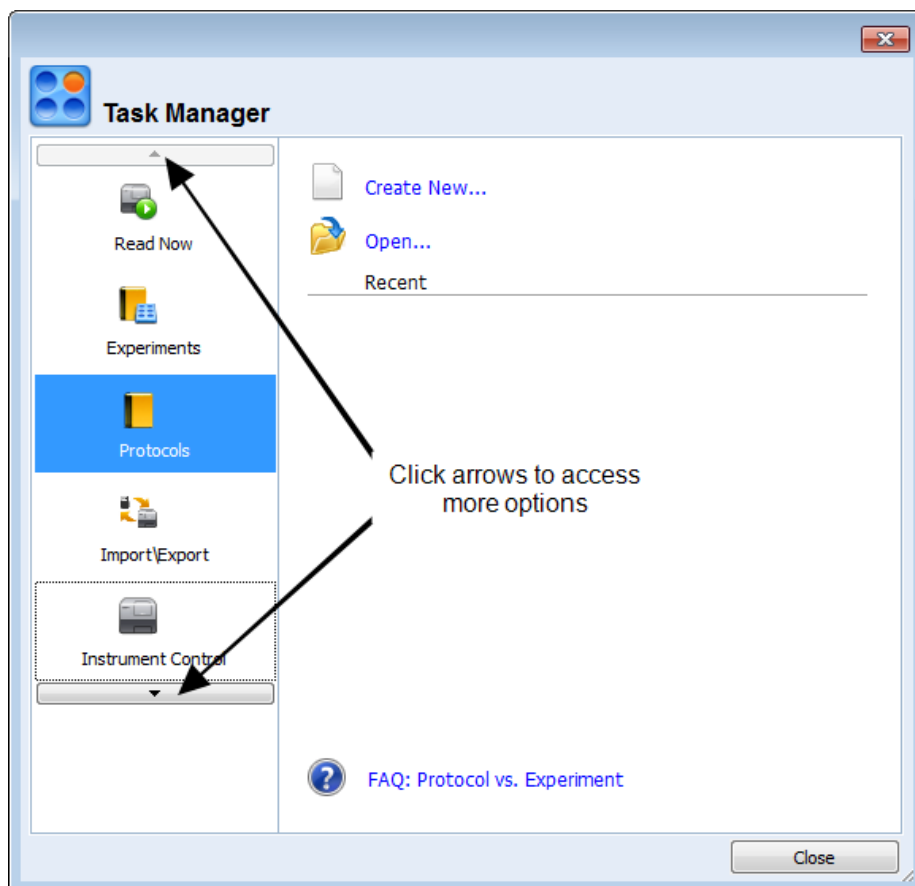
Gen5 software provides a logical interface to all automated BioTek plate reads. It is designed to flow from reading parameters, to plate layout, to data reduction, and finally to flexible data output options.

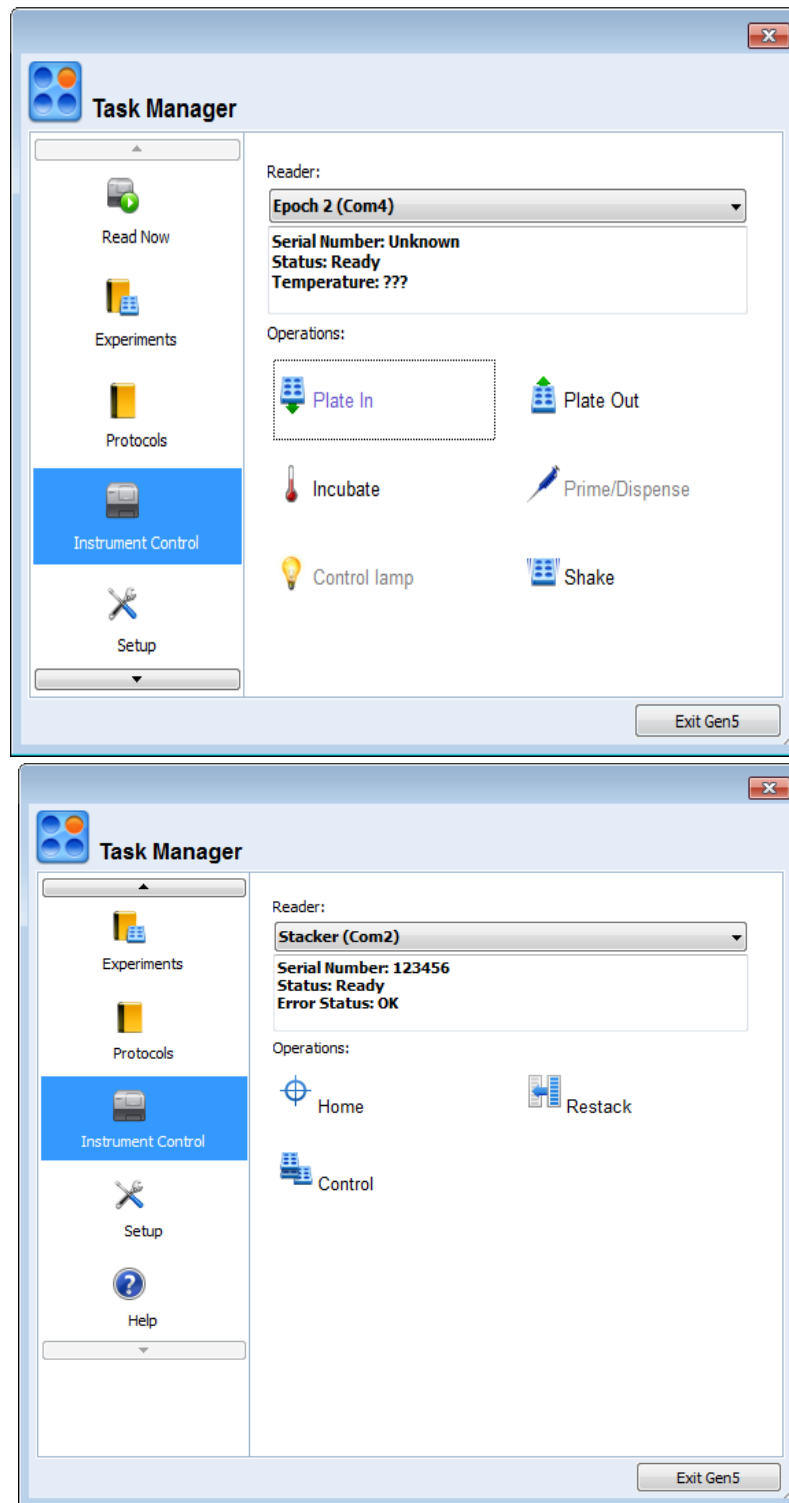
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Task Manager

Whenever you start Gen5, the Task Manager opens. You can also return to the Task

Manager by selecting **File > New Task** or . If you are in the Task Manager, selecting  returns you to the main screen.





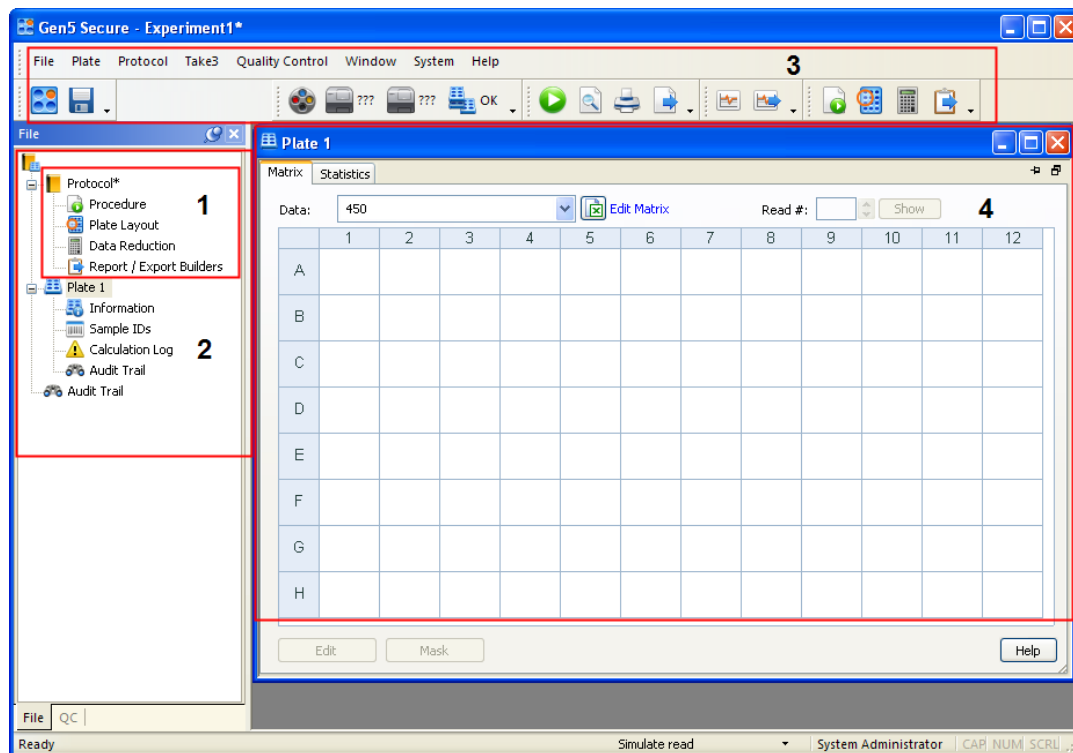
Instant Access

The Task Manager provides quick links to give you instant access to:

- Executing a new read
- Opening or creating an experiment or protocol; the most recently used protocols and experiments are listed
- Control several of the instrument's operations (e.g., incubation, shaking, plate in, stacker control)
- Accessing configuration and security settings
- Opening the System menu
- Accessing the Gen5 Help system, FAQs, and sample files

The Gen5 Workspace

Gen5 offers several controls and workspaces for developing protocols, running experiments, and viewing and reporting results:



The Quality Control menu shown above is available only in Gen5 Image+ IVD and Gen5 IVD.

1. Protocol

Every experiment is based on a protocol. The differences between a protocol and an experiment in Gen5 are described in the **Essential Concepts** chapter.

2. Menu Tree

The menu tree, docked at the left side of the workspace, provides an alternative to using the toolbars and menus.

3. Toolbars and Menus

See **Buttons and Icons** on page 43 to learn about Gen5 toolbars, buttons, and menus.

4. Plate View

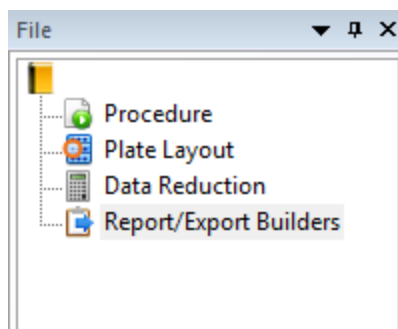
Gen5 provides a view or workspace for each plate processed (or to be processed) in an experiment. You must have an experiment, rather than a protocol, open to have a Plate View.

To open the Plate View: In an experiment, if it is not already open in the main view of Gen5, double-click the plate icon in the menu tree or select **Plate > View**.

Gen5 offers several ways to modify and customize the Plate View for on-screen display and reporting/outputting results. See **View Results** on page 65 to learn more.

Protocol Workspace

When you create a new protocol, Gen5 opens a special workspace limited to the protocol's components.



The workspace is made up of the menu tree with a branch for each of the protocol's elements. The order of the protocol elements reflects the order to follow when defining most protocols:



Defining the **Procedure** or reading parameters is the most important step to Gen5. The Procedure describes the data sets that are used in most subsequent steps to generate results output. The Plate Layout is the only other protocol element that is not affected by the Procedure; it is affected by the selected plate size.



For most protocols, it's best to define the **Plate Layout** in your second step. Gen5 automatically performs a blank-subtraction calculation when Blanks are defined in the plate layout. (You'll see this Transformation in the Data Reduction workspace.) Defining the standards and their concentrations in the plate layout is a prerequisite to generating a standard curve.







Data Reduction is one of the most powerful features in Gen5, and it requires the information provided by the two previous steps to logically offer you its capabilities. Automatically generated transformations, like pathlength correction and the ability to conduct well analysis, for example, depend on the Procedure. To plot a standard or titer curve and to validate Transformation formulas requires knowing the Plate Layout.






















Report/Export Builders is a tool for selecting and customizing the appearance of data sets that are then available for printing or exporting.






















The Menu Tree

- To keep the menu tree open in a protocol or experiment, you must click  to dock it in place. When you're working with a protocol file, the menu tree, like the toolbar, is limited to related operations.
- The menu tree provides a visual cue of the steps to follow when creating a protocol.
- All of the controls available from the menu tree can alternatively be accessed using toolbar buttons or menus.
- Click  and  next to an item to reveal or hide its components.
- Highlight an item in the menu tree and right-click for a context-sensitive menu of options, including **Read** when a plate is selected, for example.
- Asterisks (*) are displayed next to plate icons (and in the title bar) of an experiment when a change is made or an action is taken but the file has not yet been saved.
- You can move the menu tree to another corner of the workspace or let it float undocked like the Plate workspace: click the undock button , drag the title bar, and drop it in the desired location.
- When you add multiple plates to an experiment, highlight a plate and right-click for menu options to delete and renumber plates.

Buttons and Icons

| Button | Description |
|---|--|
|  | Open the Task Manager |
|  | Save the protocol or experiment |
|  | Read the plate |
|  | Print preview |
|  | Print results |
|  | Export results |
|  | Procedure |
|  | Plate layout |
|  | Data reduction |
|  | Report/Export Builders |
|  | Paneled protocols |
|  | <i>(Imaging only)</i> Go to manual mode |
|  | Set Reader Optics: Opens the Set Reader Optics dialog in which you can update the reader with new definitions for filters and mirrors. |
|  | Reader Control: Check the status, open the control panel |
|  | Stacker icon: Opens the Stacker control panel, if a Stacker is attached. |
|  | Export results to QC (Gen5 Image+ IVD and Gen5 IVD only) |
|  | Edit trended protocols (Gen5 Image+ IVD and Gen5 IVD only) |
|  | Pin the current plate view to the workspace |
|  | Duplicate a coincident display of the plate's results |

Menu Tree Icons

| | |
|---|--|
|  | Plate—not read: Put the plate in the reader and click  |
|  | Plate read successful |
|  | Plate read paused by Stop/Resume step: When you're ready, put the plate in the reader, click  , and select Resume Plate x to continue |
|  | Plate read aborted: To begin again, put the plate in the reader, click  , and select Re-Read Plate x |
|  | Plate read in progress |
|  | Plate read error, which is always preceded by an error message. The error code and message are recorded in the plate data audit trail. It is the user's responsibility to verify the integrity of the data after an error occurs. |
|  | Protocol |
|  | Procedure: Define the reading parameters |
|  | Plate Layout: Assign location of samples |
|  | Data Reduction: Set up calculations |
|  | Report/Export Builders |
|  | Plate Information: Information obtained at runtime |
|  | Sample IDs: User-defined names or IDs assigned to samples |
|  | Calculation Warning Log: Data Reduction-related errors issued by unexpected curve or calculation results |
|  | Audit trail displays any logged events |
|  | Multi-Plate protocol view of data reduction statistics and curves |
|  | Panel: Multi-protocol experiment performed on one plate |
|  | Paneled Protocols: Lists the protocols run (or to be run) in the panel experiment |

Troubleshooting

- **First Response:** Running a System Test is the best first response to an instrument error. The test may restore the instrument's initial settings and computer communication capability. **Note:** To stop the alarm, press the carrier eject button.
- **Reboot the Computer and Instrument:** When you can't run a system test, for example, Gen5 is not responding, or when running a system test doesn't resolve the issue, turn off your computer and instrument, check all the cabling (i.e., make sure your serial or USB cable is in good condition and is properly connected to the PC and instrument), and then, power on your computer and instrument. These steps should refresh the devices and reset communication parameters.

Visit BioTek's website for useful suggestions on getting the most from your reader: www.biotek.com/resources

- **Incompatible protocols:** Protocols created with one instrument are not instantly compatible with other instruments. To correct the error "Procedure was defined for a different instrument," open the Procedure and click **Validate**. If this does not correct the error, open each step in the Procedure and review it for compatibility with the current instrument.
- **Windows 7 and Windows 8.1 Missing Files:** The Gen5 installation routine attempts to avert potential file sharing issues on Windows 7 and Windows 8.1, but when multiple users share a computer, Windows may use its "VirtualStore" as the default location for file storage.

Communication Errors

FYI

- To prevent damage to the instrument, always **turn off the instrument or the computer** before removing or inserting a communications (serial or USB) cable.
- Gen5 and the instrument-communication parameters supersede the Windows settings. Windows communication port configuration settings should not need adjustment to enable proper communications.

When the computer (PC) won't communicate with the instrument

- **Confirm that the instrument passes its system self-test.** All BioTek instruments perform a self-test when turned on. Refer to the instrument's operator's manual for more details. The instrument will not communicate if it fails an internal system test. Non-keypad instruments beep continuously when the system test fails. (Press the plate-carrier button to stop the alarm.) Keypad instruments display an error

message when the test fails. Refer to the operator's manual to resolve the failure or contact BioTek TAC.

- **Make sure the serial or USB cable is in perfect condition and properly attached** to the port defined in the Instrument Configuration dialog (e.g., COM 1 or Plug & Play). Correct and reboot both PC and reader. Test communication.
- **Confirm the baud rate** (or transmission speed) defined in the Gen5 Instrument Configuration dialog matches the instrument's settings. Consult your instrument's operator's manual for the correct rate. Correct the Gen5 Instrument Settings to match and reboot both PC and instrument. Test communication.
- **Disable the Calculation Option:** The **Perform data reduction after each read** option gives Gen5 sufficient time between obtaining measurements to perform calculations.
- **Confirm that the serial/USB cable was obtained from BioTek.** Serial/USB cables are not universal. Consult the instrument's operator's manual for proper cable configuration or contact BioTek customer service to purchase a factory-tested cable. After installing a known good cable, reboot both PC and instrument. Test communication.
- **Confirm** with your computer supplier or a local PC technician **that the serial port has been enabled.** For example, the IBM Thinkpad was originally shipped with the serial port disabled. Correct and reboot both PC and reader. Test communication.
- For advanced PC users, the serial port of the instrument and PC can be independently tested using an independent serial-communication software package such as Windows Terminal, Hyper Terminal, or ProCom. BioTek does not support or sell these programs.
 - Select flow control for "XON/XOFF" and send an ASCII asterisk symbol (*) to the reader. The instrument should initiate a self-test and return the results to the PC. If the instrument fails to communicate, and Steps 1 through 5 do not resolve the problem, test the instrument on an alternative PC to confirm which device is at fault. Please contact BioTek if the instrument is diagnosed to be faulty.

Restore Optimal Performance

Numerous factors can affect your computer's performance. If you notice a slowdown in Gen5's performance, follow these suggestions:

- Close all other applications, including Internet browsers, when running Gen5.
- Do not display Gen5's Curves data in the Plate View while performing a kinetic analysis. Wait until the read step is finished before viewing the Curves data set.

Displaying the Curves data set during a Kinetic read can consume excessive resources resulting in performance degradation. You can drill down to a Well Zoom to monitor the progress of one well, then, leaving the Well Zoom open, change the Matrix Data to a numeric view.

- Disable the Calculation option **Perform data reduction after each read** to give Gen5 sufficient time between obtaining measurements to perform calculations. Select **Protocol > Protocol Options > Calculation Options**.
- Disable the auto-Save option: Save when: Interim read completed. Change the Save Options to free up resources. Select **Protocol > Protocol Options > Save Options**.

Fluorescence/Luminescence Measurements

Here's a list of potential problems, the possible cause, and a remedy:

Fluorescence/Luminescence Readings Too Low

- **Possible cause: The fixed Gain in the Read Step dialog is currently set too low**

Raise the Gain to an appropriate level. For fluorescence, the Gain is usually set between 45 and 130. For luminescence it is usually set between 100 and 200. Learn more in the Gen5 Help.

Try Automatic Gain Adjustment, using the Scale to High Well option and setting the target value to be between 20,000 and 80,000 for standard range, or 1,000,000–3,500,000 for extended range (if supported by your reader).

Some readers have extended range capabilities with flash fluorescence. These models are auto-ranging up to 10,000,000.

- **Possible cause: The wrong filters are selected in the Read Step dialog (for filter-based reads only)**

Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.

- **Possible cause: Top probe setting is not optimized**

Gen5 generally positions the top probe at the optimal height for fluorescence reads, based on the plate type selected; it focuses the beam above the well. Refer to the Gen5 Help and use the Read Height option in the Read Step dialog to make adjustments. If supported by your reader, try the Auto-Adjust feature for the probe height. The reader's operator's manual may contain additional suggestions.

Fluorescence Background Too High

- **Possible cause: Using incorrect microplates**

Solid black plates for top probe reading lower the background. Black plates with clear bottoms lower the background if bottom reading is necessary. Corning 3615 or 3614 (for cell culture) are appropriate.

- **Possible cause: The wrong filters are selected in the Read Step dialog (for filter-based reads)**

Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.

- **Possible cause: Phenol red is used in the media when exciting at 485 nm and reading at 528–530 nm**

Eliminate or replace the phenol red.

- **Possible cause: Cells, media and other contents fluoresce**

Use deionized-water blank wells as a diagnostic tool. The blank-well reading will help you determine the background value contributed by the instrument, labware, and media.

- **Possible cause: The top and/or bottom probe needs cleaning**

Refer to the operator's manual for guidance; not all readers have user-accessible internal components.

- **Possible cause: The instrument has fluorescing material spilled inside**

Refer to the operator's manual for guidance; not all readers have user-accessible internal components.

- **Possible cause: The Gain in the Reading parameters dialog is currently set too high**

Lower the Gain setting. The background should still read higher than zero. 200 is a recommended minimum and a value of 1000 takes advantage of the system's five-digit resolution.

Reader Not Achieving Desired Fluorescence Detection Limit

- **Possible cause: The wrong filters are selected in the Read Step dialog**

Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.

- **Possible cause: Using incorrect microplates**

Refer to the reader's operator's manual for information on supported plate types.

- **Possible cause: The fixed Gain is currently set too low**

Raise the Gain setting until the background wells read at least 200 RFU (1000 RFU is preferred).

- **Possible cause: Readings are taken using the bottom probe**

Try switching to the top probe, if applicable/appropriate for your reader and assay.

- **Possible cause: The solution volume is 50 μ L or less**

Increase the solution volume to 150–200 μ L, if possible.

- **Possible cause: Wrong pH**

Fluorescence is very pH dependent. Use the appropriate pH.

- **Possible cause: Phenol red is used in the media when exciting at 485 nm and reading at 528–530 nm**

Eliminate or replace the phenol red.

- **Possible cause: Top probe setting is not optimized**

Gen5 generally positions the top probe at the optimal height for fluorescence reads, based on the plate type selected; it focuses the beam above the well. Refer to the Gen5 Help and use the Read Height option in the Read Step dialog to make adjustments. If supported by your reader, try the Auto-Adjust feature for the probe height. The reader's operator's manual may contain additional suggestions.

- **Possible cause: Transfection efficiency in gene expression is too low**

Use more cells, or improve the transfection efficiency.

- **Possible cause: DNA is old or of poor quality**

Use high quality, new DNA.

- **Possible cause: Not using nuclease-free buffer in DNA quantitation**

Use nuclease-free buffer.

- **Possible cause: Poor dilution methods**

Use appropriate dilution method in tubes.

Reader Over-ranging in Fluorescence

- **Possible cause: The Gain in the Read Step dialog is currently set too high**

Lower the Gain setting. If using Automatic Gain Adjustment, try the Scale to High Well option and set the High Value in the range of 50,000 to 70,000.

Bandwidth Verification Failed

- **Error or warning messages are issued when Gen5 detects overlapping wavelengths or bandwidth**

Select/enter Filter Set wavelengths that do not overlap. Learn more about Gen5 bandwidth verification in the Gen5 Help.

Error during Auto-Sensitivity Determination

- **Reader cannot fulfill request to determine optimal Gain**

Gen5 displays an error message when the reader cannot determine the optimal Gain based on the defined reading parameters.

Luminescence integration time should be ≤ 1 sec and > 1 ms, especially when scaling to low wells.

Manually enter a Gain value or use an alternative method to determine the optimal sensitivity, if the error persists. Learn more in Gen5 Help.

System Administrator's Password

Contact BioTek Customer Care if you have lost or forgotten the System Administrator's password. Gen5 ships with the System Administrator's password set to **admin**.

Getting Technical Assistance

You can telephone the Technical Assistance Center (TAC) at BioTek World Headquarters US between 8:30 AM and 5:30 PM Eastern Standard Time (EST), Monday through Friday, excluding holidays.

Whichever method of contact you choose, please be prepared to provide the following information:

- The software version and revision numbers displayed at **Help > About Gen5**
- The license type or software level: Image+/non-Image+ versions of Gen5, Gen5 Secure, Gen5 IVD, Gen5 Reader Control, or Gen5 TS
- The specific steps that produce your problem
- Any error codes displayed

- A daytime phone number
- Your name and company information
- An email address and/or fax number, if available

BioTek US—World Headquarters

Phone: (802)655-4740

Toll-Free: (888)451-5171

Service Toll-Free: (800)242-
4685

Email:

CustomerCare@biotech.com

Service Email:

TAC@biotech.com

www.biotech.com/contact

Essential Concepts

This section will give you a good basis of information for understanding the structure and terminology of Gen5. You can find more details and answers to specific questions by using the online Help system. Select **Help > Help Topics** from the menu.

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Experiment vs. Protocol

Gen5 uses two common terms to define distinct elements of its toolkit. The distinction is subtle and will have more or less importance depending on how you use Gen5. In any case, you'll work most efficiently by understanding each role and making them work for you.

| Protocol (*.prt) | Experiment (*.xpt) |
|---|---|
| A protocol is a set of instructions designed to capture, transform, and report and/or export data. | An experiment has a copy of the protocol and at least one plate. It executes the instructions provided by the protocol to produce results. |
| Protocols are created and saved as stand-alone files. They function as a template; an unlimited number of experiments can be based on one protocol. | Although an experiment is created using an existing protocol, that protocol can be modified within the experiment. |
| A protocol consists of reading requirements, like detection method and wavelength, and reading-related actions, like shaking and incubation (procedure), plate layout, data reduction, and data viewing, reporting, and exporting parameters. | Running an experiment is the only way to process a protocol. |
| A protocol can be used repeatedly (as is or modified) within experiments. By itself, a protocol does not produce results. Protocols do not have plates associated with them. | Multiple plates can be processed in an experiment, each one considered a unique assay with independently reported or exported results. The exception is multi-plate protocols, described later. |
| .prt is the protocol's file name extension. | .xpt is the experiment's file name extension. |
| A copy of the protocol is saved within an experiment or as a stand-alone .prt file. Since protocols do not have plates, they cannot generate data outside of an experiment. | An experiment is saved as the full collection of procedures, formulas, reporting definitions, and other details. The non-imaging plate data are recalculated when the file is opened in Gen5. |

| Protocol (*.prt) | Experiment (*.xpt) |
|--|---|
| Gen5 Secure and Gen5 IVD maintain an audit trail of all activity and changes related to a protocol. All other Gen5 software levels do not support this feature. | Data acquired and transformed in an experiment is protected by an audit trail in Gen5 Secure, Gen5 IVD, and Gen5. The Reader Control edition does not support this feature. |
| Changes made to a stand-alone protocol are not reflected in any previously created experiments based on that protocol. A new experiment must be created to apply the revised protocol. | Within an experiment, you can select Save Protocol As to capture the current details of the protocol and save them as either a new protocol or as an overwrite of the original protocol. |

Gen5 also supports more complex multi-plate protocols that are not covered in this introductory material. See **Design a Multi-Plate Protocol** in the Gen5 Help system.

About File Storage

File Types

Gen5 creates multiple file types:

- Protocol = .prt
- Experiment = .xpt
- Panel = .pnl
- Imaging = .tif

The Gen5 executable file (.exe) and numerous other types of supporting files, like a Microsoft Excel template, are also installed on the computer.

Databases

Gen5 installs two databases on your system called LocalDB and SharedDB. While the databases are always used for critical, internally used files, Gen5 offers you the choice of using the Windows File System or the Gen5 (SharedDB) database for storing Gen5 protocol (.prt) and experiment (.xpt) files. This option, combined with the ability to create multiple databases, allows you to structure file storage according to your organization's requirements.

- Files may be stored on the computer's hard drive, on a network, or on a CD or other portable medium. Windows Explorer or a similar application can be used to

view the file names and locations, and to move, copy, rename, and delete files.

- Alternatively, protocol and experiment files may be stored in a secure, shared-access database. This database, initially named SharedDB.mdb, can be stored on a user's computer or on a shared-access network/computer (LAN). Gen5 provides a special file maintenance utility for viewing the file names and their locations, and for moving, copying, renaming, deleting, importing, and exporting files.
- Select the preferred method of storing protocol and experiment files at **System > Preferences > File Storage**.

Gen5 Image+ IVD and Gen5 IVD also install the QC Trending database, QCDB.mdb. It can be set up on a network for sharing among multiple users, moved, renamed, and copied. It is initially installed in a QC folder in the default database location described below. See the Gen5 Help system for more information.

File Location

During a typical installation:

- the program files are stored in this default location: C:\Program Files\BioTek\Gen5 (software edition)
- the databases are stored in this default location: C:\Program Data\BioTek \Gen5 (software edition)\(version #)\SharedDB or LocalDB
- Gen5 installs Protocol and Experiment folders in the respective File Storage locations, for example: C:\Users\Public\Documents\Protocol

The databases are critical to Gen5 functionality. Make sure they are not deleted from your system.

Image Save Options are defined in the System Preferences dialog, under Initial Protocol Settings.

Image File Management

Each saved image is saved as a TIF file. The TIF files will contain metadata, pertaining to the instrument, experiment, plate, well, and image, though this data will likely not be accessible by other TIF file readers.

Defining the Gen5 Image Library

The first time you define the Cytation 3 or Cytation 5 as a reader, Gen5 prompts you to define an Image Library location where images will automatically be saved when you run an experiment. You can change the path later in the Image Save Options dialog.

Storing Images

Warning! Each image is about 2 MB. Imaging microplates can quickly lead to generating very large amounts of data: reading only one image per well of a 96-well microplate results in 200 MB of images. Review your data storage requirements with your IT department. In addition, processed image files, such as stitched images, can be much larger.

Within the image files folder, Gen5 creates an experiment folder for each imaging experiment run. Within the experiment folder are subfolders for each plate run in the experiment. The individual image files are stored in the plate folders.

You may find that you need more hard disk space than is allocated by default. For example, running a multiplate experiment, imaging all 96 wells in a 4x4 montage in three colors will require an increase to virtual memory. Without this change, the message, "This procedure may require that you increase the size of the virtual memory in Windows," may appear. In this case, please consult with your IT group to increase virtual memory. The Gen5 Help provides instructions to change the virtual memory settings.

Best Practices

Like most software tools, Gen5 is flexible and offers several ways to accomplish a task. Here are some recommendations for saving time and using it most efficiently.

Efficiencies

- For an assay or experiment that you will run numerous times, develop a protocol to define the Procedure, Data Reduction, Data Views, and Reports required. Then you can run an experiment (from the Task Manager, **Experiments > Create using an existing protocol**) based on the protocol whenever necessary. You can fine-tune the protocol within an experiment, but remember to select **File > Save Protocol As** to update the original protocol with your improvements.
- Use **File > Save As** to give you a head start creating a new protocol based on an existing one that contains the same or similar plate layout, reading parameters, or other elements that will be repeated in your new protocol.
- Define and customize Data Views before selecting what to include in reports or export files. All the on-screen data (i.e., data views) can be reported or exported. If you use on-screen views and paper reports equally, it is most efficient to first fine-tune the Data Views, and then include them in reports/exports.
- When appropriate, assign Blanks to the plate. Blanks can be deionized (DI) water, buffer, reagent without analyte, substrate, and so on. When running fluorescence cellular assays, a DI-water blank illustrates the background contributed by the instrument and labware as separate from the cells and media. Identify the location

of the Blanks in the Plate Layout, and Gen5 will automatically create the blank-subtraction data reductions.

- Back up your database regularly: BioTek recommends once per week for most organizations. If you're using a Gen5 Database for protocol and experiment file storage, use the built-in Periodic Optimization feature.
- Take action if you get a warning message about the remaining size of your databases; see **Maintaining Files** in Gen5 Help for instructions on archiving and deleting records.
- Turn off the Multi-Read Calculation option to improve Gen5 performance. Calculation results will be the same, but your PC's resources will not be diverted for performing interim calculations. Find this option at **Protocol > Protocol Options > Calculation Options**.

Time-Savers

- **Partial Plate:** For assays using strips or partially filled plates, especially if the read steps are long or complicated, you can save time by telling the reader exactly which adjacent wells or portion of the plate to read.
- Use the Gen5 automatic Save feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 will keep all the data organized by date. Define this kind of file management setting in the Initial Protocol Settings so it will apply to all newly created protocols. Select **System > Preferences > Initial Protocol Settings > Save Options**.
- **Print Preview:** Save time and paper by viewing reports on screen before sending them to the printer.

Basic Tasks

This section provides instructions for some basic tasks.

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Quick Read

You can perform a Quick Read using the microplate reader connected to the PC to read a plate and report the results. It's called quick because it is accomplished without taking the time to set up a full protocol.

Perform a Quick Read:

1. Click **Read Now** from the Task Manager, and either select an existing protocol or create a new one for this read.
2. After you select a protocol, or define a reading procedure for a new protocol, the reader reads the plate.

When the reading is done you can report the results. If you have a full-level edition of Gen5 (Gen5 Image+, Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5, Gen5 Secure, Gen5 IVD, or Gen5 TS), you can perform data analysis.

Create a Standard Curve

Gen5 lets you create one or more standard curves for determining the concentration of test samples:

1. From the Task Manager, select **Read Now > New**.
2. In the Procedure dialog, define the Read step (and any other required steps), then click **OK**.

Gen5 performs the read and exports your results.

3. Select **Plate Layout**:
 - Select **Standard Curves** as one of the well types.
 - Define the concentrations of the Standards.
 - Assign the location of the standards, samples, and blanks (if any) on the plate.
4. Select **Data Reduction > Standard Curve**.

Gen5 may have generated a "corrected" data set: if you assigned blanks to the plate or selected Pathlength Correction or Polarization in the Read step, you'll want to select these data sets for Data In for the Y-Axis Data to plot the curve.


5. On the Data In tab, select the **Y-Axis Data**.
6. On the Curve Fit tab, choose a curve fit method.
7. Other options and requirements when defining multiple curves:
 - **Curve Name:** Replace the default “Curve” with a more meaningful or unique name.
 - **On the Data Out tab:** Replace the default “Conc” for the Data Set Name with a more meaningful or unique name.
 - **On the Data Out tab:** Define interpolations to plot on the curve.
8. Define the reporting or export requirements and save the protocol using **File > Save Protocol As**.

Imaging Module: Manual Mode

Applies only to models with the imaging module.




The following sections **briefly** describe how to use the Gen5 imaging module in manual mode. See the Gen5 Help for more complete instructions and descriptions of these features.

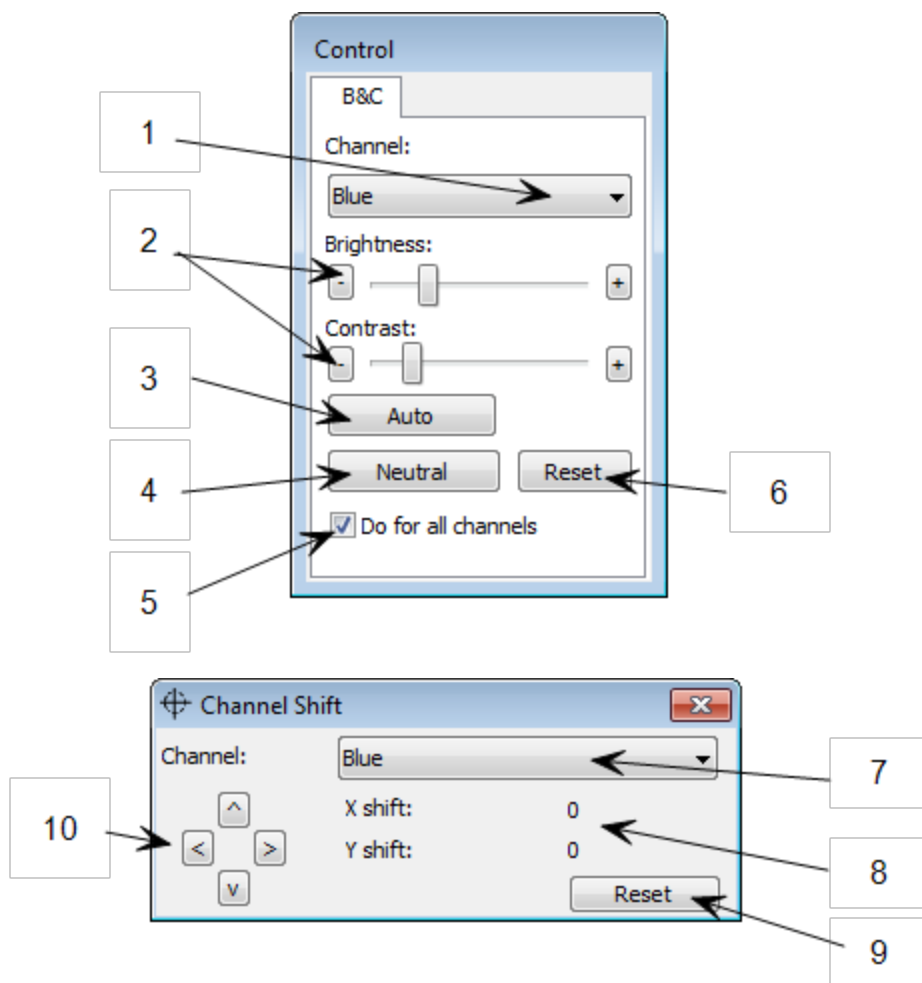
Focusing the Camera

1. From the Task Manager, select **Read Now > Run Imager Manual Mode**.
Alternatively, from the main Gen5 screen, click .
2. Click **Find Image**. Gen5 automatically sets the exposure and focuses on the image. If the displayed image requires additional fine-tuning:
 - Click **Auto Expose**, or manually adjust the Exposure settings until you see an image.
 - Click **Auto Focus**, or click the arrow buttons until the image is in focus.
 - Repeat these two steps until the image is exposed and focused to your liking.

Capture and Save an Image

After you have exposed and focused an image, you can capture and save it for later analysis.

1. When an image in the Capture dialog is exposed and focused to your liking, click  to capture it.
2. To save the image or images you capture, click **Review**, click  to perform any additional brightness and contrast adjustments or  to manually align overlaid images to compensate for pixel shift, then click **Save**.



| # | Description |
|---|---|
| 1 | Select a color channel |
| 2 | Use the sliders or +/- buttons to adjust brights and contrast |
| 3 | Have Gen5 automatically adjust brightness and contrast |
| 4 | Set to neutral settings (similar to the default settings) |
| 5 | If selected, changes to brightness and contrast will apply to all color channels of the image |

| # | Description |
|----|---|
| 6 | Reset to default settings |
| 7 | Select a color channel |
| 8 | Gen5 displays the number of pixels the channel is shifted |
| 9 | Reset to default settings |
| 10 | Adjust the shift using the arrows |

Any adjustments made using the B&C (brightness and contrast) dialog and the Channel Shift dialog are for display purposes only; the changes do not affect the data measurements from the images.

Edit the Image Processing Step

In manual mode, you can modify or add steps to your imaging process.

1. In the Capture screen, after you have acquired an image, click **Review/Save**.
2. In the review screen, click **Add step** in the Image Processing area. Gen5 presents a list of processes, such as stitching or digital phase contrast, that are relevant for your image.
3. Select the desired step. Gen5 performs the step on your image.

To display the processed image without the added processing step applied, click **Starting image** in the Image Processing area.

Analyze Captured and Saved Images

1. With your captured or saved images displayed in the left panel of the Capture dialog, click **Review**.
2. In the Review dialog, you can further adjust the brightness and contrast of the image, if necessary.
3. Click **Analyze**.
4. In the Analyze Tool, select which type of analysis you want to perform and define the parameters for the analysis, then click **Start**. The results are displayed in the right pane.

Imaging Module: Experiment Mode

Applies only to models with the imaging module.

The following sections **briefly** describe how to use the Gen5 imaging module in experiment mode. See the Gen5 Help for more complete instructions and descriptions of these features.

Perform an Image Read

The following steps briefly describe how to set up a protocol with an imaging read step. See the Gen5 Help for more detailed instructions.

1. Create a new protocol.
2. Select **Image** as the Read Method.
3. (Optional) Enter a step label or unique name for this step. Data sets of the reading results will use the label in online views, reports, and export files.
4. Select the Objective.
5. Define whether to read a full plate or specific wells.
6. Define up to four channels to read, defining the color filter and exposure settings and selecting which well are to be read for each color channel.

If you select **Auto** for Exposure, you must define which well or wells will be used to determine the exposure.

7. Define the horizontal and vertical offset from the center of the well.
8. Select whether to create a single image for each well, an image montage, or a Z-stack.
9. Gen5 provides default settings for image montage and Z-stack reads. You can enter your own values, if desired.
10. Define advanced options, if needed.




After you have defined the protocol, you can create an experiment and read the plate:


1. Open a new experiment.
2. Select the protocol you created with the imaging read step, and click **OK**.

3. Select a plate in the menu tree and read it.
4. When the read is complete, images and measurement values appear in Gen5. Select the desired data set from the Data list.
5. To add image processing steps, such as digital phase contrast, to the experiment, either add them through the Data Reduction dialog or open the Image zoom, click **Process**, and then click **Add Step** when finished.
6. To add image analysis steps, such as cellular analysis, to the experiment, either add them through the Data Reduction dialog or open the Image zoom, click **Analyze**, and then click **Add Step** when finished.
7. Select **File > Save** and give the file an identifying name.

View Results

You can instantly view the results of an experiment in the Gen5 main workspace using the Plate View:

- In the upper-right corner, click  to pin a plate view in the workspace. Pinning a plate view allows you to have multiple plates' plate views open simultaneously. If a plate view is not pinned, when you open another plate, it opens in the same plate view.
- In the upper-right corner, click  to open another instance of the Plate View. Use this feature to view the raw data results of a reading in one window and simultaneously display a curve plotted from the results in another window, for example.
- After reading the plate (or otherwise acquiring data), in the Plate View use the Data list to display the raw data and any data reduction results. You can also select **Create new Matrix** to define a new view.
- Click **Edit Matrix** next to a data set to customize the view's appearance. This feature is also available in the Data Views dialog.
- Click  to instantly open the current view in Microsoft Excel.
- Asterisks (**) are used to signal a change: In the Gen5 title bar an asterisk indicates the current file has been changed but not yet saved. When a data set is enclosed by asterisks, it has become invalid. Generally this is because a Read step or Data Reduction step has been altered. Edit custom-made data views to select valid data sets.

-  384- and 1536-well plates require resizing to effectively see the data. Gen5 adds a button to the Plate View to zoom in on the top-left quadrant of the plate and zoom out to view the entire plate. After zooming in, use the scroll bars to bring the other quadrants into focus.
- Multi-index readings offer another viewing option. Kinetic and scanning reads generate views based on the number of intervals, wavelengths, or positions defined. Use the buttons or enter the desired read index and click **Show** to display it. Gen5 displays the time, wavelength, or position of the selected read number.
- Kinetic and Scanning protocols can generate Well Analysis data sets labeled Curves. In the Matrix tab, open the Curves data set and click on a well for a Well Zoom. (384- and 1536-well plates show a magnifying glass in the well in lieu of a curve.)

Starting at the Curves data set, you can display multiple well zooms simultaneously by holding Ctrl while selecting wells.

- Select the **Statistics** tab to view a table of data reduction results.
- Select the **Graphs** tab (when available) to view any standard curves.
- Select the Results List tab (when available) to view the values or results of the cutoff or validation formulas.
- Review the description of the Gen5 naming convention for the raw data/results.

Important Notes

- Gen5 may not display some data points by default; to see them you must create your own Data Views. If you expected to see certain results that are not currently displayed, try creating your own views.
- All data views are also available for reporting and/or exporting.
- Modify a data view to change the way results are reported, including the number of decimal places and significant digits.
- Gen5 always uses your computer's regional settings to display and input data.

Print Results



Click the **Print** button to print the results of an experiment.

Prerequisite

First, you must select the specific content for the report using the Gen5 Report/Export Builder (**Protocol > Report/Export Builders**).

Reporting in an experiment is done on a per-plate basis:

- Highlight a plate in the menu tree and select **Print/Print Preview**.
- In a multi-plate experiment: You can select multiple plates by holding the Ctrl key while highlighting them, or to select contiguous plates, highlight the first plate, hold the Shift key, and select the last plate. Then, click **Print**.



Gen5 offers enormous flexibility in report output. After defining the report elements, use the Print Preview option to view the report on screen before printing it to paper. Unneeded columns and other individual report elements can be removed or modified to improve the appearance and usefulness of the report.

In Gen5 Help you can find step-by-step instructions for creating and customizing reports.

Test the Instrument

System > Diagnostics > Run System Test

Most BioTek instruments perform a self-test every time they're turned on, but when you want to view and/or print the results of a system test:

1. From the Gen5 main window, select **System > Diagnostics > Run System Test**.

When there is more than one instrument attached to the PC, select the desired one and click **OK**.

2. When the test is completed, fill in the text fields—User, Company, Comments—to be included in the report of the test results, then click **OK**.
3. Print the report to retain a hard copy for your records.
4. Click **Save As** to convert the results to a text file. This is especially useful when troubleshooting an instrument. You can email the text file to BioTek TAC.

Test History

Gen5 keeps the results of System Tests when they are performed using the menu controls.

To review or print the results, select **System > Diagnostics > Test History**.

Set Up a Protocol

This section walks through the basic steps to creating a protocol. You'll find more details in the Gen5 Help. If you haven't already done so, read the differences between experiments and protocols in Gen5 in the Essential Concepts section.

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Design a Protocol

In the Task Manager, click **Read Now > New** to get started.

1. Define the procedure, then click **OK**. Gen5 performs the read and displays the resulting data.
2. Define the plate layout (for all except Gen5 Reader Control).
3. Define the data reduction requirements (for all except Gen5 Reader Control).
4. Define the reporting and exporting requirements.
5. Save the protocol (**File > Save Protocol As**).

Follow this sequence of tasks when developing a protocol to take advantage of the automatically created Gen5 data reduction events. For example, when you add Blanks to the Plate Layout, Gen5 automatically creates a Blank-Subtraction data set.

You can find instructions for developing specific types of protocols in the Gen5 Help.

Define the Reading Procedure

Protocol > Procedure

Procedure grayed out? When more than one plate has been read in an experiment, the procedure cannot be changed for the current experiment. If this isn't the case, your System Administrator may have restricted your ability to modify the protocol elements.

Grayed-out buttons mean the action cannot be performed by the current reader or because previously defined steps—for example, a kinetic loop—limit the function or your level of software limits the feature.

How to:

For all procedures, define the Plate Type and then:

1. Click a link to add that step to the procedure. Most links open a screen for defining the parameters of that step, for example, **Read** lets you define wavelengths.

When defining a kinetic or synchronized well/plate mode analysis, add the Kinetic and Synchronized Mode steps **first**. Kinetic and Synchronized Mode steps form a

loop or block. Put the Read and other valid steps to be performed inside the loop, between the Start and End. Monitor Well is similar: First add the Monitor Well step and then add a Read step inside the Monitor Well loop.

2. Define the details of the step and click **OK**.
3. Click **Validate** to check the selection and sequence of the steps.

Your instrument must be communicating with Gen5 for it to fully validate the procedure: Make sure it is turned on, not busy, and properly connected to the PC.

StepWise Procedure Features

- You can drag and drop steps in the procedure to change their sequence order.
- Highlight a step in the procedure, and then click an action button to add a step before it.
- Double-click a step to open it for editing.
- Select a step in the sequence and right-click for additional options.
- Click **Validate** at any time to verify the reader's ability to perform the current sequence of steps.
- Highlight a step and press **Delete** to remove it from the procedure.
- Kinetic analysis, Synchronized Mode processing, and Monitor Well functions are set up in a loop or block. First define the function, for example, add the Kinetic step, and put the read and other steps inside the loop

Drag and drop is limited in Synchronized Modes. For example, you cannot drag and drop a step into or out of a Well Mode block.


Define the Plate Layout

Protocol > Plate Layout

The Plate Layout Wizard appears only when no plate layout has been defined (including Preferred Well IDs) and the wizard is not disabled in Do Not Show Preferences.

1. In the Plate Layout Wizard, select the well types you want to define for the plate, then click **Next**. The Wizard prompts you to define each type of well you have selected. When all well types are defined, the Plate Layout dialog opens.
2. Select a well ID in the left pane, then click in a well, or drag over contiguous wells, to assign the selected well type to the plate layout.

Some readers support random well selection.

- The well assignment starts with the well ID you select in the left pane. For example, if you select Well SPL1:4 in the left pane, when you click in a cell in the plate layout, that cell will be assigned SPL1:4.
- Use the Auto Select and Replicates options to speed up your work: set the options and click and drag to fill multiple wells at once. Click a column or row header to fill it.
- Use the Serial Assignment tools () to quickly assign replicates to the plate layout in a horizontal or vertical line or in square groupings. Select one of the directions (toggle through to access horizontal, vertical, or square) then click or drag in the plate layout.
- You can export a plate layout for use in multiple experiments, or import an existing plate layout.

The type of plate, such as 96-well, is defined in the procedure and displayed in a representative matrix or grid format in the Layout and Transformation screens.

Helpful Hints

- Set up your preferred default Well IDs at **System > Preferences > Plate Layout Well IDs**. For example, you can define PC (for Positive Control) instead of CTL1 for Assay Controls. Well IDs defined in the System Preferences are available when defining the plate layout for all newly created protocols/experiments.
- Click **Undo** at the bottom of the screen to undo the last action. Up to 10 previous actions can be undone.
- To clear the grid and start over, right-click and select **Empty Layout**. To clear selected cells, set the Type of Well Settings to **Empty** and select the cells you want to clear.

- You can print the plate layout. 384-well plates print out in two sections, columns 1–12 and 13–24. 1536-well plates print in eight sections to fit all 48 columns and rows from A to Z and AA to AF.
- For Samples (unknown test specimen) Gen5 lets you assign and track data points, such as age or gender, in addition to the Sample ID. You can create additional identification fields.
- To copy the contents of the grid to the Windows virtual clipboard to paste into a text/external file, right-click and select **Copy Layout**. Open the receiving file, for example, Microsoft Word or Excel, then right-click and select **Paste**. Generally, plates larger than 96 wells do not fit completely in a standard-size Word or text file; a spreadsheet is required.
- Each instance of a Sample and Sample Control Well ID and each Assay Control group can have a unique concentration/dilution value. Gen5 assigns a dilution index to the Well ID to keep track each instance.
- Well selection must be compatible with the Replicate, Auto Select, and Filling option settings.
- You can resize the plate view in the standard Microsoft Windows way: click and drag the outer borders of the view, or click the maximize button in the top-right corner.
- You can resize the rows and columns: hover your mouse over a grid line between two numbered columns or alpha-labeled rows until the cursor changes to a separator, then click and drag.
- When running an experiment with a cuvette, the plate layout is mapped on a 96-well plate.

Set Up Data Reduction

Protocol > Data Reduction

There are several options available for interpreting the results of your experiment. Gen5 automatically creates the most commonly applied data reduction steps.

Top Six Things to Know about Data Reduction

1. Reductions are StepWise, that is, they can be built one upon another based on their sequence in the Data Reduction dialog. For example, a data set created in a previous step can be used in a later transformation, curve, cutoff, and so on, if applicable.

2. Gen5 creates some data reduction steps automatically:
 - When blanks (BLK) are assigned to the plate, Gen5 automatically creates a Blank Subtraction data set. Gen5 subtracts the mean of the blanks from all other wells on the plate.
 - A Well Analysis step is created when a multi-read index Read step is defined: Kinetic, Spectrum, Area, or Linear Scan. If applicable, blank-subtraction results are used to perform the well analysis.
 - A Pathlength-Corrected data set is created when this option is selected in an Absorbance read step.
 - The Fluorescence Polarization (FP) transformation is performed automatically when this reading mode is selected in the Read step. If applicable, blank-subtraction results are used to perform the FP reduction. In kinetic experiments, well analysis is based on the FP transformation results.
 - For Imaging reads other than Z-stack reads, Gen5 automatically creates an Image Statistic data reduction step.
3. Once the Data Reduction dialog is opened and saved, that is, **OK** is selected to close it, Gen5-created reductions are no longer added, deleted, or changed. Keep this in mind when modifying a protocol or experiment.
4. Plate-specific Data Reduction Variables can be collected from users when they read the plate so Gen5 can use them in data reduction calculations. First, define the variable in the Runtime Prompts, then write a formula using the variable. At runtime, when the measurements and variables are obtained, Gen5 performs the calculation using the input value.
5. Raw data sets used in Data Reduction steps are named according to the Gen5 Data Set Naming Convention, which is based on the number of Read steps defined in the procedure. When the number of read steps is changed, any previously defined Data Reduction steps are voided, because the data set name is also changed. When you add or remove a Read step, you must update the effected Data Reduction steps.
6. In the Data Reduction dialog, you can:
 - Drag and drop Data Reduction steps to change their sequence order.
 - Select a step and right-click to delete it.
 - Double-click an event to open it for modification or deeper review (or right-click and select **Edit**).

About Exporting Results

Gen5 provides the following exporting tools:

- **QuickExport:** to instantly export the current view to a Microsoft Excel worksheet
- **Export to Excel:** to export selected data to Excel using the Power Export feature
- **Export to Text:** to export selected data, excluding curves, to a text file (for use in another software application) using the File Export feature
- **Right-Click Menu Options:** **Copy to Clipboard** and **Save As**; to copy or save the current selection for use in another software application

Prerequisites

For the QuickExport and Power Export features, you must have Excel 2003 or higher installed on your PC. Use File Export or right-click options if you do not have Excel.

About the Export Tools

- The Power Export and File Export methods require selecting the content you want included in the output file **before** executing the export for a designated plate.
- You can save your export selections with the protocol to reuse them every time you run an experiment based on that protocol.
- Exporting data is like generating a report; it is done individually for each plate*. Although you can select the export content in a protocol, you must run (or execute) the export in an experiment (after selecting a plate or multiple plates).
[* except in multi-plate assays]
- In an experiment, to run the export, you can select a plate in the menu tree and right-click for a menu that offers the **Export** option.

Export Multiple Plates to One File

When you run multiple plates in an experiment you can export all the data to one file:

1. In the menu tree, select/highlight multiple plates (by holding down the Ctrl key).
2. Right-click and select **Export**.

Make sure the File Export Settings are defined to automatically append the data.

About Reports

Protocol > Report/Export Builders > New Report

You can use the Report/Export Builders to define exactly:

- What to include in the report
- How to format an item in the report
- Where to place the item in the report

Defining Report Elements

1. When setting up the protocol, after customizing the Data Views, select **Report/Export Builders** from the Protocol menu tree.
2. From the Report/Export Builders dialog, click **New Report**.
3. Define your settings in the Properties, Content, and Options screens, then click **OK**. If you are running Gen5 Image+ Secure, Gen5 Image+ IVD, Gen5 Secure, or Gen5 IVD, Gen5 prompts you to add a comment to the Audit Trail event.

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