GloMax® 96 Microplate Luminometer

Instructions for Use of Products
E6501, E6511 and E6521

GloMax® 96 Microplate Luminometer

1. Description

The GloMax® 96 Microplate Luminometer is an easy-to-use, highly sensitive microplate luminometer with a broad dynamic range. It features two optional injectors and reads both glow- and flash-luminescent reactions in 96-well plates. Its superior sensitivity and dynamic range makes the GloMax® 96 Microplate Luminometer an ideal laboratory instrument for a wide range of luminescent assays, including Promega bioluminescent reporter, cell-based and biochemical assays. This manual will guide you through the installation, setup, and operation of your instrument.

1.A. Inspection

When you receive your luminometer, please inspect the package carefully to make sure all accessories are present.

Power Supply Kit

Luminometer

White, 96-Well Plates

Data Cable (9-pin serial cable)

USB Serial Adapter

CD-ROM

Figure 1. Standard accessories with the GloMax® 96 Microplate Luminometer (all models). Protocol (TM278) and Quick Start Guide are not shown**.**

Additional accessories for Cat.# E5611 and E5621 also include:

Reagent Bottle Holder
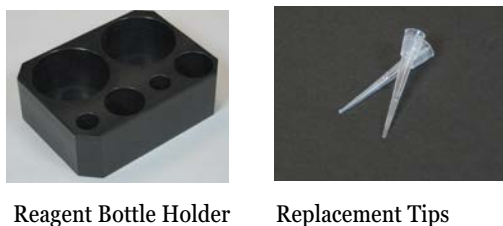
Replacement Tips

Figure 2. Additional accessories for GloMax® 96 Microplate Luminometers Cat.# E6511 and E6521.

1.B. Precautions and Computer Requirements

The GloMax® 96 Microplate Luminometer contains sensitive optical components and precision-aligned mechanical assemblies. It is intended for indoor use only. Avoid rough handling. Wipe up spills immediately.

The maximum volume for the microplate is 300μl/well. Using an injector with a bent or damaged tip or overflowing the microplate will cause fluid to leak onto the sample tray cover. The residue can cause the optical head to malfunction. If any moisture appears on the sample tray cover, clean the optical head and the interior of the luminometer (see Sections 12.A and B).

Do not perform injection runs with bent or damaged tips. If the injector tip appears damaged, replace it (see Section 12.F).

If the instrument is ON, the optical head must remain in the home position when the lid is open. Attempting to move the optical head when the instrument is ON exposes it to ambient light. Ambient light will damage the sensitive electronics in the optical head.

The instrument can be connected to a computer using either an RS-232 cable or USB/RS-232 adaptor. The GloMax® 96 software is compatible with the following:

*   32-bit and 64-bit PCs using Windows® 7 Professional Service Pack 1 and Microsoft® Office 2010 Home and Business Edition, 32-bit version.
*   Windows® XP
*   Microsoft® Office 2003

Compatibility with the Microsoft® Office Home Premium and Ultimate Editions, and other combinations of Microsoft® Office and Windows® versions has not been tested. Microsoft® Office 2010 Home and Business Edition has 32-bit and 64-bit versions. The 32-bit software version is recommended by Microsoft® for use on both 32-bit and 64-bit Windows® PCs. Therefore, the 32-bit version of Microsoft® Office Home and Business Edition was tested with a 64-bit Windows® 7 PC and found to be compatible with GloMax® 96 software. The 64-bit software version was not tested. Refer to: **www.microsoft.com** for more information.

Avoid using any other software in the background while running the GloMax® 96 software, as this can affect perfor-mance. While collecting data using GloMax® software any power saving options, screensavers, and computer scans (e.g., automatic updates, virus scans, etc.) should be disabled. If using a laptop, connect the laptop to a power supply, not the battery. The GloMax® software uses a Microsoft® Excel spreadsheet. Do not use Excel® for any other purposes while conducting an experiment.

Operating the GloMax® 96 software over a network is not supported.

2. Product Components

PRODUCT	SIZE	CAT.#
GloMax® 96 Microplate Luminometer	**1 each**	**E6501**
GloMax® 96 Microplate Luminometer with Single Reagent Injector	**1 each**	**E6511**
GloMax® 96 Microplate Luminometer with Dual Reagent Injectors	**1 each**	**E6521**

Includes:

* 1 Luminometer
* 1 Power Supply Kit
* 1 Data Cable (9-pin serial cable)
* 1 USB Serial Adapter (with CD-ROM containing USB drivers)
* 5 White, 96-Well Plates
* 1 GloMax® Software CD-ROM
* 1 Quick Start Guide (FB082)
* 1 Reagent Bottle Holder (Cat.# E6511 and E6521 only)
* 1 pkg. Replacement Tips (Cat.# E6511 and E6521 only)

Available Separately

PRODUCT	SIZE	CAT.#
GloMax® Microplate Luminometer Light Plate	**1 each**	**E6531**

Materials to Be Supplied by the User

* PC with Windows® 7 Professional Service Pack 1 with Microsoft® Office 2010 Home and Business Edition, 32-bit version. Also compatible with Windows® XP and Microsoft® Office 2003. (See Section 13.E for details of operating system and MicroSoft® Office version requirements).

3. Hardware Overview

Instrument lid

Injector tip assembly

Outlet tubing

Tubing guide

Injector syringe

Inlet tubing

Waste container

Injector(s)

Reagent bottle holder

Injector tip holder

Tip drip pan

Optical head

Sample tray cover

Waste tray

Sample tray latch

LED status lights

5498MA
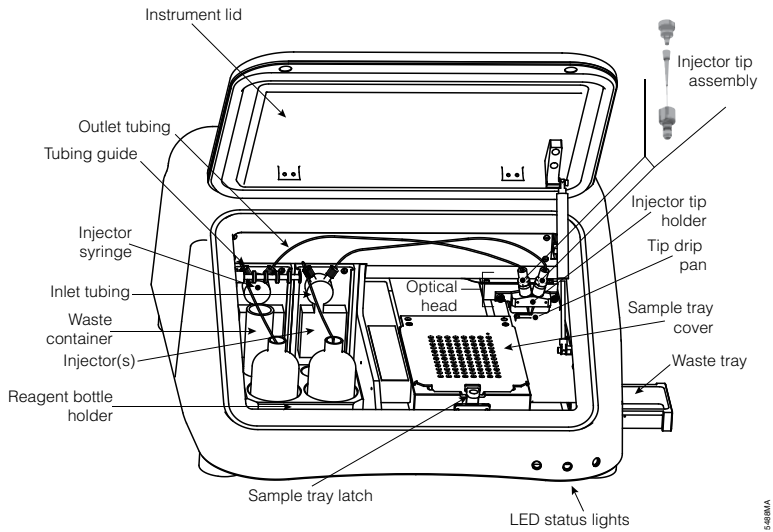
Figure 3. Interior view of the GloMax® 96 Microplate Luminometer.

Serial port (connect to the serial port of your computer)

Power connection
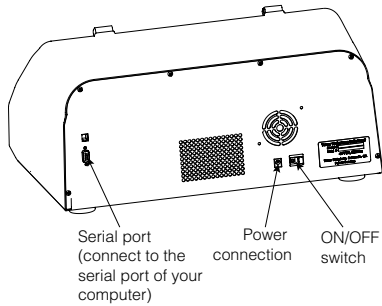
ON/OFF switch

5491MA

Figure 4. Rear view of the GloMax® 96 Microplate Luminometer.

4. Software Installation and Setup

Please refer to the Computer Requirements in Section 13.E before installing the software to ensure compatibility with your operating system.

1. Position the luminometer on a level surface. Leave enough clearance above the luminometer to open the instrument lid (approximately 7in or 18cm).

2. Remove foam packing that prevents optical head from moving during shipping.

3. Plug the AC adaptor into the power connection of the luminometer. Plug the power cord into an outlet.

4. Connect the 9-pin serial Data Cable between the luminometer and your computer. The male 9-pin connector attaches to the luminometer. The female 9-pin connector attaches to your computer. If necessary, use the USB serial adaptor to connect the serial cable to a USB port. For installation instructions refer to the *USB Serial Adaptor Operating Manual* that accompanies the USB serial adaptor shipped with your luminometer.

   Note: When using the Tripp-Lite USB to Serial Adaptor (U209-000-R), the driver should be detected by Windows® automatically. The driver is also provided on the CD accompanying the GloMax® 96 Instrument and from the Tripp-Lite website: **www.tripplite.com**. Be sure to install the drivers (32- or 64-bit) appropriate for your PC operating system.

5. Turn ON luminometer using the ON/OFF switch on the rear of the luminometer (Figure 4).

6. Insert the GloMax® Software CD from the accessory kit in the CD-ROM drive. The software CD will automatically launch an installation wizard to assist you.

   Note: For Windows® XP, only an administrator may successfully install the GloMax® Software. If you are unsure of your user privileges, contact your internal computer support department. For Windows® 7, we recommend the Windows® 7 Professional Service Pack 1 and Microsoft® Office 2010 Home and Business Edition, 32-bit version. Other combinations of Windows® and Microsoft® Office have not been tested. Therefore, we cannot guarantee compatibility with other versions.

7. Allow the installation wizard to launch the program immediately after the wizard tool completes the installation.

8. The GloMax® Software will attempt to establish communication between the computer and the luminometer. If the default COM port is not available, the COM port selection box will appear. Select the COM port that corresponds to the serial port for your computer. If using the USB serial adaptor, choose the COM port that corresponds to the USB port for your computer. A yellow light indicates no connection.

9. If the software cannot establish communication with the luminometer, check the power and serial data cable, and then click "OK." The software will again attempt to establish communication. If you wish to access the software without a luminometer, click "Cancel."

5.   Using the GloMax® 96 Software

5.A. Overview

The GloMax® 96 Microplate Luminometer software is easy to use. This direct-to-Excel-based software gives the user flexibility in data retrieval, storage, and analysis. The software comes preloaded with protocols for Promega luminescent assays. It is also possible to create, store and retrieve user-defined protocols.

5.B. Software Operation

1.   To launch the GloMax® 96 software, click on the GloMax® icon on the desktop, or select the program from the Start menu of your computer. The *Welcome to GloMax*® dialog box will appear (Figure 5).
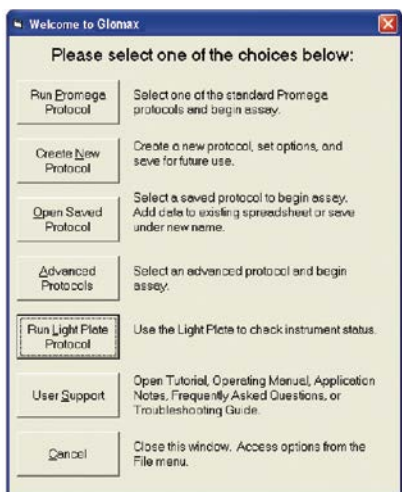

Figure 5. The *Welcome to GloMax*® Dialog Box.

2.   New users should click "User Support" to see an electronic copy of this Technical Manual. To start running assays, select one of the five protocol options.

5.C. Choosing a Protocol

The five protocol options available in the *Welcome to GloMax®* dialog box are "Run Promega Protocol," "Open Saved Protocol," "Create New Protocol," "Advanced Protocols," and "Run Light Plate Protocol."
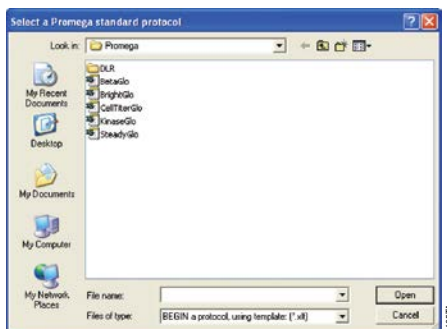

Figure 6. Selecting a Promega standard protocol.

Promega Standard Protocols

To run a Promega assay, select "Run Promega Protocol." A dialog box will appear, allowing you to browse the available Promega protocols (Figure 6). Select a Promega protocol. Some protocols are contained inside a folder. The protocol you select will depend on your choice of injectors. Open the specific Promega protocol that corresponds to your assay. The default settings for Promega templates are optimized for reagent performance. You do not need to change the settings.

The Light Plate Protocol

The optional GloMax® Light Plate protocol provides a quick way to verify instrument performance. The Light Plate (Cat.# E6531) comprises three highly stable light sources that simulate luminescent samples at signal levels spanning over four decades. The Light Plate Protocol automatically analyzes the results of the Light Plate run and gives you a clear indication of the instrument status. Save the file after you run the Light Plate Protocol. Refer to the Light Plate instruction card for detailed instructions on using the Light Plate.

5.C. Choosing a Protocol (continued)

Opening a Saved Protocol

You may open an existing protocol to recall previous assay conditions or to add new data. To open an existing protocol, select "Open Saved Protocol" from the *Welcome to GloMax®* dialog box, and select the apppropriate protocol from the window that appears (Figure 7).
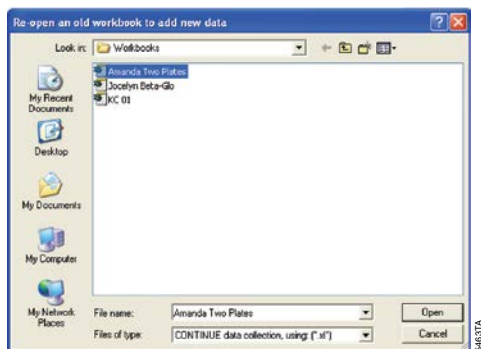
Figure 7. Opening an existing protocol.

Running an Advanced Protocol

Section 9 describes several advanced protocols including Kinetic Measurement, Automatic Injector Options and Super Protocols.

5.D. Creating a New Protocol

1. If you wish to select all of the options manually, then click on "Create New Protocol" to launch the "Protocol Setup Wizard." It will guide you through a series of steps to customize your protocol (Figure 8).

Figure 8. The Protocol Setup Wizard.

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2. The wizard tool will allow you to specify the parameters for your new protocol:

Injectors: You may choose to use zero, one, or two automatic injectors. If you select one or two injectors, you can also choose to measure your plate before injection.

Delay before measurement: You can set a delay before the luminometer begins a run. The delay is useful for dark-adapting samples and microplates to reduce background.

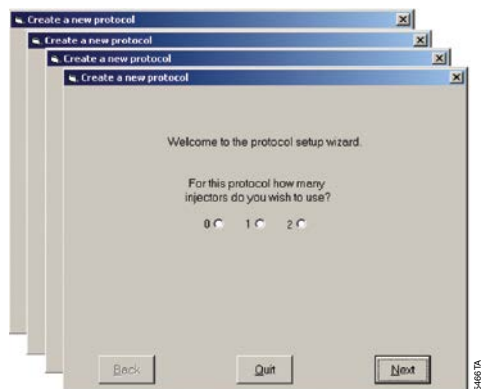**Number of runs:** If you choose a noninjection run, you may set up to 999 automatic repeats of the run. This option is useful for measuring a change in luminescence over an extended period of time.

Delay between runs: For repeated runs, you may set a rest period between each run. **Note:** Do not open the instrument lid during the delay between repeated runs.

Injection Volume: The volume injected per well ranges from 25−250µl. The maximum volume capacity per well is 300µl. Determine the volume of your sample per well before selecting injection volume. Overfilling a well will cause flooding and impair performance.

Delay after injection and before measurement: Setting a delay after the injection will allow flash-type luminescence to fully actualize before a reading.

Integration Time: Adjust the measurement time per well according to your assay protocol. In the case of flash-type luminescence, a longer integration time ensures an accurate reading of the entire peak of luminescent output.

Selecting Wells to Measure: To select or deselect all of the wells for measurement, click the "All" button at the top left corner of the grid (Figure 9). Alternatively, you can click on a letter to select or deselect the indicated row. Click on a number to select or deselect that column. You may also select individual wells by clicking on the well you wish to measure. The instrument reads only the wells that are selected. Selected wells are blue on the grid. Unselected wells are gray. (Unselected wells are marked with an "X" in the Excel spreadsheet.)

3. After you click "Finish," the wizard will automatically record your choices into the "Options" menu. To access your settings, click "Options" from the Main Dialog Box.
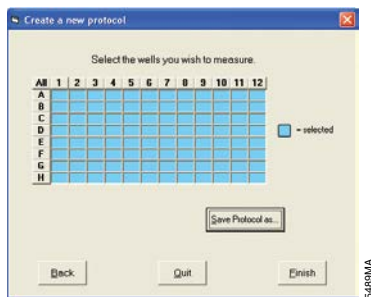

Figure 9. Selecting wells to be measured for a new protocol.

4. After setting up your new protocol with the wizard, you may store the protocol for future use. Click on "Save Protocol As" to save the protocol as an Excel template. Saving the new protocol automatically closes the wizard and takes you to the Main Dialog Box (Figure 10). You are ready to begin measurements.

6.   Operating Injectors

The "Main Dialog Box" (Figure 10) allows the user to start runs, change setup options, establish communication between the luminometer and a computer, prime injectors, flush injectors and reverse purge injectors.

Figure 10. The Main Dialog Box.

6.A.   Priming Injectors

If you need injectors for your assay, you must prime the injectors before running the assay.

1.   Fully insert the inlet tubing into the reagent bottle. Secure the tubing to the bottle with the tubing guide.

2.   Click the "Prime" tab in the Main Dialog Box. Select the injectors you wish to prime (see Figure 11).
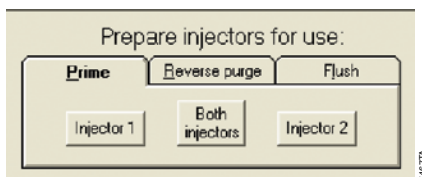

Figure 11. Selecting injectors for priming.

3.   Click "Prime" to start priming the injectors. An automatic priming sequence will begin. Each priming sequence uses 700µl of reagent. Approximately 300µl will be dispensed into the waste tray. Refer to Section 12.D for instructions on cleaning the waste tray.

4.   Click "Exit window to start a run" to return to the Main Dialog Box.

6.B. Reverse Purging Injectors

After you finish your assay, you may retrieve any unused reagent by clicking the "Reverse purge" button in the Main Dialog Box. This will push any reagent left in the injector and the tubing back into the reagent bottle.

6.C. Flushing Injectors

Failure to flush injectors after every run will cause injector clogging, and the injectors may not be serviceable in the field, requiring costly repairs. To flush the injectors and maintain your system, follow these steps:

1. Select the "Flush" tab in the Main Dialog Box. Select the injectors you wish to flush.

2. Flush the injectors three times each with deionized water, 70% ethanol, then deionized water again and finally air.

7. Measuring your Samples

1. To start a run, fill in the information you would like in the "Experiment," "Operator," "Plate Number," and "Notes" text boxes of the Main Dialog Box. This step is optional.

2. Open the instrument lid and gently press the sample tray latch to release the sample tray cover (see Figure 3).

3. Insert your 96-well plate into the sample tray and close the sample tray cover. The plate should be oriented so that the A1 well is at the back right corner.

4. Close the instrument lid and click "Start" in the Main Dialog Box. The Excel spreadsheet will occupy the lower portion of the computer screen. Data will appear in the Excel spreadsheet.

   Note: Do not open the instrument lid while a run is in progress.

   If you need to stop or cancel a run, click on the "Cancel" button in the Main Dialog Box before you open the instrument lid and remove the plate.

5. When the run is complete, the Main Dialog Box will display the message, "The plate completed run successfully." We recommend removing the plate and saving the data immediately after the run is finished.

8. Instructions for Using the Data Analysis Macro

The GloMax® 96 Microplate Luminometer software includes a data analysis macro for the Promega Dual-Luciferase® Reporter Assay with one or two injections.

8.A. Running the Dual-Luciferase® Reporter Assay

1. Open the GloMax® 96 software and select "Run Promega Protocol."

2. Open the "DLR" folder and select "DLRwithOneInjection" or "DLRwithTwoInjections", as appropriate.

   Note: Promega does not recommend performing the DLR™ Assay without injectors. To do so, you will need to create a custom protocol.

3. Open "Options" from the Main Dialog Box and select wells for measurement.

4. Prime the injector or injectors (see Section 6.A).

5. Insert the microplate into the sample tray, close the lid and click "Start" to begin the protocol.

Analyzing Data

6. After the luminometer finishes reading samples in the microplate, go to the "Excel Serving GloMax®" window. The first spreadsheet entitled "Measurements" contains the data as reported by the luminometer. The second spreadsheet entitled "Analysis" includes a data analysis tool that generates the ratio between the firefly and *Renilla* signals. Select the "Analysis" spreadsheet to view the raw ratio of firefly to *Renilla* signals. The data analysis macro also provides simple charts for the firefly and *Renilla* signals. Scroll to the right to view these charts.

   Background Subtraction: You can perform background or blank subtraction on the "Analysis" spreadsheet.

7. Enter the term "BLK" for any well in the microplate map that represents a background measurement.

8. Click on "Analyze Data" to perform the background subtraction. The data analysis macro averages the signal from BLK wells and subtracts this value from all other samples for both the firefly and the *Renilla* signals. The data analysis macro fields are copied below the previous field, and the adjusted ratio appears in the new data analysis macro field.

Sample Groups

You can assign unique Sample IDs to track experimental or unknown samples or sample groups.

9. Enter the Sample IDs into the microplate map. Remember to enter this information into the most recent microplate map. Repeat the Sample ID for each well containing the same sample group.

10. Click on "Analyze Data." The data analysis macro fields are copied below the previous fields. The software will take the average for any group of identical sample IDs and calculate the average, standard deviation, and coefficient of variance for the group.

Controls

11. Use the control option to distinguish your controls from your unknown samples. If the microplate contains positive or negative controls, you may designate these wells as CTL on the microplate map.

8.B. Multiple Analyses of the Same Microplate

New data analysis macro fields appear after each analysis. You can make adjustments to the microplate map and re-analyze the data. However, you must make any adjustments in the most recent microplate map. In most cases, you will need to scroll down on the "Analysis" spreadsheet to find the most recent microplate map.

8.C. Multiple Microplates

When the GloMax® 96 Microplate Luminometer runs multiple microplates in the same protocol, new microplate data sets appear in the measurement spreadsheet. You can analyze all of the microplates on the "Analysis" spreadsheet. However, you must analyze one run or microplate data set at a time. To analyze the next microplate data set, click "Step to Next Run's Measurements" on the top of the "Analysis" spreadsheet. After you proceed to the next microplate, you can not make adjustments to previous microplates. To match the analysis with the microplate data set, go to the "Measurements" spreadsheet. The highlighted microplate data set is the same set undergoing analysis.

8.D. On-the-Fly Analysis

Advanced users with multiple microplates containing identical sample and background mapping can enable On-the-Fly Analysis. The On-the-Fly Analysis option automatically analyzes each new microplate data set according to the initial microplate map. Enter in the analysis information into the microplate map before starting the protocol. Then click "Enable On-the-Fly analysis." Click "Start" on the Main Dialog Box to begin the protocol. After the luminometer finishes reading the microplate, the data analysis macro will immediately analyze the data.

9. Advanced Protocols

The "Advanced Protocols" folder contains "Kinetics," "Automatic Injector Options," and "Super Protocols" (Figure 12).
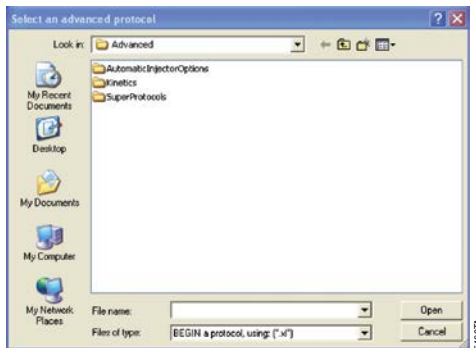

Figure 12. The Advanced Protocols window.

9.A. Kinetic Measurement

1. Kinetic protocols are available for measurement with and without injection. Open the "Kinetics" folder and choose the protocol of interest.

2. Click on "Options" to select wells for measurement. During the kinetic protocol, the luminometer will collect data for each well and send the data to the Excel spreadsheet. The results for all kinetic runs appear in column format.

3. Select the "Other Options" tab to adjust the frequency of data point collection as well as the number of total measurements per well (Figure 13).

   Notes:

   The dynamic range for kinetic measurements is limited when compared to the dynamic range of discreet or normal measurements.

   Extremely bright samples may saturate the luminometer in "Kinetics" mode.

Figure 13. Plate Setup and Options window.

9.B.   Automatic Injector Options

The "Automatic Injector Options" folder (Figure 14) contains a variety of automatic injection sequences with and without measurement. The title of the protocol reflects the sequential order of injection and measurement. For example, the protocol entitled "Inject1Inject2Measure" programs an injection by Injector 1 followed by an injection by Injector 2 immediately followed by a measurement of the well. This sequence repeats for each well selected. Use "Options" to adjust the volume for each injection and the measurement time.
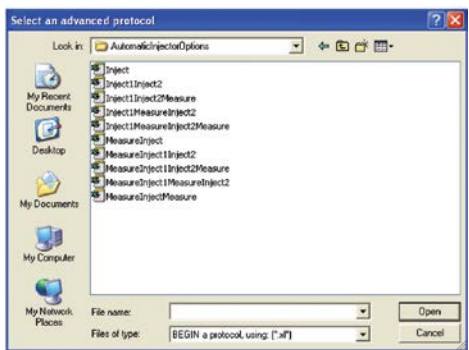

Figure 14. Automatic Injector Options folder.

9.C. Super Protocols

Super Protocols allow you to run multiple individual protocols in a user-preferred sequence. For example, a properly designed Super Protocol might execute an injection-only run followed by a 5-minute incubation period and finally a measurement-only run.

1. Before setting up a Super Protocol, set up the individual subprotocols and save each protocol with a descriptive file name.

2. Close the subprotocols, then open the Super Protocols folder in the "Advanced Protocols" folder. Standard and column formats are available for reporting data from Super Protocols (Figure 15).
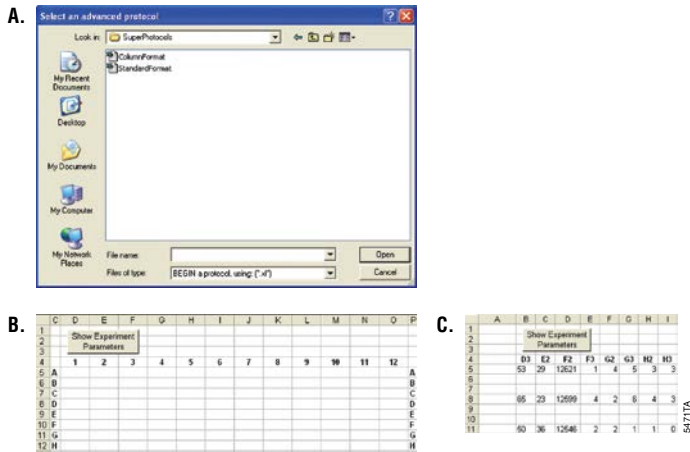

Figure 15. The Super Protocols Folder (Panel A) and data report formats: standard (Panel B) and column (Panel C).

3. Click on "Options" then the "Other Options" tab to assemble the order of execution for each individual sub-protocol (Figure 16).
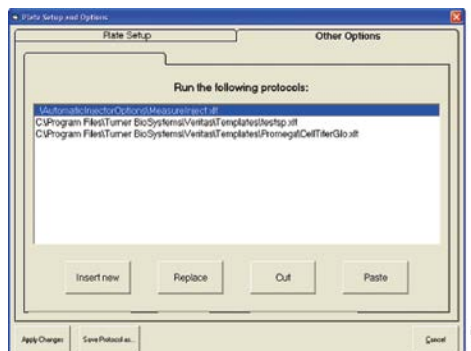
Figure 16. Selecting the order of execution for each subprotocol of a Super Protocol.

The following rules apply to Super Protocols:

1. Kinetic measurements are not compatible with Super Protocols. A Super Protocol cannot be successfully completed if a subprotocol executes kinetic measurements.

2. The luminometer only executes actions on wells selected in both the subprotocol and Super Protocol. Select all wells in the subprotocols, then use the Super Protocol to select individual wells, rows or columns for measurement.

3. Protocols constructed in earlier software versions may not work for Super Protocols. Reconstruct older protocols using the "New Protocol" wizard tool.

4. Multiple injections into a well that flood a microplate may cause serious mechanical problems as well as increase the background readings. Clean up spills immediately (see Section 12).

10. Saving Templates and Data

To save a file as a template for future measurements, choose all the necessary options from the Plate Setup and Options screen, then click "Save Protocol As". Alternatively, you can click "Apply Changes", run the plates, then save the Excel data file by choosing "Save a copy as" from the "File" menu in the Main Dialog Box. Both templates and data files can be opened later by clicking on "Open Saved Protocol" on the *Welcome to GloMax®* dialog box.

11. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

11.A. Software

Please refer to the Computer Requirements in Section 13.E to ensure you are using Windows® 7 and Microsoft® Office versions compatible with the GloMax® 96 Software.

Symptoms	Causes and Comments
The software does not respond.	The computer is not connected to the instrument. Check the yellow status light on the front of the instrument. If illuminated, the luminometer and computer are not communicating. Make sure the data Cable connects the luminometer with the computer.
	The luminometer is not on. Check the green status light to see if the instrument is ON. If it is not illuminated, check the power supply and the ON/OFF switch.
Communications Error: GloMax® is not communicating with the computer.	The 9-pin serial Data Cable is not fully connected to the instrument or computer. Check the female and male connectors of the 9-pin serial Data Cable to ensure complete connection.
	The USB Serial Adaptor is not fully connected to the 9-pin Data Cable or computer. Check the USB Serial Adaptor for proper connection. Refer to the USB Serial Adaptor operating manual for installation instructions.
	The luminometer is not on. Check the green status light to see if the instrument is ON. If it is not illuminated, check the power supply and the ON/OFF switch.
	Retry until you get a user prompt to change your selection of COM port.
	There is a firmware problem. Check the red status light. If illuminated, turn OFF the luminometer and restart your computer. Open the GloMax® software, then turn ON the luminometer. If the red status light is still illuminated see Section 13.A for information.
Error Message: GloMax® Run-time error '8018': Operation valid only when the port is open.	There is a software problem. Uninstall the GloMax® Software then reinstall it. (See Section 4).
Communications Error: Cannot open COM port; connection. Use the Settings menu.	The communication between the luminometer and the computer was disrupted. Restart the GloMax® software.

Symptoms	Causes and Comments
Communications Error: USB Drivers not loaded for USB serial adapter.	Load drivers from the CD-ROM provided with the USB Serial Adapter.
Error Message: GloMax® can't exit yet. A run is still in progress.	Another program has reserved the serial port. Contact your computer support department to determine the availability of serial ports on your computer.
The luminometer does not read the microplate when you click "Start."	The luminometer is already reading a microplate. Click "OK" to return to the Main Dialog Box. If necessary, click "Cancel" from the Main Dialog Box to stop a run in progress. To close the GloMax® Software, go to the Task Manager on your computer and use "End Task" to close the software. You may lose data by closing the software from your Task Manager.
	Instrument is waiting through a user-set delay time. Inspect the Status Bar on the Main Dialog Box to see if a delay time is set. Cancel the run and select "Options" to reset the delay time.
The luminometer does not respond when you click "Start."	There is a software problem. Close the GloMax® Software. Restart your computer and turn the luminometer ON. Open the GloMax® Software and reprime injectors (if necessary) before starting the run.
Data appears in the wrong spreadsheet and overwrites saved data.	There are no wells selected for measurement. Select "Options" from the Main Dialog Box to choose the wells you want to measure. Selected wells are blue. Unselected wells are gray.
The data fields have a yellow background.	The GloMax® Software will enter data into any Excel spreadsheet opened in the "Excel Serving GloMax®" window. After the luminometer finishes the run, cut and paste the new data into a new Excel spreadsheet. Close the old Excel spreadsheet but DO NOT SAVE changes. In the future do not open another Excel spreadsheet in the "Excel Serving GloMax®" window.
The data fields have a red background and zero RLU values.	The samples are too bright. Dilute the samples and rerun the microplate or switch to a black microplate.
	A run was canceled or an error occurred during the run. Premature attenuation of a run may cause loss of data. Reprime injectors if they are in use, and start a new run.

11.B. Troubleshooting, Injectors

Symptom	Possible Causes and Comments
The injectors are not available under the Options menu.	Injectors were not chosen when the user set up a new protocol. You cannot add automatic injectors to an existing noninjection protocol. Choose "Create new protocol" from the *Welcome to GloMax®* dialog box, and select the injectors you wish to use.
	The luminometer does not have injectors installed (Cat.# E5601). Contact Promega Corporation to ask about automatic injectors on the GloMax® 96 Microplate Luminometer.
Error Message: Pump 1, needed for this run, is not primed. Prime it, then click "Start" again.	The injector was not primed or the instrument was power-cycled after the injector was primed. Select the Prime tab from the Main Dialog Box, and prime the injectors. Remember to prime into a waste container.
Injectors will not inject.	Air bubbles are blocking the line, or the end of the inlet tubing is not in the reagent. Make sure that the inlet tubing is completely inserted into the reagent bottle. Reprime the injectors.
	Reagent residue is clogging the tubing. Replace the tubing after use to prevent reagent residue build up inside the injector, tubing and tip.
	Reagent residue is clogging the valves. Contact Promega Corporation.
Injector do not prime or flush properly.	The tray is not in the Home position. Restart the software. Select "Eject Tray" to return tray to the Home position.
Injector tips are damaged or bent.	See Section 12.F for maintenance instructions for changing the ejector tips.
Injections sputter, drip or are otherwise weak.	Air bubbles are blocking the line, (see above) or reagent residue is clogging the tubing. Replace tubing. Always flush the tubing after use to prevent reagent from building up inside the injector, tubing and tip.
Injector leaks.	The inlet or outlet tubing is not properly connected to the injector syringe. See Section 12.G. for instructions on removing and replacing tubing.
Injector tips do not sit properly in the injector tip holder.	Reagent residue has built up inside injector tip holder. See Section 12.A for instructions on cleaning the optical head.

11.C. Miscellaneous Troubleshooting

Symptoms	Causes and Comments
Grinding noise during run.	Reagent residue has built up on optical head mask. See Section 12.B for instructions on cleaning the interior of the luminometer.
	The sample tray cover is open during the run. Close the sample tray cover and secure with the sample tray latch. Make sure plate holder is in home position.
Red LED status light is ON.	The luminometer is not operational. If the software does not initiate an automatic firmware download, turn OFF the luminometer. Close the software and reboot the PC. Turn ON the luminometer, then open the software. This power cycle may restore the luminometer.
The sample tray cover does not close.	The microplate is not sitting flat in the sample tray. Gently push the tray cover up to a 90° angle with the sample tray to completely open the tray. Set the microplate down inside the tray.
Head or tray motion failure	The mechanical components of the luminometer are not moving properly. This can be caused by reagent residue on the sample tray cover, the optical head mask, or on the rails along which the tray assembly moves. The only remedy for this problem is to thoroughly clean these components. For cleaning instructions, select "User Support" from the Help menu. Click on "Operating Manual"on the left side of the window and select "Appendix A-Maintenance" from the Table of Contents.
Moisture appears on top of the sample tray cover after a run.	The injector tips are bent. See Section 12 for directions for changing the injector tips and cleaning the optical head.
	The microplate has overflowed inside the luminometer. See Section 12.A for directions for cleaning the optical head. Check the wells selected for injection and measurement. Check the total injection volume from the Options menu. Check the volume of the sample inside the well. The maximum volume per well is 300μl. Thoroughly clean the interior of the instrument.
Reagent has spilled inside the luminometer	Wipe up spills immediately using an absorbent paper towel or delicate task wipe. If necessary, use a 70% ethanol solution to remove reagent residue.

11.C. Miscellaneous Troubleshooting (continued)

Symptoms	Causes and Comments
Light plate reads very low or blank.	The light plate was not ON before the run. Press "Start" to turn the light plate ON. The green status light will flash. The light plate will auto-matically turn OFF after 5 minutes.
	The wells selected do not corrleate with the light plate. Select the "Light Plate Protocol" option from the *Welcome to GloMax®* dialog box. The light plate sample wells are at B2, D2 and F2.
Error Message: Run #1 canceled. Lid opened—Recipe canceled.	The lid was opened during the run. Reprime the injectors if they are in use. Close the lid, and do not open the lid until the run is complete.
	The optical head is stuck or jammed. See Section 12.A for directions for cleaning the optical head.
Error Message: Run #1 canceled. Move to plate sense position not done.	The optical head is stuck or jammed. See Section 12.A for directions for cleaning the optical head.
Error Message: Run #1 canceled. Move failed.	The optical head is stuck or jammed. See Section 12.A for directions for cleaning the optical head.
Error Message: Cannot START with the lid OPEN. Please close the lid and try again.	Check for any obstruction that may prevent the lid from closing completely (e.g., reagent bottles). The lid should close easily. Do not force the lid.
Error Message: Cannot START without a microplate in the instrument.	The microplate is not in the luminometer. Insert the microplate and click "Start".
	A clear plate is in the sample tray. Use a white or black opaque microplate.
Readings are extremely low for positive control samples.	Injection failure. Determine if the correct volume was injected into the well. Make sure that the inlet tubing is fully inserted into the reagent bottle. Look for air bubbles in the outlet tubing. Reprime the injectors before the next run.
	The wrong wells were injected or measured. Check the wells selected from the Options menu. Check the plate orientation.

Symptoms	Causes and Comments
High background for negative control or empty wells.	White microplates can phosphoresce inside the luminometer. Dark-adapt the microplate by setting a delay time (Options menu) before measuring samples.
	The light detector is saturated with ambient light. Remove the microplate and keep the lid closed for 30 minutes to allow the light detector to recover.
	Bright samples can cause cross-talk interference. The luminometer reads from left to right (rows A−H). On injection runs, position your negative controls at the beginning of the plate.
RLU readings appear inconsistent with expected results.	Ambient temperature is too high. The ambient temperature should be ≤23°C for best results.

12. Maintenance

The GloMax® 96 Microplate Luminometer requires the following maintenance tasks every 30 days: Cleaning the Optical Head, Cleaning the Interior, Cleaning the Injectors (for Cat.# E6511 and E6521), and Cleaning the Waste Tray.

12.A. Cleaning the Optical Head

1. Turn OFF the luminometer. Disconnect the power supply. DO NOT attempt to clean the optical head while the instrument is ON.

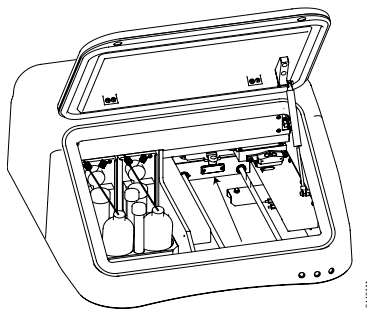2. Push the tray from the Home position to the back of the luminometer (Figure 17).

Figure 17. Push the tray from the Home position to the back of the luminometer.

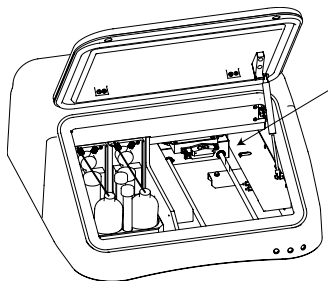3. Push the optical head from the Home position toward the center of the instrument (Figure 18).

Figure 18. Push the optical head toward the center.

4. Remove the injector tips, if possible. If not, remove the tubing from the injector syringe. Go to Section 12.E for further instructions.

5. Remove the injector tip holder from the optical head. Facing the front of the luminometer, push upward, then leftward (as viewed from the bottom of the holder to release the holder from the optical head (Figure 19).
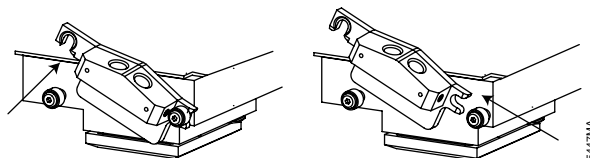

Figure 19. Removing the injector tip holder.

6. Place your hand underneath the optical head. Grasp the sides of the optical head mask and pull the mask toward you (Figure 20).

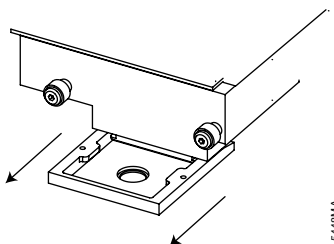(!) **Note:** Do not touch the bottom surface of the optical head.

Figure 20. Removing the optical head mask.

7. Soak the injector tip holder and the optical head mask in a 70% ethanol solution for 30 minutes to dissolve reagent residue. Rinse with deionized water.

8. Allow the injector tip holder and optical head mask to air dry completely before proceeding. Alternatively, blot the optical head mask and injector tip holder dry with a lint-free, delicate task wipe.

12.A. Cleaning the Optical Head (continued)

9. Return the optical head mask to the optical head. Align the mask with the optical head and gently guide the mask onto the optical head (Figure 21).
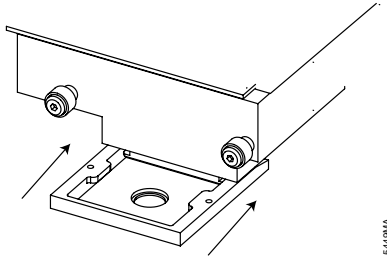

Figure 21. Replacing the optical head mask.

10. Return the injector tip holder to the optical head. Position the holder just above the front pins on the optical head. Push the holder toward the right to lock the holder securely to the right pin of the optical head. Then push down and lock the holder on the left pin (Figure 22).
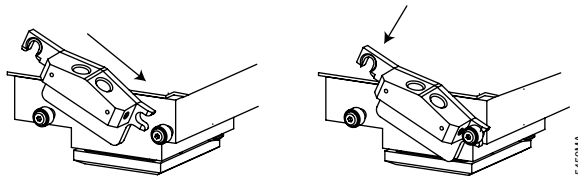

Figure 22. Replacing the injector tip holder.

11. Insert injector tips into the injector tip holder. Snap injector tips in place (Figure 23).

Injector tip holder
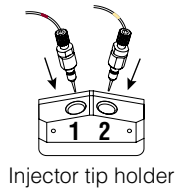
Figure 23. Inserting the injector tips.

12. Return the optical head to the home position by pushing the optical head from the center of the instrument all the way to the right (Figure 24).
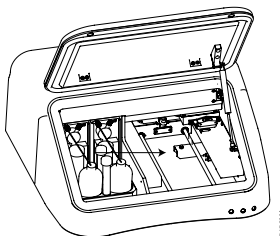

Figure 24. Placing the optical head in the Home position.

13. Pull the sample tray forward to the Home position.

14. Reconnect the power supply. It is now safe to turn the luminometer ON.

12.B. Cleaning the Interior

Note: Failure to properly maintain a clean interior may cause a mechanical error and the irretrievable loss of data. Clean the interior every 30 days.

The accumulated residue from various luminescent substrates may inhibit proper movement of the optical head. We recommend thoroughly cleaning the interior of the luminometer every 30 days and immediately after any spills.

1. Turn OFF the luminometer.

2. Use a laboratory tissue dampened with 70% ethanol to wipe the sample tray cover. Repeat.

3. Push the sample tray to the back of the luminometer, exposing the two steel guide rails upon which the sample tray glides (Figure 17). Use a laboratory tissue and 70% ethanol to clean the guide rails.

4. Push the optical head to the center of the luminometer, exposing the black metal shelf on the right side of the luminometer (Figure 18).

5. Clean the black shelf with a fresh laboratory tissue dampened with 70% ethanol. Be sure to clean the rest position for the optical head.

6. Return the optical head to the Home position (Figure 24).

7. Close the lid and turn the luminometer ON.

8. Use the "Eject Tray" button to automatically return the sample tray to the Home Position.

12.C. Cleaning the Injectors

For Cat.# E6511 and E6521, we recommend thoroughly cleaning the injectors every 30 days.

1. Prepare a solution of 70% ethanol and use it to flush the injectors three times.

2. Allow the solution to sit in the fluid path for 30 minutes before flushing the injectors nine times with deionized water.

3. Flush the injectors three times with air. A small volume of water will remain in the injector after the air purge.

12.D. Cleaning the Waste Tray

A removable waste tray prevents flooding of the luminometer interior. The capacity of the waste tray is approximately 100ml. There is a time delay between the departure of liquid from the tip and the arrival of the liquid in the waste tray. Place an absorbent paper towel underneath the luminometer while emptying the waste tray.

12.E. Removing or Inserting Injector Tips

1. Grasp the injector fitting. Pull upward in a single, smooth motion. Do not use excessive force.

2. To replace the injector tip, carefully align the tip with the injector holder. Hold the tip by the fitting.

3. Gently push the injector tip into the injector tip holder. Continue with a steady push until the injector snaps into place. Do not force the tip farther than allowed by the injector tip holder. An internal clip locks the tip into place.

12.F. Changing Injector Tips

Replacement injector tips are available from Promega Corporation (Cat.# E4861).

1. Gently remove the injector tips from the injector tip holder.

2. Twist the tip fitting counterclockwise to release the fitting from the tubing (Figure 25).
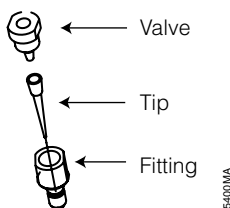

Figure 25. The injector tip assembly.

3. Untwist the tip fitting to release the tip.

4. Discard tip.

5. Insert a new tip into fitting.

6. Twist the fitting clockwise to complete the injector tip assembly. Twist the fitting until tight. A small gap (approximately 1mm) between the two fittings is normal.

7. Twist the assembled fitting with the new tip clockwise onto the tubing.

8. Insert the tip into the injector tip holder on the optical head.

12.G. Removing or Replacing Injector Tubing

Replacement tubing (Cat.# E4822) is available from Promega.

1. Grasp the injector tubing fittings located on the top of the injector syringe.

2. Disconnect the inlet and outlet tubing from the injector syringe by twisting the fitting counterclockwise.

3. Discard the used injector tubing.

4. Twist the fitting of the replacement tubing to secure the tubing onto the syringe.

12.H. Additional Maintenance

Periodically wipe off the outside of the instrument with a damp cloth. Do not use solvents or abrasive cleaners to clean the instrument. Avoid spilling liquids into the sample tray.

If a spill has occurred:

1. Unplug the instrument.

2. Wipe up any moisture inside the sample tray.

3. Use a laboratory tissue dampened with a 70% ethanol solution to clean the tray.

4. Plug in the instrument and turn ON the power. Allow the instrument to warm up for a few minutes or until it is completely dry inside.

5. If moisture appears on the sample tray cover, refer to Section 12.A.

> If you suspect that fluids have spilled onto the light detector, please contact Promega Corporation for cleaning instructions.

13. Appendix

13.A. LED Status Lights

There are three LED status lights on the front panel of the luminometer. All three illuminate briefly after you turn the luminometer ON, but only the green light should remain illuminated. It indicates that the instrument is powered and ready.

If the yellow light remains lit, the computer and the instrument are not communicating. A communication failure can occur if:

• the luminometer is ON, but the software is not open on the computer.

• the luminometer is ON, but the 9-pin serial Data Cable is not connected to the computer.

• the luminometer is connected to an unusable COM port.

The red LED light indicates other types of errors. When the red LED light is illuminated, the software may initiate an automatic firmware download. Follow the instructions as they appear on the screen. It may take several automatic downloads to fully restore the luminometer. While the red LED light is illuminated, the luminometer is nonoperational. If the software does not initiate an automatic firmware download, turn OFF the luminometer. Close the software and reboot the PC. Turn the luminometer ON, then open the software. This power cycle may restore the luminometer.

13.B. Save and Restore Parameters

The Utilities menu features two options regarding parameters. Each instrument contains its own unique parameters that aid in its ability to measure light. Under normal operation, it is not necessary to save or restore parameters. In certain cases, restoration of parameters will resolve erratic behavior. Contact Promega Technical Services for more information.

13.C. Firmware Upgrades

The GloMax® 96 Microplate Luminometer can download new firmware to the instrument from a computer. This capability is reached through the Utilities menu. This is a password-protected function to prevent accidental use. The purpose of firmware upgrades is to add new capabilities to the system. When a new version of firmware is developed in the future, Promega Corporation will send you specific instructions on how to perform the upgrade.

13.D. Warranty and Service

The GloMax® 96 Microplate Luminometer comes with a one-year warranty from Promega. Additional warranty is available. For more information contact Promega Technical Services. Contact information is available on the web at: **www.promega.com.** E-mail: **techserv@promega.com**

To obtain service during the warranty period, please take the following steps:

1. Contact Promega Technical Services.

2. Carry out minor adjustments or tests as suggested by your Technical Service contact.

3. If it is determined that the instrument should be returned for repair, Promega Technical Services will arrange for service by an authorized GloMax® service agent. You will be issued a Promega return authorization number.

    YOU MUST OBTAIN a Promega return authorization number before returning a luminometer for service.

4. Before returning the luminometer, you will be responsible for cleaning it and providing a Certificate of Decontamination (see Section 13.F).

13.E. Specifications

Performance Specifications

Sensitivity: 3×10^{-21} moles luciferase
Linear Dynamic Range: > 9 decades
Cross-Talk: Better than 3×10^{-5}
Precision: CV < 3%
Detector: Photomultiplier Tube (PMT)
Spectral Response Range: 350−650nm
Peak Wavelength: 420nm
Plate Format: 96-well
Injectors: One or Two Injectors (optional)
Injection Volume: Selectable between 25 and 250μl (±1μl)
CV% < 1%

Technical Specifications

Computer Interface: RS-232 port
User Interface: Windows® 7 Professional Service Pack 1 with Microsoft® Office 2010 Home and Business Edition, 32-bit version. Also compatible with Windows® XP and Microsoft® Office 2003. See computer requirements below.
Power: 0.5A @ 100−240V, 50−60Hz (universal)
Dimensions: 19.44" D x 18.75" W x 9.28" H (49.38 x 47.63 x 23.57cm)
Weight: 28lb (12.7kg)
Operating Temperature: 60−105°F (15−40°C)
Warranty: One year
Approvals: CE

Computer Requirements

The GloMax® 96 software is compatible with 32-bit and 64-bit PCs using Windows® 7 Professional Service Pack 1 and Microsoft® Office 2010 Home and Business Edition, 32-bit version. It is also compatible with Windows® XP and Microsoft® Office 2003. The computer can be connected to the instrument using either an RS-232 cable or USB/RS-232 adaptor.

Compatibility with the Home Premium and Ultimate Editions, or with other combinations of Microsoft® Office and Windows® versions, has not been tested. To ensure compatibility with the GloMax® 96 software please follow the PC requirements recommended in this manual.

Microsoft® Office 2010 Home and Business Edition has 32-bit and 64-bit versions. The 32-bit software version is recommended by Microsoft® for use on both 32-bit and 64-bit Windows® PCs. Therefore, the 32-bit version of Microsoft® Office Home and Business Edition was tested with a 64-bit Windows® 7 PC and found to be compatible. The 64-bit software version was not tested. Please refer to: **www.microsoft.com** for Windows® 7 Professional and Office 2010 Home and Business Edition system requirements.

13.F. Certificate of Decontamination

Disinfection and decontamination are required before shipping the instrument and instrument accessories for repair. Instruments returned for service must be accompanied by a signed and dated Certificate of Decontamination, which must be attached to the outside packaging of the instrument.

1. Clean the optical head according to the instructions in Section 12.A

2. Clean the interior of the luminometer accoring to the instructions in Section 12.B.

3. Clean the injectors according to instructions in Section 12.C.

4. Disinfect the Waste Tray.

Failure to confirm disinfection and decontamination will result in decontamination charges before the instrument will be serviced.

Select either (A) or (B):

A. I confirm that the returned items have not been contaminated by body fluids or by toxic, carcinogenic, radioactive, or other hazardous materials.

B. I confirm that the returned items have been decontaminated and can be handled without exposing personnel to health hazards.

Circle the type of material used in the instrument:

       Chemical   Biological   Radioactive**

Briefly describe the decontamination procedure performed:

_____

_____

_____

Date: _____

Place: _____

Signature: _____

Name (block capital letters): _____

** The signature of a Radiation Safety Officer is also required if the instrument was used with radioactive materials.

This instrument is certified by the undersigned to be free of radioactive contamination.

Date: _____

Place: _____

Signature: _____

Name (block capital letters): _____

Promega

13.G. Related Products

Luminometers and Accessories

Product	Size	Cat.#
GloMax® 96 Tubing Replacement Kit for Injectors	1 each	E4822
GloMax® Injector Tips Replacement (30)	1 pkg	E4861
GloMax® 96 Microplate Luminometer Light Plate	1 each	E6531
GloMax® 20/20 Luminometry System	1 each	E5311
GloMax® 20/20 Luminometry System with Single Auto Injector	1 each	E5321
GloMax® 20/20 Luminometry System with Dual Auto Injector	1 each	E5331

Dual-Luciferase® Assay Systems

Product	Size	Cat.#
Dual-Glo® Luciferase Assay System	10ml	E2920
Dual-Luciferase® Reporter Assay System	100 assays	E1910

Available in additional sizes.

Firefly Reporter Assays

Product	Size	Cat.#
Luciferase Assay System	100 assays	E1500
Bright-Glo™ Luciferase Assay System	10ml	E2610
Steady-Glo® Luciferase Assay System	10ml	E2510

Available in additional sizes.

Renilla Reporter Assays

Product	Size	Cat.#
EnduRen™ Live Cell Substrate	0.34mg	E6481
ViviRen™ Live Cell Substrate	0.34mg	E6491

Available in additional sizes.

Luminescent Cell Viability Assay

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570

Available in additional sizes.

Luminescent Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	10ml	G8091
Caspase-Glo® 8 Assay	10ml	G8201
Caspase-Glo® 9 Assay	10ml	G8211

Available in additional sizes.

Luminescent CYP450 Screening Systems

Product	Size	Cat.#
P450-Glo™ CYP1A2 Screening System	1,000 assays	V9770
P450-Glo™ CYP2C9 Screening System	1,000 assays	V9790
P450-Glo™ CYP3A4 Screening System	1,000 assays	V9800
P450-Glo™ CYP2C19 Screening System	1,000 assays	V9880
P450-Glo™ CYP2D6 Screening System	1,000 assays	V9890

Available in additional sizes.

Luminescent Enzyme Assays

Product	Size	Cat.#
MAO-Glo™ Assay	200 assays	V1401
Pgp-Glo™ Assay System	10ml	V3591

Available in additional sizes.

Luminescent Kinase Assays

Product	Size	Cat.#
Kinase-Glo® Luminescent Kinase Assay	10ml	V6711
Kinase-Glo® Plus Luminescent Kinase Assay	10ml	V3771

Available in additional sizes.

Luminescent Protease Assays

Product	Size	Cat.#
DPPIV-Glo® Protease Assay	10ml	G8350
Calpain-Glo™ Protease Assay	10ml	G8501

Available in additional sizes.

13.H. Summary of Changes

The following change was made to the 1/16 revision of this document:

1. Updated catalog numbers for GloMax® 96 Tubing Replacement Kit for Injectors and GloMax® Injector Tips Replacement.