

Gel Doc™ EZ System with Image Lab™ Software

Instruction Manual



BIO-RAD

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BIO-RAD RESOURCES AND REFERENCES

Bio-Rad Laboratories provides many resources for scientists. The following websites contain useful information about running electroporation experiments:

- **Life Science Research website (discover.bio-rad.com)**
This site includes links to technical notes, manuals, and product information.
- **Life Science Support website (support.bio-rad.com)**
This site includes links to technical support.

In the United States, you can reach Bio-Rad Laboratories at the following:

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For information concerning Bio-Rad Laboratories, Inc., and its products, visit our website at **www.bio-rad.com**.

Safety and Regulatory Compliance

The Gel Doc™ EZ imager is intended for laboratory use only. This instrument is meant for use only by specialized personnel who know the health risks associated with the reagents normally used with this instrument.

The UV light source is computer controlled, and proper interlocks are implemented to avoid users' accidental exposure to UV radiation.

If the Gel Doc EZ imager is used in a manner not specified by Bio-Rad Laboratories, the protection provided by the Gel Doc EZ imager may be impaired.

To help you make informed decisions about safety, we have provided comprehensive operating procedures and safety information in this manual and on labels affixed to the imager. This information will alert you to any potential hazards. It is the user's responsibility to read and understand the safety information and use it for safe operation of the system.

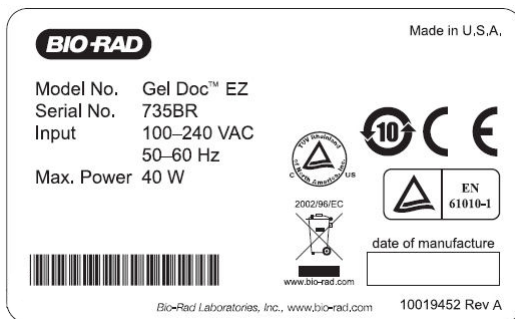
Safety Use Specifications and Compliance

The Gel Doc EZ imager is designed and certified to meet EN61010, the internationally accepted electrical safety standard, and EN61326 Class A EMC regulations. Certified products are safe to use when operated in accordance with the instruction manual.

This instrument should not be modified or altered in any way. Modification or alteration of this instrument will:

- Void the manufacturer's warranty
- Void the regulatory certifications
- Create a potential safety hazard

Gel Doc EZ imager certification label





For easy customer access, the serial number information appears in two locations on your instrument: on the back panel and inside the front door.

Instrument Safety Warnings

Notes, cautions, and warnings are used to highlight certain operating procedures and recommendations. The table below describes how each is used in this document.

Notes, Cautions, and Warnings

Icon	Meaning
Note:	Note: A note indicates a special procedure, an exception to normal operation or something else of specific interest to the reader. Notes are preceded by the word Note .
	Caution: A caution precedes an operational step that could damage the instrument or destroy data unless the operator takes certain precautions. Cautions located in the main text are preceded by the word Caution and are accompanied by the Caution symbol in the left margin.
	Warning: A warning precedes an operating procedure that could cause injury to the operator if not followed correctly. Warnings located in the main text are preceded by the word Warning and are accompanied by the Warning symbol in the left margin.

NOTICE

The Gel Doc EZ imaging system is intended for laboratory use only. This system is meant for use by specialized personnel who know the health risks associated with reagents normally used in electrophoresis. The UV light source is computer controlled, and proper interlocks are implemented to avoid users' accidental exposure to UV radiation. Bio-Rad Laboratories, Inc., is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended or for instrument modifications not performed by Bio-Rad Laboratories, Inc., or an authorized agent.

WARRANTY

Each Gel Doc EZ imaging system is protected by a comprehensive instrument warranty agreement. Please read this manual thoroughly so that you fully understand the coverage provided and are aware of your rights and responsibilities. One of the responsibilities of system ownership is regular maintenance. Following the maintenance instructions provided with this manual will help keep your system and peripherals functioning optimally and will protect your investment. Please also keep in mind that Bio-Rad offers a range of comprehensive service agreements that can be tailored to meet your specific needs. Bio-Rad Laboratories is dedicated to your total satisfaction and will be pleased to answer any questions you may have.

CAUTIONS

Caution: With the exception of cleaning or replacing light bulbs, refer all servicing to qualified Bio-Rad personnel or their agents. If you experience technical difficulties with the instrument, contact Bio-Rad to schedule service. The instrument should not be modified or altered in any way. Alteration voids the manufacturer's warranty and may create a potential safety hazard for the user.

Caution: If the case interlock is defeated, there is a possibility of UV-B radiation hazard due to UV-B light exposure. Caution must be exercised when servicing the instrument.

Caution: Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than that for which it is intended, or by the modification of this instrument when not performed by qualified Bio-Rad personnel or their agents.

Caution: Disconnect the AC power cord before removing the instrument cover.

WARNINGS

Warning: This instrument must be connected to an appropriate AC voltage outlet that is properly grounded.

Warning: There are hazardous voltages on the inside of the Gel Doc EZ. Do not remove the system cover when the instrument is connected to AC power.

Warning: Do not defeat instrument interlocks; they are designed to prevent user injury.

Warning: The Gel Doc EZ imager contains high voltage circuits. The user must disconnect the power cord prior to opening the system cover or removing the lamp modules for bulb replacement.

Warning: It is mandatory for users to turn off the imager and disconnect AC power from the unit before performing disassembly, as is needed to replace the lamps.

Warning: Do not remove the instrument cover when power is supplied to the instrument or defeat the UV safety interlock. Attempting to operate the unit with the cover removed may damage the instrument and expose the operator to hazardous voltages and UV radiation.

Warning: Use of controls or adjustments or performance of procedures other than those specified herein may result in exposure to hazardous UV radiation.

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1 System Overview

This manual describes the Gel Doc™ EZ system, which consists of the Gel Doc EZ imager and Image Lab™ software.



The Gel Doc EZ system supports multiple applications, including Coomassie and ethidium bromide staining, blue excitation for nondestructive DNA visualization, and stain-free gel imaging.

When coupled with the stain-free gel tray, the Gel Doc EZ system represents the next generation in stain-free imaging. It features one button acquisition, yields quick results with higher image quality, and is so simple to use it requires no training, even for users who are not familiar with image analysis systems. The Gel Doc EZ system's compact size frees up benchspace for running your experiments.

The Gel Doc EZ system is a reproducible and fast label-free SDS-PAGE/native PAGE system that eliminates time-consuming staining and destaining steps. Image Lab image acquisition and analysis software works with the Gel Doc EZ imager to create an automated and time-saving system to image and analyze electrophoresis gels. Data can be viewed, modified, and reported using Image Lab software.

Image Lab software protocols can automate gel image acquisition, analysis, and report generation at the push of a single button on the imager.

Researchers can run default protocols or easily design their own custom protocols. Image Lab software enables you to view analyzed data, adjust the analysis, and produce customized reports that show precisely the settings that were applied, assuring repeatable results.

Gel Doc EZ Imaging System

The Gel Doc EZ imaging system is a dedicated low-noise detection camera and UV transilluminator-based system with a small footprint. Designed for economy, automation, and ease of use, the user interface consists of a set of four sample trays and a one-touch button to image your gel using a protocol.



Gel Doc EZ imaging system, catalog #170-8270

Four sample trays are available for the Gel Doc EZ imaging system. Each tray supports a different set of applications, as shown in the table.

Sample Tray Types and Applications

UV Tray Catalog #170-8271	White Tray Catalog #170-8272	Blue Tray Catalog #170-8273	Stain-Free Tray Catalog #170-8274
Ethidium bromide	Fast Blast™ DNA stain	SYBR® Green	Stain-free gels
SYBR® Green	Coomassie Blue stain	SYBR® Safe	Stain-free blots
SYBR® Safe	Copper stain	SYBR® Gold	
SYBR® Gold	Zinc stain	GelGreen	
GelGreen	Silver stain		
GelRed			
Flamingo™ fluorescent gel stain			
Oriole™ fluorescent gel stain			
Coomassie Fluor Orange			
SYPRO Ruby			
Krypton			

Image Lab Software Capabilities

Image Lab software runs automated protocols on the Gel Doc EZ system for routine gel imaging documentation and analysis.

GEL DOCUMENTATION

Gel electrophoresis is a common way to separate, identify, and purify proteins or nucleic acids. The Gel Doc EZ system enables you to image and print gel images for documentation in laboratory notebooks or to export images for publication or presentation.

MOLECULAR WEIGHT ASSESSMENT

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and agarose gel electrophoresis are used to separate proteins or nucleic acids according to their size. Protein or nucleic acid molecular weight standards provide a reference for estimating the molecular weight of the proteins or nucleic acids being tested.

QUANTIFICATION

The components in a sample (bands) are quantified to determine relative amounts of each component in a sample or to compare the amount of a sample component relative to a standard. Assessing the purity of a sample requires the quantification of all components of a sample relative to each other. The results are expressed either as a percentage of all bands identified (band %) or as a percentage of all signal in the sample lane (lane %). Determining expression levels of a protein or nucleic acid requires quantification among samples. Data can be reported as either relative values, if the quantity is unknown, or as an absolute value, if a standard of known quantity is present.

2 Configuring the System

Configuring the system requires the following steps, all of which are described in this chapter:

1. Install Image Lab™ software on the PC or Mac computer you intend to use with the imager.
2. Connect the Gel Doc™ EZ imager to the computer.
3. Configure the imager.

Steps 1 and 2 are described in detail in the *Gel Doc™ EZ Installation Guide*. This topic walks you through the wizard steps for configuring the imager.

Installing Image Lab Software

Windows

1. Place the Image Lab software CD in your CD-ROM drive. The installation should start automatically. If it does not, open My Computer and click the CD drive icon.
2. Double-click setup.exe. The setup program installs Image Lab software on your computer.

The Image Lab software icon appears on your desktop. Follow the instructions in the next section to connect your system.

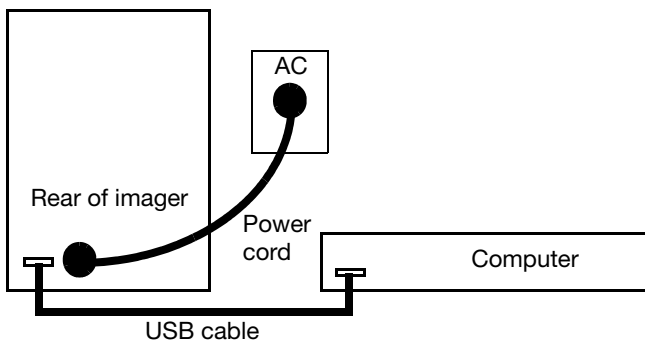
Mac

1. Place the Image Lab software CD in your CD-ROM drive.
2. Double-click the CD icon on your desktop to see the folder contents.
3. Double-click the file Image Lab.dmg.
4. Drag the Image Lab application icon into the Applications folder.

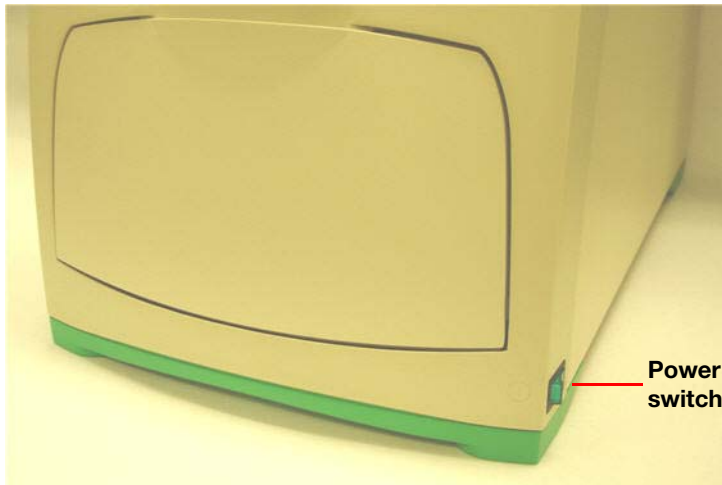
Follow the instructions in the next section to assemble your system.

Connecting the Imager to the Computer

1. Install or upgrade the Image Lab software before connecting the Gel Doc EZ imager to a computer.
2. Connect the imager to the computer using the USB cable provided with the system.
3. Connect the Gel Doc EZ imager to AC power using the power cord provided.



4. Turn on power to the imager using the switch on the side of the instrument.



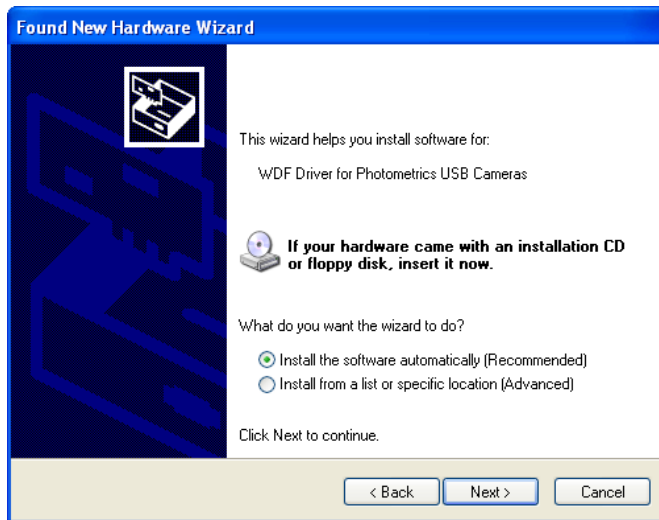
If Your Computer is Running Windows XP

After the power is turned on, the PC automatically recognizes the imager, and the software prompts you to install the drivers. The screen below appears:



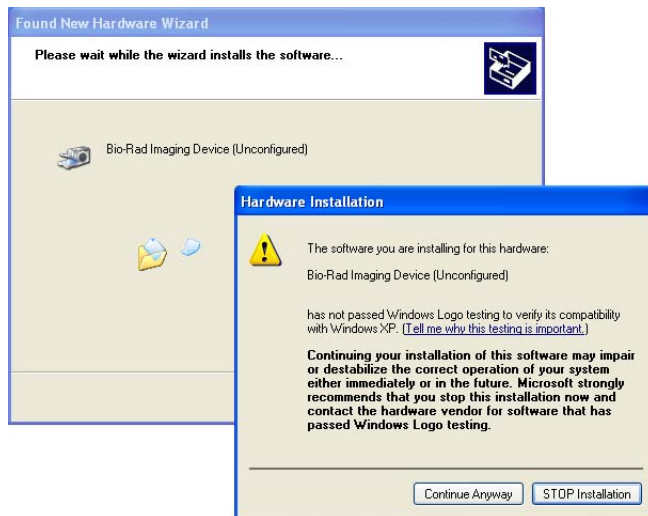
5. Select No, not this time, and then select Next.

The following screen appears:



6. Install the software automatically is selected by default. Click Next.

The following screen appears:



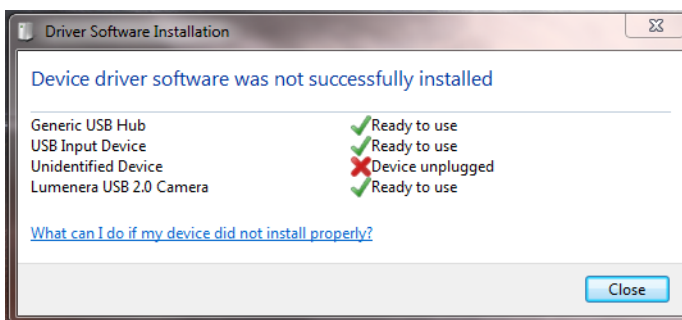
7. Click Continue Anyway. Windows installs the driver.

- Click Finish when it appears. A message appears briefly in the lower right corner of the screen: Your new hardware is ready to use.

Note: Windows XP may prompt you to go through the driver installation a second time. If this happens, repeat steps 5 through 8 until you receive the message “Your new hardware is ready to use.”

WINDOWS 7

During the installation process the following warning may appear. This warning can be ignored because it appears even when the driver has been installed correctly.



Configuring the Imager

To use the imager with Image Lab software, the imager must first be configured. Imager configuration consists of initializing the sample trays and collecting the dark image.

The imager is configured in the setup wizard, which appears the first time the imager is connected to a computer.

To configure the imager:

- Turn on the imager and start Image Lab software. The setup wizard appears.
- Complete the steps in the wizard, which are described next.

Note: You must go all the way through the wizard or the imager will not be usable.

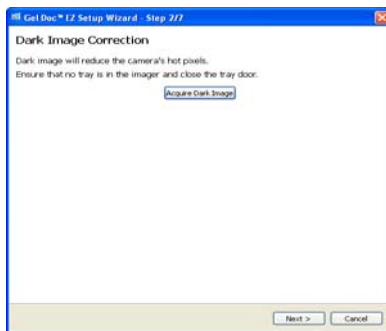
The first page of the setup wizard indicates that a new instrument has been found on the system and displays the instrument's serial number.

1. Click Next.



The Dark Image Correction page appears. Correcting the dark image reduces dark current noise generated from the charge-coupled device (CCD). Dark current noise is typical of all CCDs and is a result of the accumulation of charge in the absence of light.

2. If a tray is in the imager, remove it and close the tray door.
3. Click Acquire Dark Image.

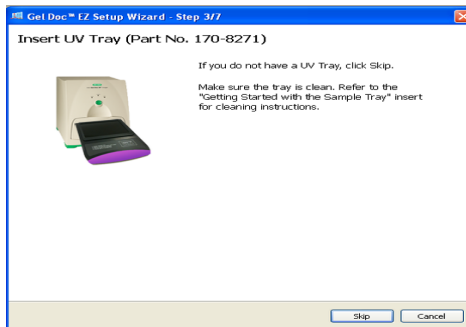


A progress indicator reports the acquisition of the dark image.

When the dark image has been acquired, the wizard prompts you to initialize the UV tray.

4. If you do not have a UV tray, click Skip and go to step 7.
5. Make sure the UV tray is clean. Then insert the tray into the imager and close the door.

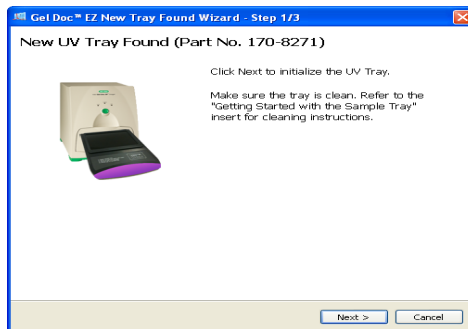
A progress indicator reports the initialization of the tray.



Connecting the Imager to the Computer

When the tray has been initialized, the next page confirms that the tray has been found and initialized and lists the applications you can run with this tray.

6. Remove the UV tray from the imager. Then click Next.

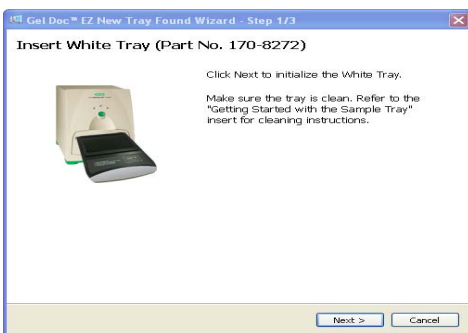


The wizard prompts you to initialize the white tray.

7. If you do not have a white tray, click Skip and go to step 10.

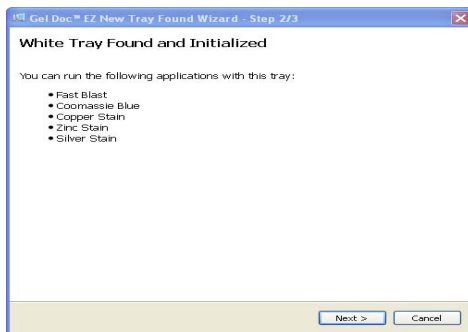
8. Make sure the white tray is clean. Then insert the tray into the imager and close the door.

A progress indicator reports the initialization of the tray.



When the tray has been initialized, the next page confirms this and lists the applications you can run with the tray.

9. Remove the white tray from the imager. Then click Next.



10. The wizard prompts you to initialize the blue tray. If you do not have a blue tray, click Skip and go to step 13.

11. Make sure the blue tray is clean. Then insert the tray into the imager and close the door.

A progress indicator reports the initialization of the tray.

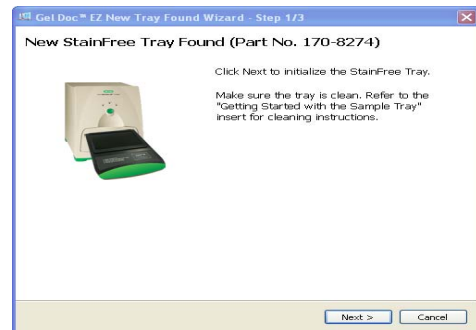
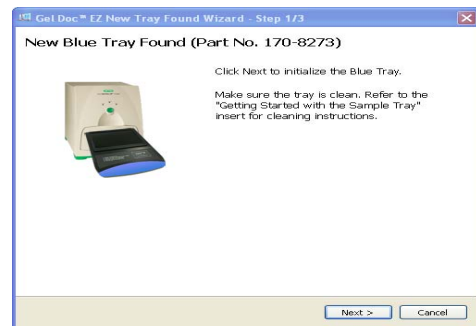
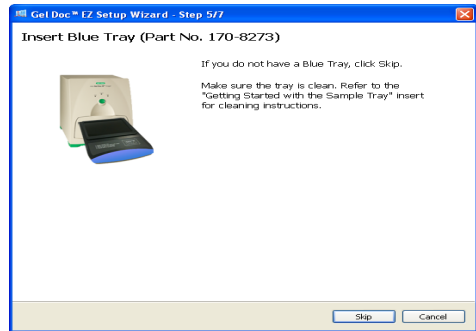
When the tray has been initialized, the next page confirms this and lists the applications you can run with the tray.

12. Remove the blue tray from the imager. Then click Next.

13. The wizard prompts you to initialize the stain-free tray. If you do not have a stain-free tray, click Skip and go to step 16.

14. Make sure the stain-free tray is clean. Then insert the tray into the imager and close the door.

A progress indicator reports the initialization of the tray.



Connecting the Imager to the Computer

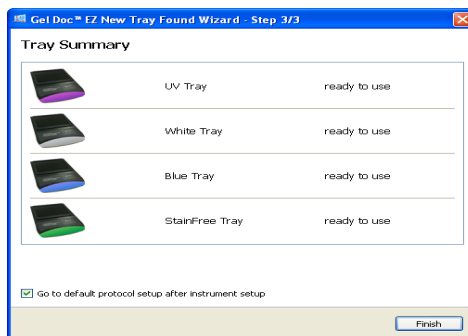
When the tray has been initialized, the next page confirms this and lists the applications you can run with the tray.

15. Click Next.



A tray summary page lists all the trays and their status. Trays you initialized appear as ready to use.

16. Click Finish to exit the wizard.



See Chapter 4, Acquiring Images to configure the default protocols that run when the green button on the imager is pressed.

Initializing an Additional Tray

After you configure the imager, you can add another tray to the system when you need it.

To initialize an additional tray:

1. Insert a new type of tray in the imager. The New Tray Found wizard opens.
2. Complete the steps in the wizard.

The wizard identifies the new tray and asks whether you want to initialize it.

1. Click Next.

The wizard begins initializing the tray. A progress indicator reports the initialization of the tray.



When the tray has been initialized, the wizard displays a list of the applications you can run using the tray.

2. Click Next.



The wizard displays the Tray Summary, which shows the highlighted, initialized new tray.

3. Do one of the following:

Click Finish to leave the wizard and display the Default Protocol Setup window, where you can start creating a default protocol for the new tray.

Or

Clear the checkbox at the bottom of the wizard page to skip setting up a default protocol. Then click Finish to leave the wizard.



3 Image Lab Software Overview

Image Lab image acquisition and analysis software works with the Gel Doc™ EZ imager, stain free gels, and other gels that are not stain-free to create a reproducible, automated, and time-saving workflow for imaging and analyzing gels.

In Image Lab software, a protocol is any combination of imaging, analysis, and report settings that has been saved to run as a single workflow. Researchers can run one protocol repeatedly or easily design a wide range of protocols.

With Image Lab software you can view analyzed data, edit the analysis, and produce customized reports that show the precise settings that were applied to ensure repeatable results.

Two types of files are generated by Image Lab software:

- Protocol files describe the parameters for imaging and analyzing gel images. Protocol files are saved with a .ptf extension
- Image files contain the imaged gel, annotations, and analysis performed on the gel. Image files are saved with an .scn extension

An imaged gel, run according to a protocol file, generates an image file.

Imaging Process Overview

You can image your gel on the Gel Doc EZ imager by running a default protocol or a custom protocol. You can set up default protocols for the gels, applications, and analyses you are likely to run repeatedly. Then you can access them with a touch of a button on the front of the imager. For gels and applications you run less frequently, you can set up custom protocols that you can call and run with a software command. Both default and custom protocols can be saved and reused.

Default Protocols

Image Lab software supports four default protocols, one for each type of available sample tray. First, set up a default protocol for the kind of application you want to run. Then, run the default protocol, which activates and images the gel with one touch of a button. See *Setting Up a Default Protocol*, on page 33 for detailed instructions.

To run a default protocol:

1. Open the imager door and remove the sample tray.
2. Place your gel on the sample tray and insert it into the imager until the magnet grabs the tray.
3. Close the door.
4. Press the green Run button on the front panel of the imager. The default protocol runs automatically.

Run button

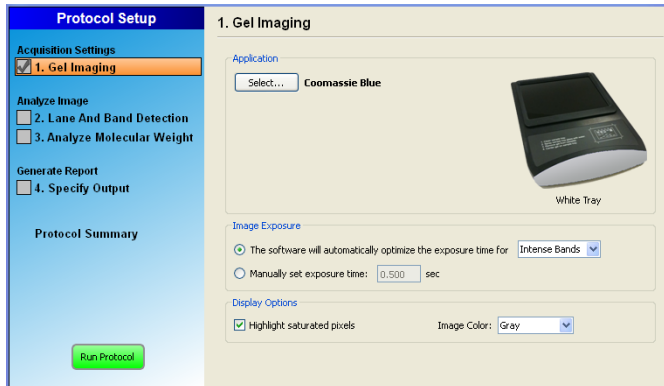


Custom Protocols

You can set up additional custom protocols based on your exact requirements. Setting up a custom protocol is similar to setting up a default protocol.

To run a custom protocol:

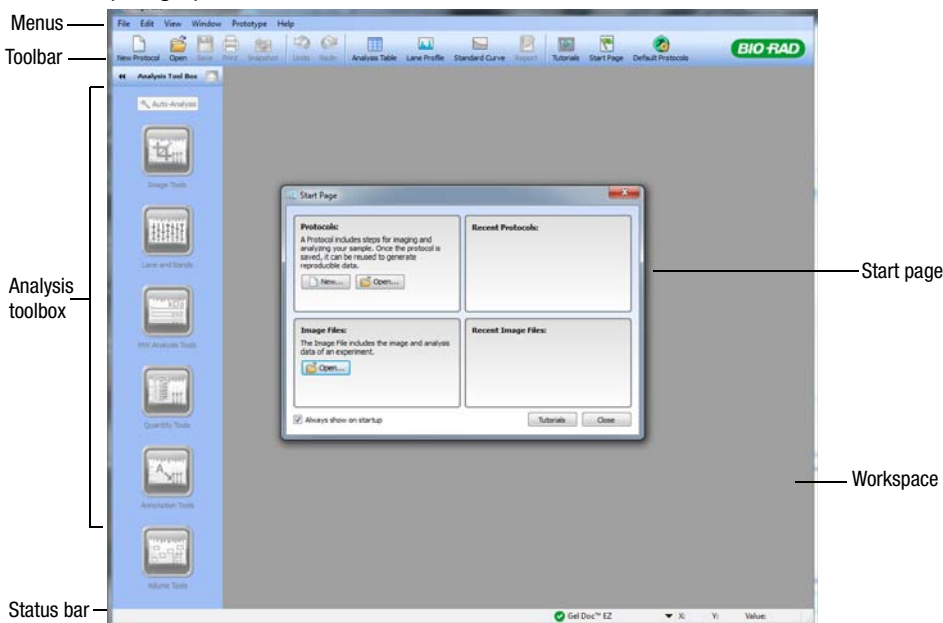
1. Open the imager door and remove the sample tray.
2. Place the gel on the sample tray and insert it into the imager until the magnet grabs the tray.
3. Close the door.
4. Click New in the Protocols box on the Image Lab Start Page, or click New Protocol in the toolbar.
5. Select the parameters for your protocol. See Running a Custom Protocol, on page 47 for detailed information.
6. Click the green Run Protocol button in the left pane of the Protocol Setup window to run the protocol.



Using the protocol you specified, the software controls the imager to activate the sample, analyze the image, and then generate and print a report.

Interface Overview

The following screen shows the Image Lab software main window. The paragraphs below describe the main software elements.



Main Window

Image Lab software displays a single main window. All image and protocol windows that present choices open in the workspace, which is the gray area of the main window.

If many screens are open in the workspace, you can make one visible by clicking the title bar at the top of the selected screen. A list of open protocols and image files also appears in the Window menu; you can select one to bring it to the top.

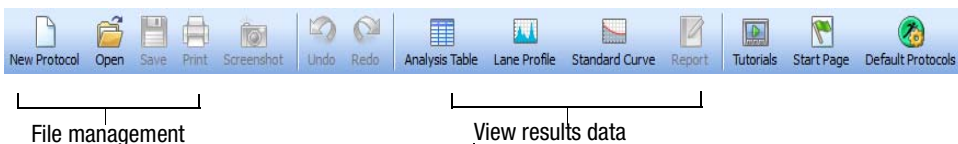
You can view the complete analysis for an image or protocol at one time or compare image results by arranging the screens in the workspace.

Toolbar

Many Image Lab software tools can be selected by clicking toolbar icons. The Screenshot tool enables you to send a screen capture of your image to the clipboard or to save it as a file. You can view demonstrations of various functions by clicking Tutorials. The unlimited Undo and Redo buttons enable you to correct missteps easily.

Clicking the green Default Protocols button at the right end of the toolbar displays the Default Protocol window for setting up, viewing, and editing default protocols.

Additional tools for viewing protocols and results data presented in various forms are labeled below. These tools are described in detail in Chapter 5, Viewing Images, on page 49.



Results Data

Results data associated with gel images can be viewed as an analysis table, a lane profile, a standard curve, or in a report. These views always display the analysis for the selected image.

Buttons to toggle these views on and off are located in the main toolbar. All of them can be viewed at once. See Displaying Data, on page 56 for details.

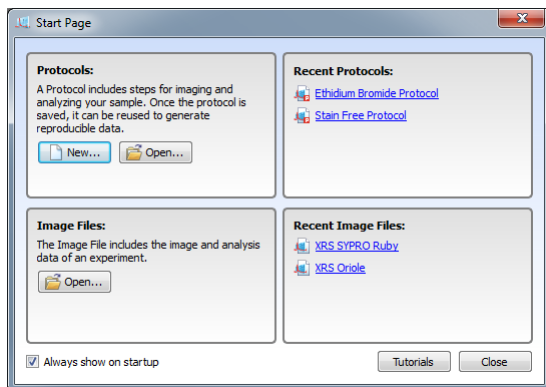
Display Toolbox

The Display Toolbox near the top of every image enables you to display your images in the most useful ways. See *Displaying Gel Images*, on page 50 for details.



Start Page

The Start Page guides you through creating, opening, and viewing protocols and images.



Analysis Tool Box

The Auto Analysis button quickly analyzes images. The remaining tools customize the analyzed data.

Note: An image file must be selected (on a Windows computer, the title bar is dark blue) to make analysis tools available.



Image Tools enable you to flip, rotate, and crop images, and to transform the image files.

Lane and Band controls the detection function, enabling you to resize, adjust, and bend lanes, and to detect, adjust, add, or delete bands.

MW (Molecular Weight) Analysis Tools enable you to choose standard samples, assign standard lanes, and choose a regression method.

Quantity Tools enable you to automatically quantitate bands using either relative or absolute values.

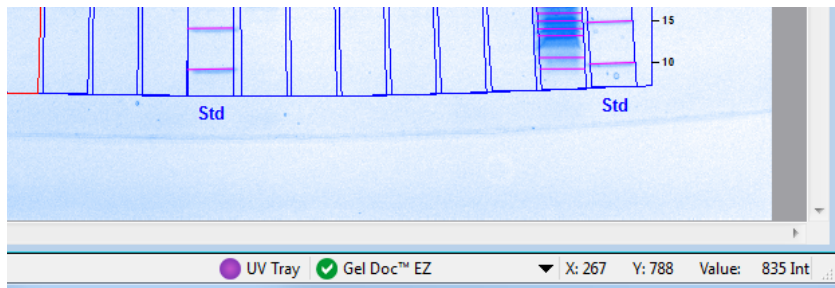
Annotation Tools are useful for drawing attention to any area of a gel of interest.

Volume Tools enable you to manually quantify an object inside a boundary that you define.

Analysis tools are described further in , on page 63.

Status Bar

The status bar in the lower right corner of the main window shows the tray and imager in use and the X and Y values for the cursor position on an image file.



If a default protocol has been set up for the tray in use, its name also appears in the status bar next to the tray name. The Int (intensity) value shows a numerical value for the image intensity at the mouse position. The maximum data range using the Gel Doc EZ imager is 0–4,095, but the ranges vary depending on the values contained within each image.

Menu Commands

The following section describes all menu commands in the File, Edit, View, Window, and Help menus. Many commands are also available on the toolbar or the Start Page.

File Menu Commands

New Protocol enables you to create a new protocol that contains the necessary steps and choices. See Chapter 4, Acquiring Images, on page 31, for detailed instructions. Protocols can also be altered and stored for reuse.

Open browses your file system to retrieve a previously saved protocol file or image file.

Recent Images gives you the choice of opening a recent image file.

Recent Protocols gives you the choice of opening a recent protocol.

Save enables you to save a protocol or image file once the protocol or image is named.

Save As enables you to name and store a protocol or image. Protocols are stored with a .ptl file extension. Image files are stored with an .scn extension.

Close closes the active screen.

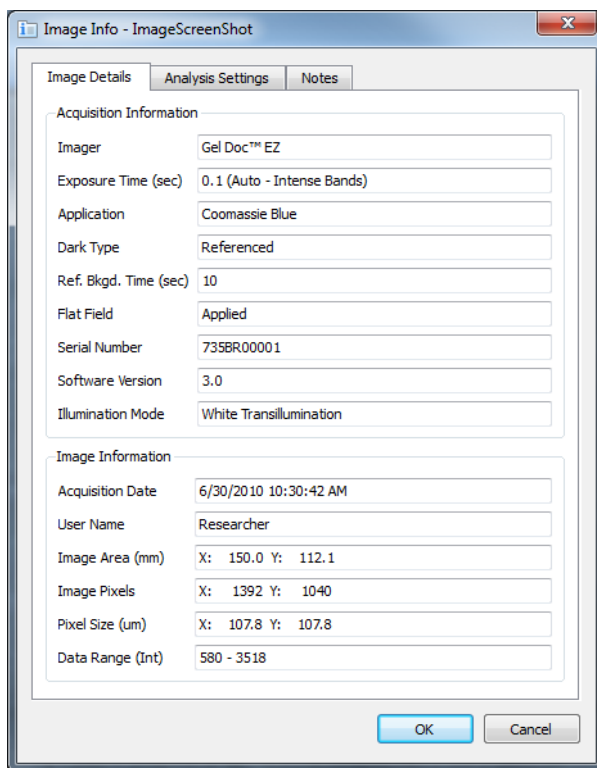
Close All closes all the screens.

Export enables you to export gel images or analysis tables with the following options:

- **Export for Publication**—exports a displayed image to a file. You can select from .bmp, .png, .jpg, and TIFF formats. The gel displays with any lanes, bands, and annotations that appear on the screen. See Exporting Gel Images for Publication, on page 90 for more information
- **Export for Analysis**—creates a TIFF formatted file that retains all gel image data. Analysis data are not included. Use this option to analyze the image in other software such as Quantity One[®], FPQuest[™], or InfoQuest[™]FP. See Exporting Gel Images for Analysis, on page 91 for more information
- **Export for PulseNet**—reduces the image to an 8-bit TIFF image file. Resolution is limited and file size is restricted to 300 dpi
- **Lane and Band Table to Excel**—if Excel (or Numbers, on a Mac) is installed on your computer, it launches with your lane and band table displayed as a spreadsheet
- **Lane and Band Table to File**—exports as a CSV (comma-separated values) file so your lane and band table can be opened in a database application
- **Volume Table to Excel**—if Excel (or Numbers on a Mac) is installed on your computer, it launches with your volume table displayed as a spreadsheet
- **Volume Table to File**—exports as a CSV (comma-separated values) file so your volume table can be opened in a database application

See Chapter 8, Exporting Results, on page 89 for detailed information about exporting files.

Image Info displays information about individual gel and blot images such as acquisition date, data range, and image capture details such as the exposure time and illumination source used in three tabs (Image Details, Analysis Settings, and Notes).



Page Setup contains print controls such as orientation (landscape or portrait), margins, printer used, and paper size.

Print displays a print preview of the gel and the header information, which includes the filename of the image, the user's name, and date and time it was printed. The usual Windows Print screen is available as well; it enables you to select a printer and the number of copies.

Exit closes Image Lab software (after asking you to save changes to your protocols or images).

Edit Menu Commands

Undo undoes your last action.

Redo restores your last action after an Undo.

Screenshot enables you to take a screen capture of the Lane Profile Window, the Standard Curve Window, or the default choice, Current Image View. This capture can include the name of the image, and it can be placed on the clipboard or saved in a file.

Default Imager enables those who own two or more different model imagers to switch among them.

Instrument Setup enables you to review the instrument name and serial number, camera serial number, firmware version, and the flat field version. Available sample trays are shown with the names of associated default protocols. Dark Image time and date are also shown. You can reset the dark image. You can reset the flat field only for the white sample tray.

Report Settings enable you to configure reports. This dialog box displays three tabs. All the checkboxes are selected by default; clear the boxes for information you do not want to include in reports. Your selections apply to all reports until you change them again.

- The General tab has options for excluding or reporting information about the gel image
- The Lane and Band Table tab enables the researcher to choose whether to include all or selected lanes with appropriate identifiers. Lane profiles can also be included
- The Volume Table tab enables you to choose appropriate identifiers for the volume table and provides the option of excluding the table from reports

Preferences displays two tabs.

- The Protocol tab shows presets for naming your image files. You can choose to include a designated Prefix, User Name, Date and/ or Time in the name of your image files
- The Colors tab enables you to choose colors for the graphic elements in your gels such as Lane Frame, Lane, Band, Band Attribute, and MW Legend. This functionality ensures that these elements are visible, regardless of the color of the gels

View Menu Commands

Image Overview opens a small window that shows the entire gel image with a red rectangle outlining the area visible in the larger main window. This is useful when you zoom into a small section of your image.

Image Transform opens a window showing a histogram you can use adjust the light and dark values of your gel image. This adjustment does not change your data, only the way the data display on your monitor.

Operations History opens a window showing the sequence of actions performed.

Window Menu Commands

The Window menu controls enable you to show and hide multiple open image files in your workspace. A list of all currently open images and protocols displays in this menu.

Tile aligns all open image files so all are visible at once.

Tile Horizontal places all open image files from top to bottom.

Tile Vertical places all open image files from left to right.

Cascade stacks all open image files and protocols with overlapping title bars so that each one can be easily chosen for view.

Imitate Zoom changes the zoom setting of all open images to the same zoom setting as the current image file.

Imitate Transform changes the brightness and contrast of all open images to the same transform settings as the current image file.

Next cycles through all open image files from oldest to newest.

Previous cycles through all open image files from newest to oldest.

Help Menu Commands

Image Lab Help displays the help system.

User Guide displays this instruction manual in .pdf form.

About displays Image Lab software version information and release date.

4 Acquiring Images

Acquiring an image requires a set of steps in a workflow called a protocol. In Image Lab™ software, a protocol can include any combination of settings for imaging, analyzing, and reporting that has been saved to run as a single workflow.

Users can link a protocol to a tray type by default. Such a default protocol is associated with:

- A specific type of sample tray
- The green Run button on the front of the Gel Doc™ EZ imager
- The user's computer system login ID

The advantage of a default protocol is that it can be run with a single press of the green Run button on the imager. Custom protocols are run by clicking a software button in Image Lab software. In all other respects, default protocols and custom protocols are similar.

Typically, a default protocol is set up for the applications you run most often with a particular tray. Each user can set up four default protocols (one for each type of sample tray) to run applications. A custom protocol can be set up for less frequently run applications to capture the setup steps for later use.

Once set up in Image Lab software, a default protocol is run by placing a gel in a sample tray, inserting the sample tray, and pressing the green Run button on the front of the imager. The imager automatically runs the default protocol assigned to the type of sample tray you inserted.

If you have only one sample tray, it will automatically be considered the default sample tray. For example, suppose you have only a blue tray. Once the blue tray has been associated with the imager, Image Lab software will list all applications that can run on the blue sample tray.

Selecting a Sample Tray

The following tables list preset applications and the appropriate sample tray to use with each. Refer to these tables to select a sample tray for your application. If your application is not listed here and you are unsure of the appropriate sample tray to use for your application, contact Bio-Rad Technical Support for assistance.

Applications for Nucleic Acid Gels

Detection Reagent	Primary Tray	Alternate Tray
Ethidium bromide	UV	None
SYBR® Green	Blue	UV
SYBR® Safe	Blue	UV
SYBR® Gold	Blue	UV
GelGreen	Blue	UV
GelRed	UV	None
Fast Blast™ DNA stain	White	None

Applications for Protein Gels

Detection Reagent	Tray
Stain-free gels	Stain-free
Stain-free blots	Stain-free
Coomassie Blue stain	White
Copper stain	White
Zinc stain*	White
Flamingo™ fluorescent gel stain	UV
Oriole™ fluorescent gel stain	UV
Silver stain	White
Coomassie Fluor Orange	UV
SYPRO Ruby	UV
Krypton	UV

*Recommended for imaging only.

Setting Up a Default Protocol

Setting up a protocol consists of three major processes:

- Acquisition settings
- Analyze image
- Report settings

Each process comprises a set of steps. The appropriate options appear as each protocol step is selected.

Important: You must configure Acquisition Settings (steps 1 through 6 in the following procedure) to run an application. However, it is not necessary to configure Analyze Image and Report Settings unless you want to calculate the molecular weight automatically and generate a report.

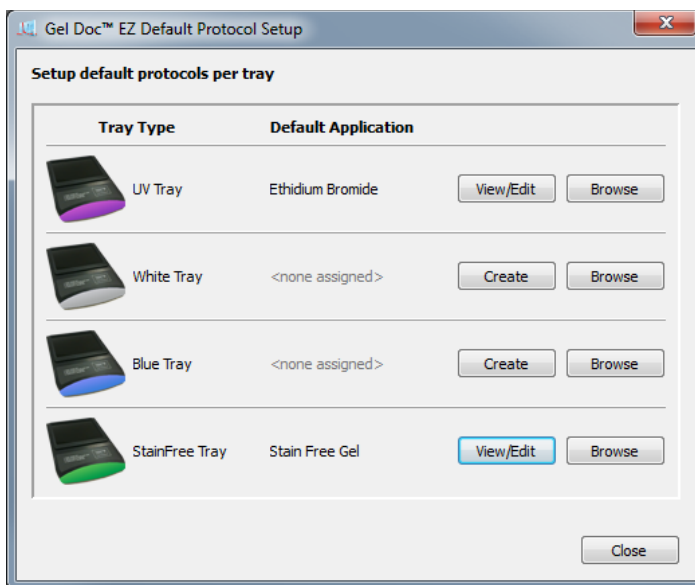
To set up a default protocol:

1. Click the Default Protocols button in the toolbar to set up a default protocol.

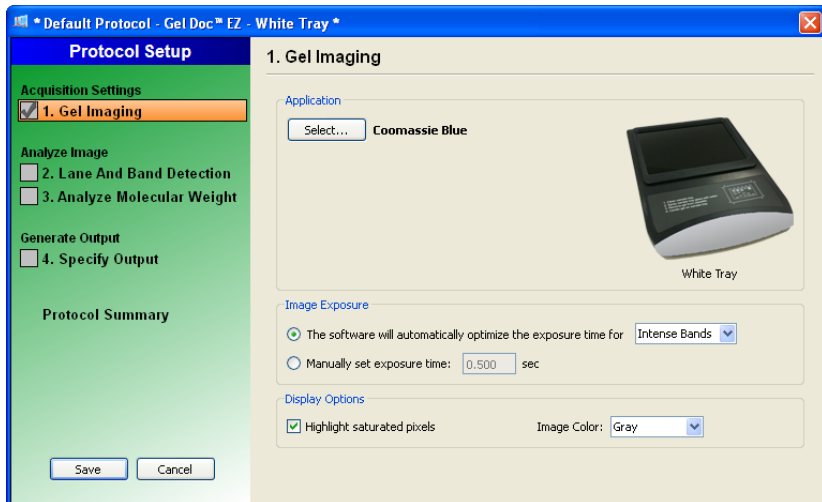


Caution: If the imager is not connected to your computer, a message appears asking whether you want to run a simulation of the imager. Click No. Turn on the imager. Do not set up a protocol in simulation mode. Protocols set up in simulation mode cannot be run.

A Default Protocol Setup window displays each sample tray type as well as buttons that enable you to create a protocol or browse an existing protocol. Trays that have not been initialized appear dimmed. Note that once you create a default protocol, its Create button changes to View/Edit so you can review or change the protocol settings.



2. Click Create next to the sample tray type for which you want to create a protocol. The first Protocol Setup window appears. The title bar identifies the protocol as a default protocol. The imager type and sample tray type are also shown.



The three major processes are listed in the left pane. Numbered major steps in each process appear under these headings. To select options under a protocol step, select the accompanying checkbox. Options appear in the right side of the window. To disable any step, clear its checkbox.

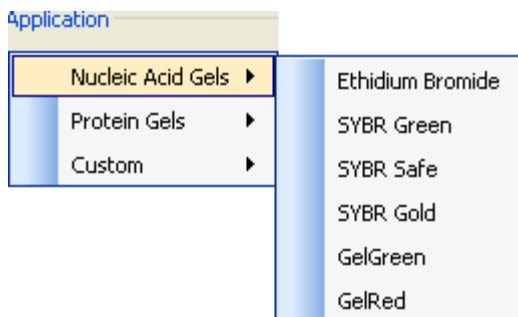
Acquisition Settings

STEP 1. GEL IMAGING

The Gel Imaging checkbox is already selected. The right side of the Protocol Setup window displays Gel Imaging options.

APPLICATION OPTIONS

3. **Select an application:** Click Select and choose an application from the drop-down menus.

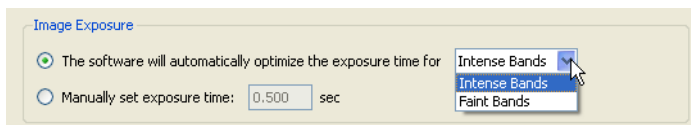


The menu list only applications appropriate for the selected sample tray. When you choose an application, any required filter or illumination source displays in the Protocol Setup window.

Note: Custom applications are available only for custom protocols. See Setting Up a Custom Protocol, on page 45 for more information about custom applications.

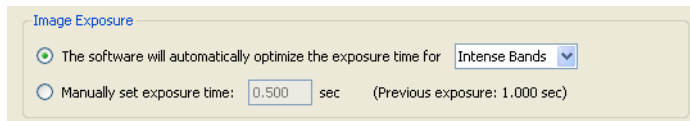
4. **Set the image exposure**

If you select the first image exposure option, Image Lab software acquires an image using the optimal exposure time. This option ensures the best use of the dynamic range. You can automatically optimize for intense or faint bands.



- If you choose Intense Bands from the drop-down menu, exposure is optimized for all bands
- Choosing Faint Bands from the drop-down menu results in a longer exposure where faint bands are more visible, but more prominent bands may be overexposed
- Select manual exposure to override automated imaging. Exposure time can range from 0.001 to 10 sec

After a gel is imaged with automatic exposure optimization, the used exposure time displays in the protocol as a guide so you can manually adjust the exposure time if necessary.



You can also view the exposure time of the image later in the Image Info window (refer to Image Info, on page 54).

5. Set the Display Options:

Highlight saturated pixels—Select the Highlight saturated pixels checkbox to see any saturated pixels in red, which shows how much of the gel image is saturated. Saturated pixels are beyond the maximum quantifiable range of the imaging system. This option can be changed later by selecting View > Image Transform.

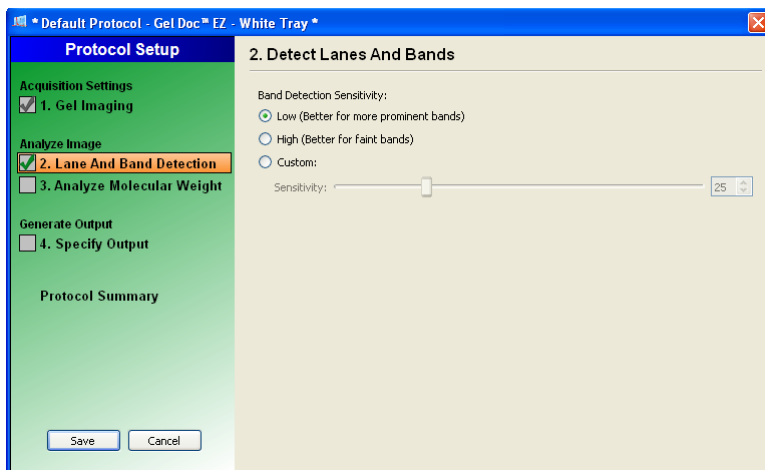
Image Color—Select color choice to display the sample image. Viewing the image with a different color scheme can make it easier to see all of the elements. See Image Colors, on page 53 for the color choices.

Analyze Image

STEP 2. DETECT LANES AND BANDS

To analyze the gel automatically, Image Lab software must detect lanes and bands on the image. Lanes are detected automatically, and then the background is automatically subtracted. Refer to Lane and Band Tools on page 68 for details. You can customize band detection with the following options.

6. Select the Lane and Band Detection checkbox in the left pane of the Protocol Setup window. The Protocol Setup window displays lane and band detection options.



Low Band Detection Sensitivity— This option sets detection at a low level for images with prominent bands. Faint bands are not detected.

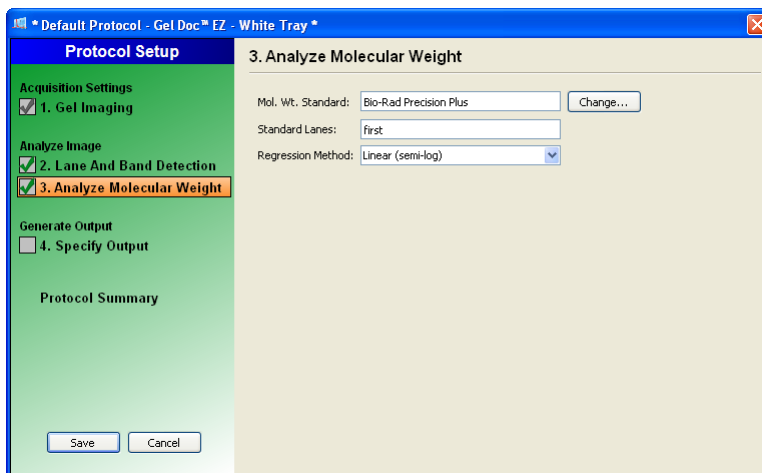
High Band Detection Sensitivity— This option sets detection at a higher level for images that are faint. Extraneous bands can be removed later using the Band Tools in the Analysis Tool Box. See Lane and Band Tools, on page 68.

Custom— Select a numerical value between 1 and 100 to choose the best detection sensitivity for your sample.

Note: When low or high band detection sensitivity is used, these numerical values are set: low sensitivity = 25; high sensitivity = 75.

STEP 3. ANALYZE MOLECULAR WEIGHT

7. To analyze molecular weight automatically, select Analyze Molecular Weight in the left pane of the Protocol Setup window. When this checkbox is selected, the software calculates the molecular weight for each band based on the specified standard.
8. To estimate the size of the molecules in the bands of your gel, enter the standards you are using and specify the lanes in which the standards are placed.



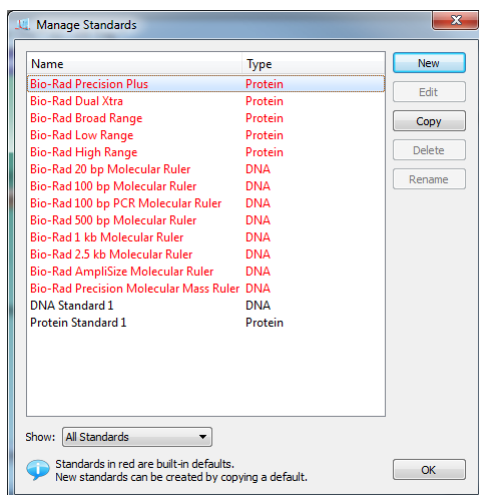
STANDARDS

Determining molecular weight depends upon selection of the proper protein standards. Many protein standards are available from Bio-Rad, including:

- Precision Plus Protein™ standard
- Prestained SDS-PAGE standards, broad range
- Prestained SDS-PAGE standards, low range
- Prestained SDS-PAGE standards, high range

Bio-Rad standards cannot be edited. However, these standards can be copied, and the copies can be renamed and edited.

The Change button opens the Manage Standards dialog box in which you can add third-party standard samples and edit your list.



STANDARD LANES

9. Choose the lane that contains your standards by typing lane numbers or the words First and Last in the Standard Lanes field. The format is xx, xx, ... where xx is the lane number. For example, if you run an 18-well gel and want your standards in lanes 1, 10, and 18, enter First, 10, and Last.

Note: Lane detection works best when standards are placed in the first and last lanes.

REGRESSION METHODS

A regression method is used to calculate the molecular weight of the unknown bands. The software uses the relative front and molecular weight values of the standard bands to calculate the standard curve. The standard curve is then used to calculate the values of the unknown bands. The shape of the standard curve is based on the selected regression method. Choose one of four regression methods.

The methods and the minimum number of standard bands required for each type are shown in the following table.

Regression Method	Minimum Number of Standard Bands
Linear (semilog)	2
Point-to-point (semilog)	2
Logistic	5
Cubic spline	5

If you do not have enough data points for the selected method, the molecular weight of the unknown bands is not calculated.

10. Select the appropriate method for the gel type:

- **Gradient gels:** The linear (semilog) regression method works well for these gels because the mobility of the bands is linear to the log of their molecular weight. As an alternative, the point-to-point (semilog) method can be used if the R^2 value is not sufficient
- **Fixed percentage gels:** These gels have a nonlinear relationship between the mobility and the molecular weight. For these gels, choose the logistic or cubic spline regression method

You can check how well each regression method fits the data in the standard curve window (for more information, see Standard Curve, on page 60). The linear (semilog) regression method provides a measurement that describes how well the standard curve fits the data R^2 value. The closer the R^2 value is to 1.0, the better the data fit the standard curve.

The molecular weight of each band is displayed in the Analysis Table in the Mol. Wt. column. Refer to page 72 for more information about molecular weight.

For information about the calculations behind the regression methods, see Regression Calculation Methods.

Output Settings

Image Lab software prints to the default printer, unless you select otherwise.

You have three choices for specifying output:

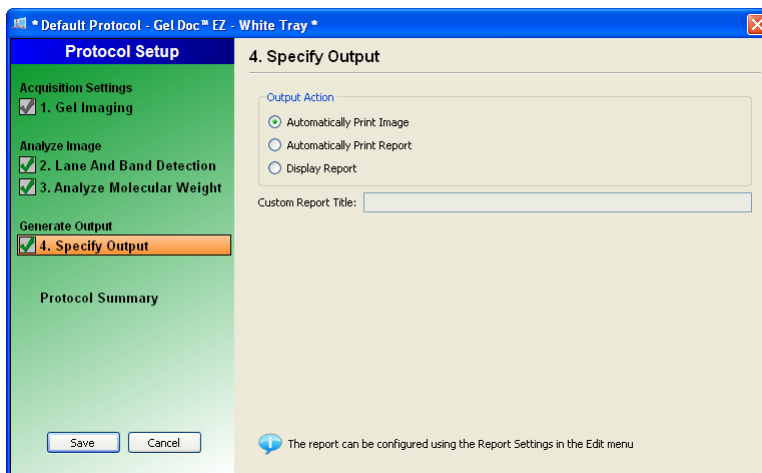
- You can automatically print the image; this is useful for gel documentation, especially if a thermal printer is connected. See Mitsubishi P93/P95 Thermal Printer Settings for instructions on setting up the Mitsubishi P93/P95D thermal printer
- You can automatically print the report, including all accompanying analysis information

Note: You cannot print a report on a thermal printer.

- You can display the image on your compute

STEP 4. SPECIFY OUTPUT

11. Select Specify Output in the left pane of the Protocol Setup window to display output options.



12. In the right pane, you can choose whether to automatically display or print an image or a report after a protocol runs.

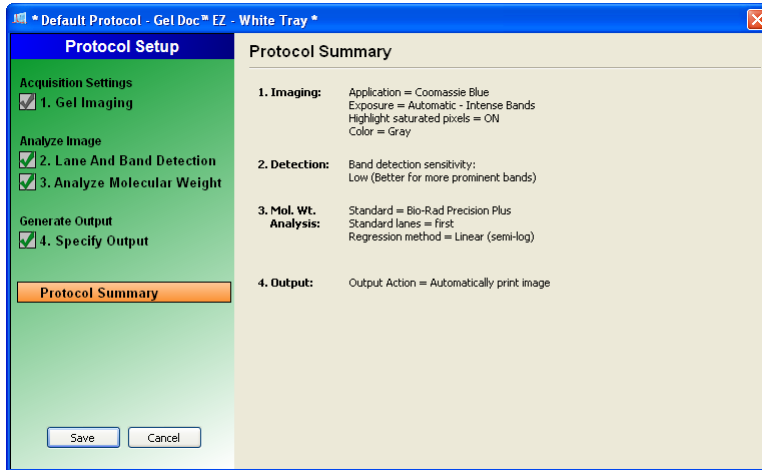
The report can be customized with the options in Edit > Report Settings.

For more information about reporting options, see Generating Reports, on page 85.

Review Protocol Settings

PROTOCOL SUMMARY

13. Click Protocol Summary in the left pane of any Protocol Setup window to see a quick review of all protocol settings.



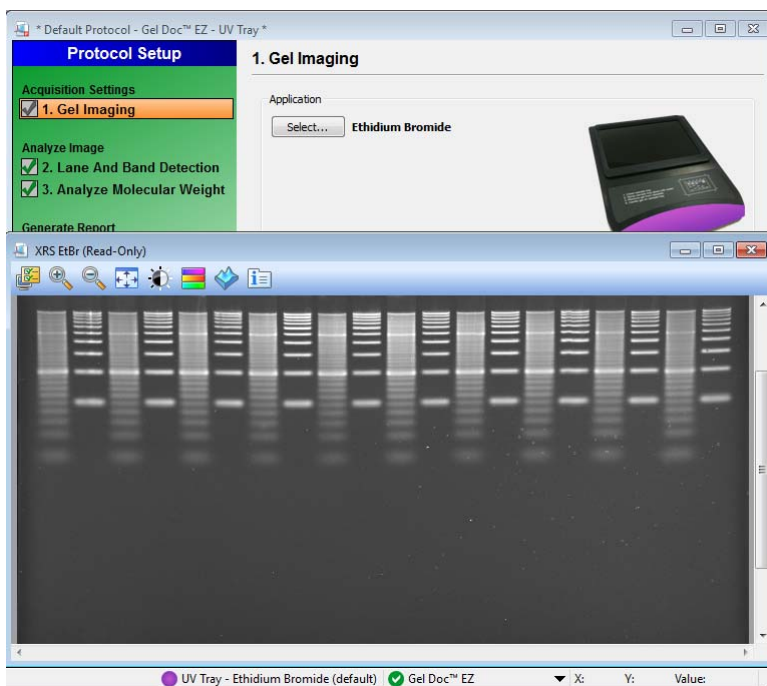
14. Click Save in the left pane to save the default protocol.

Running a Default Protocol

To run a default protocol:

1. Place a gel on the appropriate sample tray and insert the tray into the imager until the magnet grabs the tray.
2. Close the door.
3. Press the green Run button on the front of the imager. The default protocol runs automatically.

The default protocol appears with a green background. After the protocol runs, the resulting image pops up and the protocol window remains open behind it. The application name appears in the status bar below the gel preview window.

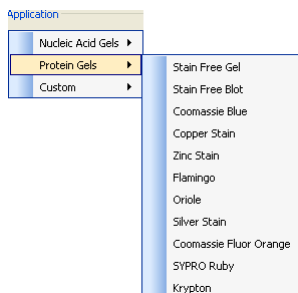


Setting Up a Custom Protocol

Creating a custom protocol is similar to creating a default protocol.

To create a custom protocol:

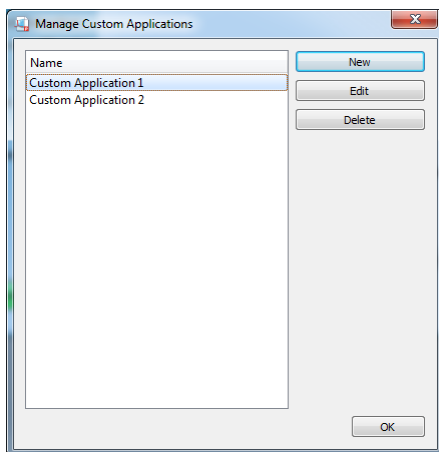
1. Choose New in the Protocols box on the Start Page or click New Protocol in the toolbar. The Protocol Setup window appears.
2. Select an application from the Application drop-down menus.



The menu list all predefined applications you can run on the Gel Doc EZ imager.

Note: If you want to image a gel using a detection reagent not listed in the menus and do not find an equivalent reagent in the menus, contact Bio-Rad Technical Support for protocol recommendations.

Choose Custom to run an existing application with a new name or when you have an application unlike any existing applications. Selecting Custom > Manage Custom Applications in the Application menu displays the Manage Custom Applications dialog box. Custom applications you save are listed in this dialog box. An Edit button enables you to make changes to custom applications.

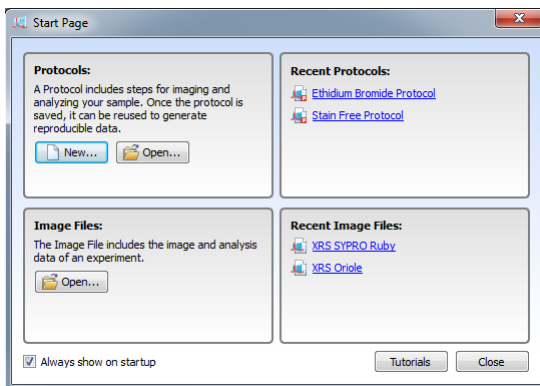


Note: You cannot create custom applications for the stain-free tray.

3. To create a custom application, click New. The Create Custom Applications dialog box appears.
4. Enter a unique application name.
5. The remainder of the setup instructions are the same for both default and custom profiles. Go to step 4, **Set the image exposure** in Setting Up a Default Protocol on page 34 and follow the instructions there.

Selecting a Custom Protocol

Use the same protocol repeatedly by clicking Open in the Protocols box on the Start Page or by clicking Open in the toolbar. You can also select recently used protocols or image files from the lists on the Start Page.



Running a Custom Protocol

To run a custom protocol:

1. Open a protocol or create a new one.
2. Place your gel on the appropriate sample tray and insert the tray into the imager until the magnet grabs the tray.
3. Close the door.
4. Click the green Run Protocol button in the left pane of the Protocol Setup window. The custom protocol runs automatically.

After the protocol runs, the resulting image pops up and the Protocol window remains open behind it. The application name appears at the bottom of the gel preview window.

Editing Protocols

Protocols can be changed or renamed using the many tools in Image Lab software.

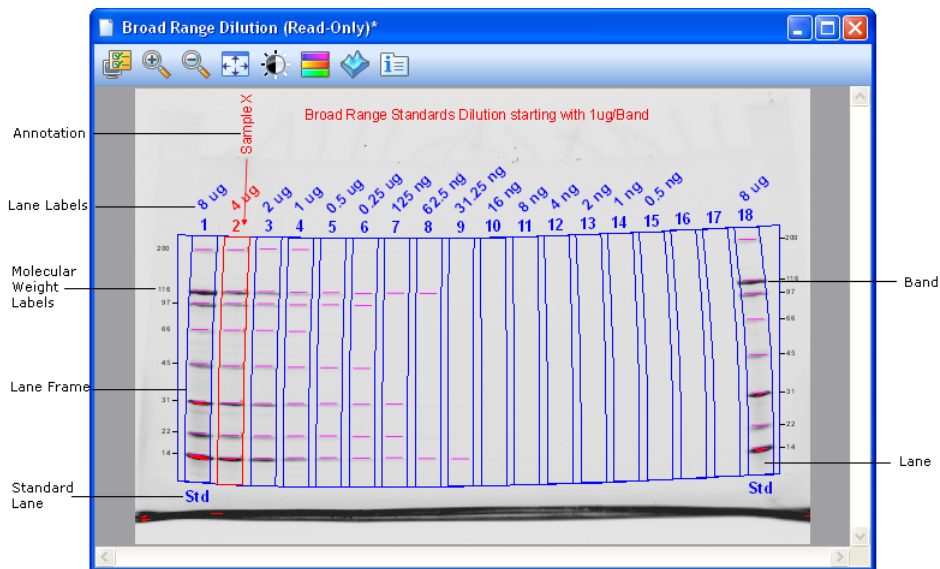
Open any saved protocol by choosing File > Open or by clicking Open in the Protocols box on the Start Page. You will be presented with the same set of dialog boxes and choices described in the section, Setting Up a Custom Protocol, on page 45.

Make and save your changes without renaming the protocol to replace an older one. Save the edited protocol with a different name to make a second protocol.

5 Viewing Images

Once a gel has been imaged, the image appears in the workspace. Many controls are available to optimize viewing and to analyze the image.

Results Overview



The screen shows a gel image with band and lane detection as well as annotations. The labels show overlays that you can show or hide.

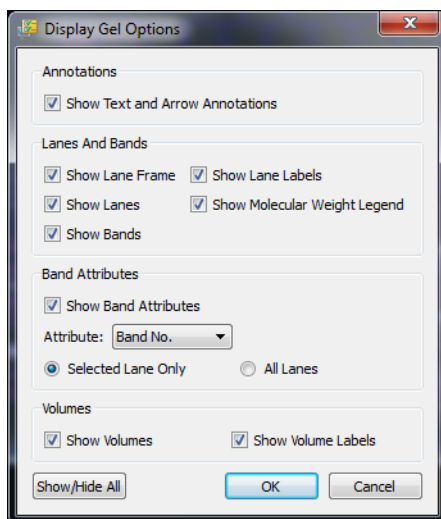
Refer to Display Gel Options on page 50 for more information.

There are many ways to view the data associated with the results. You can view data as an analysis table, a lane profile, a standard curve, and as a report.

Displaying Gel Images

Toolbar buttons appear above the gel image. Each of these tools is described in the following sections.

Display Gel Options



ANNOTATIONS

You can choose whether or not to show text and arrow annotations that have been drawn on the image.

LANES AND BANDS

You can turn on or off any image overlays, such as lane frames, lanes, bands, lane labels, and molecular weight legends.

BAND ATTRIBUTES

You can show the following attributes for selected lanes or for all lanes.

- Band number
- Band label
- Molecular weight
- Relative front
- Volume
- Abs. Quant.
- Rel. Quant.
- Band %
- Lane %

VOLUMES

If you have drawn volume boundaries on the gel, you can display the volume boundaries and their volume labels.

Zoom Tools



The zoom tools resize the gel image. click the magnifying glass with the plus to make the image larger; the minus to make the image smaller.

You can also zoom with the right mouse button. Click and drag to select an area you want to magnify. Right-click again to return to the original view.

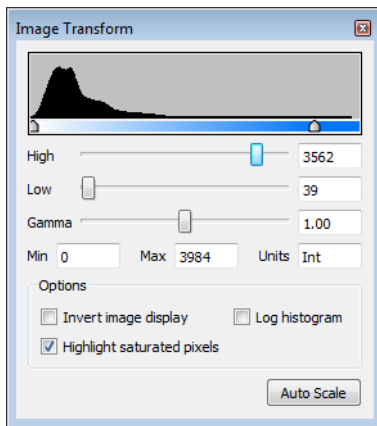
You can also resize the image with the scroll wheel on your mouse.

Fit in Window



When you zoom in on an area of an image, clicking this button brings the entire image into view.

Image Transform



Use the Image Transform dialog box to adjust image brightness and contrast, optimizing the image display so faint detail can be seen. The minimum to maximum range varies, depending on the light and dark values present in the image. These adjustments do not change the data; they change only the way the data are displayed. The human eye cannot see as great a range as the image contains.

The frequency distribution histogram shows the total data range in the image and the amount of data at each point in the range.

Auto Scale determines an optimal setting for the image automatically. The lightest part of the image is set to the minimum intensity, and the darkest is set to the maximum.

- The High progress indicator determines which intensity value is shown at the maximum grayscale (or other color) in the gel image
- The Low progress indicator determines which intensity value is shown at the minimum grayscale (or other color) in the gel image
- The Gamma progress indicator changes the grayscale curve. A value of 1 is linear. A value < 1 redistributes a greater proportion of the grayscale to the first half of the intensity values. A value > 1 redistributes a greater proportion of the grayscale to the second half of the intensity values

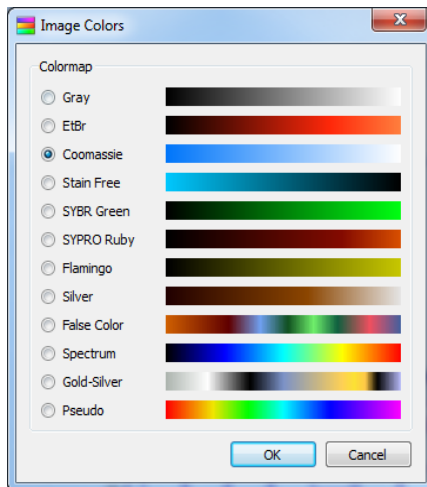
You can also type numerical values in the text boxes next to the progress indicators. Clicking anywhere on the progress indicator bars moves the progress indicator incrementally.

Options:

- **Invert image display**—Inverts dark bands on a light background to light bands on a dark background and vice versa

- **Highlight saturated pixels**—When this checkbox is selected, areas of the image with saturated signal intensity (above a measurable range) are highlighted in red
- **Linear or Logarithmic histogram**—This adjustment changes the y-axis on the histogram to display the number of pixels at each intensity value using either a linear or a logarithmic scale

Image Colors



You can choose a colormap for an image results file. Viewing the image with a different color scheme can make it easier to see all of the elements in the image, but it does not change the data.

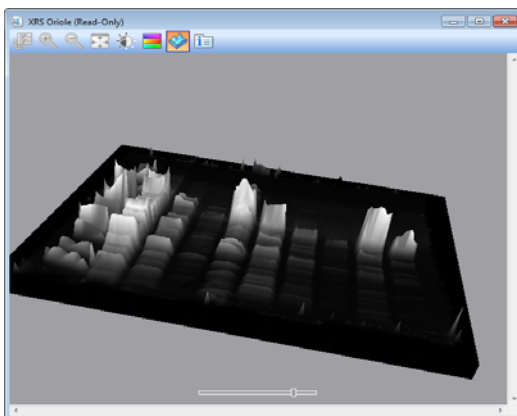
The first eight color choices imitate the colors of stained gels. The remaining choices supply enough color variation to highlight small differences in the image data.

Available colors include:

- Gray
- EtBr (Ethidium bromide)
- Coomassie
- Stain Free
- SYBR Green
- SYPRO Ruby
- Flamingo
- Silver

- False Color
- Spectrum
- Gold-Silver
- Pseudo

3-D Projection



Clicking 3-D transforms the gel image into a solid 3-dimensional model spinning in space with x, y, and z dimensions. Accentuate or diminish the relative heights of data points by pulling the slider at the bottom of the window to the right or left.

To view the intensity of various bands:

1. Click 3-D in the display toolbox above the image.
2. Click and drag the model to rotate it into your preferred view.
3. Bring the window into focus by clicking the image.
4. Press C to display a green cone, which can be dragged around to evaluate the intensity of various bands.

Image Info



The Image Info dialog box provides information about an active image. Click Image Info in the display toolbox to display three tabs of information about the image.

IMAGE DETAILS

Acquisition and image information appear in this tab.

ANALYSIS SETTINGS

The settings that were used when the gel was analyzed are displayed here, for example, Band Detection and Molecular Weight Analysis, if they were performed.

NOTES

You can add notes, make custom labels for each of the lanes, point out the types of samples used, and add any other information about the results.

The screenshot shows a dialog box titled "Image Info - ImageScreenshot" with three tabs: "Image Details", "Analysis Settings", and "Notes". The "Image Details" tab is active and contains two sections of input fields:

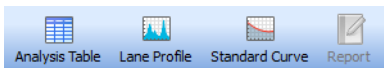
Acquisition Information	
Imager	Gel Doc™ EZ
Exposure Time (sec)	0.1 (Auto - Intense Bands)
Application	Coomassie Blue
Dark Type	Referenced
Ref. Bkgd. Time (sec)	10
Flat Field	Applied
Serial Number	735BR00001
Software Version	3.0
Illumination Mode	White Transillumination

Image Information	
Acquisition Date	6/30/2010 10:30:42 AM
User Name	Researcher
Image Area (mm)	X: 150.0 Y: 112.1
Image Pixels	X: 1392 Y: 1040
Pixel Size (um)	X: 107.8 Y: 107.8
Data Range (Int)	580 - 3518

At the bottom of the dialog box are "OK" and "Cancel" buttons.

Displaying Data

The results from analyzed data associated with the gel images can be visualized using an analysis table, a lane profile, a standard curve, or a report.



Buttons to toggle these views on and off are located in the main toolbar. All of the views can be seen at one time.

Analysis Table

Numerical data associated with an analysis can be viewed in the analysis table. Data from the Lane and Band analysis can be viewed in the Lane and Band tab. If volume analysis was performed, this data can be viewed in the Volume Table tab.

Clicking Analysis Table opens a tabular display of the data extracted from the results. The buttons above the table offer several ways of displaying and exporting analysis table data.

Lane 1									
Band No.	Band Label	Mol. Wt. (kDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %	
1		140.0	0.313	230,040	9.0	0.55	8.9	3.5	
2		96.9	0.403	243,120	9.5	0.58	9.4	3.7	
3		35.4	0.650	258,000	10.1	0.62	10.0	4.0	
4		22.1	0.766	1,623,240	63.7	3.91	63.1	24.9	
5		9.4	0.975	218,460	8.6	0.53	8.5	3.3	

Lane 2									
Band No.	Band Label	Mol. Wt. (kDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %	
1		134.7	0.323	318,600	12.5	0.77	16.9	9.9	
2		68.0	0.427	110,620	4.4	0.27	5.9	3.4	
3		49.6	0.573	313,260	12.3	0.75	16.6	9.7	

Volume Table

SET WINDOW SIZE

To change the size of your Analysis Table window, move to the top of the window until the cursor changes into a double-headed arrow. Left-click and drag the edge of the window until you can see all of the data.

Note: Resizing the Analysis Table window is restricted when a Protocol window is open.

DISPLAY DATA OPTIONS

This dialog box displays three tabs: Measurements, Display, and Export.

Measurements—Choose the measurements you want displayed by clicking the arrow buttons to move them from the Displayed to the Not Displayed field.

Default display settings—Move selected lane to top is on or off, depending on whether the checkbox is selected.

Per Measurement Precision—You can set the precision (decimal places) for the measurements in both the Lane and Band table and the Volume table.

Example Precision—This field shows how your measurement will display, with the number of decimal places you choose in Per Measurement Precision.

Export formatting—This field offers two checkboxes to include lane headers (Lane and Band table tab only) and/or column headers in the exported file.

Export delimiter—This field offers three delimiter options for the exported file.

- CSV (comma-separated values)
- Tab delimited
- Other user-defined delimiter

CHANGE ANALYSIS TABLE ORIENTATION

This button toggles between two table orientations.

Horizontal—Displays the lanes/volumes beside each other, so the user can scroll through the table from left to right.

Vertical—Displays the lanes/volumes on top of each other, so the user can scroll through the table from top to bottom.

COPY ANALYSIS TABLE TO THE CLIPBOARD

Click this button to copy the analysis table to the clipboard so you can paste the analysis table into word processing or presentation applications. It is best to use the vertical table orientation when copying to an 8-1/2 x 11-inch page, to allow the columns enough room to display.

EXPORT ANALYSIS TABLE TO A FILE

When exported as a CSV (comma-separated values) file, the data file can be opened in a database application.

EXPORT ANALYSIS TABLE TO A SPREADSHEET

You can use Excel's sorting and formula functions to manipulate data. If you have Excel (or Numbers, on a Mac) installed on your computer, it opens with your spreadsheet displayed.

Lane and Band Definitions

Band Number—each band in a lane has a unique number, sorted from top to bottom.

Band Label—you can assign a custom label to each band by clicking the Band Label field of the Lane and Band table.

Molecular Weight—the molecular weight of the band is calculated based on the user-defined standard and regression method. Italic values indicate extrapolated values. When using nucleic acid gels, the size of the band is displayed in base pairs.

Relative Front—values between 0–1 indicate the relative movement of the band from top to bottom.

Abs. Quant.—absolute quantification of the band.

Rel. Quant.—relative quantification of the band compared to the reference band.

Band %—percentage of the band volume compared to all band volumes in the lane.

Lane %—percentage of the band's volume compared to the entire volume of the lane.

Volume Definitions

Volume Number—a unique number is assigned to each volume.

Volume Label—software-generated labels for different types of volumes (U—Unknown, B—Background, S—Standard) can be changed in Volume Properties.

Volume—the sum of all the intensities within the band boundaries.

Adjusted Volume—the background-adjusted volume.

Mean Background—the mean value of the background.

Absolute Quantity Volume—the quantity of the volume based on the standard volumes and the regression method.

Relative Quantity Volume—the ratio of the adjusted volume and the adjusted volume of the reference volume.

Pixels—number of pixels inside the volume boundary.

Minimum Value—intensity of the pixel with the minimum intensity inside the volume.

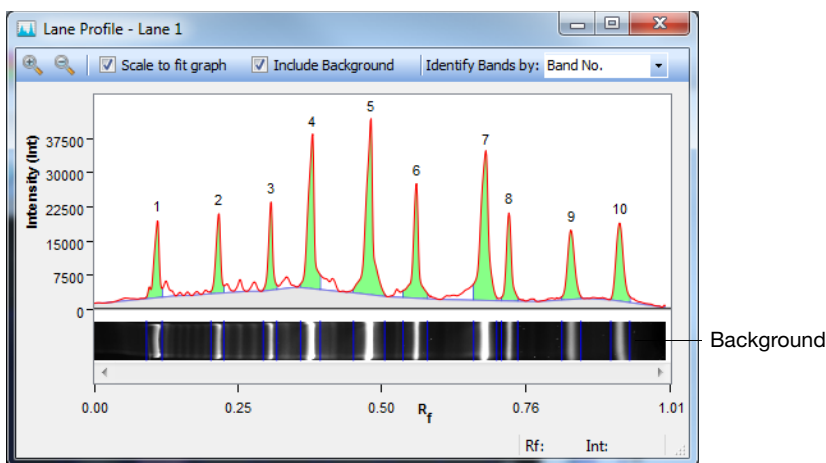
Maximum Value—intensity of the pixel with the maximum intensity inside the volume.

Mean Value—mean value of all pixels inside the volume boundary.

Standard Deviation—standard deviation of all pixels inside the volume boundary.

Area—area of the volume in mm².

Lane Profile



The Lane Profile option shows a cross-section of the selected lane, rotated 90 degrees. You can select other lanes by clicking them while the Lane Profile window is open.

When the Include Background checkbox is selected, the Lane Profile window shows the subtracted background under the blue line. The area used for band quantification appears in green under the red line.

You can see the current Rf (relative front) value and the intensity at the cursor in the lower-right corner of the Lane Profile screen.

You can change how the bands are labeled by choosing from the options in the drop-down list. By default, the bands are labeled with the band number.

SCALE TO FIT GRAPH

You can choose the highest point of the display to define the range of the graph. This provides the best view of the lane profile.

You may want to clear the Scale to Fit Graph checkbox to display the entire range of possible intensity values in the graph. Doing so allows valid comparisons between different lanes.

INCLUDE BACKGROUND

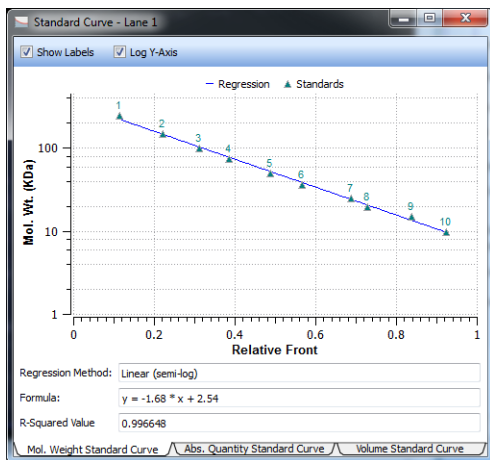
When you clear the Include Background checkbox, the area of the lane profile that represents the background of the image does not display.

IDENTIFY BANDS BY

You can display call outs on the Lane Profile, which indicate these attributes:

- Band Number
- Band Label
- Molecular Weight
- Relative Front
- Volume
- Abs. Quant.
- Rel. Quant.
- Band %
- Lane %

Standard Curve



The Standard Curve dialog box displays the best curve fit for the defined standards and the bands relative to this curve for the lane selected in the image. The tabs display the standard curves for three different analyses.

Standards appear in green; unknown bands appear in red. You can change the molecular weight display on the y-axis between linear and log scale by clicking the Log y-axis box at the upper left. The regression method you chose in Molecular Weight Analysis Tools appears, as well as the formula (if applicable) and the R^2 value of the regression method.

Tabs in this window display the Mol. Weight Standard Curve, the Abs. Quantity Standard Curve, and a Volume Standard Curve.



See Generating Reports on page 85 for information about reports.

6 Analyzing Images

Analysis Tool Box tools are enabled once an image file is opened and in focus. An active or “in focus” window has a darker blue menu bar on a Windows PC. On a Mac, the window control icons display more brightly when a window is active. This distinction helps you to identify the active window among many open image files in your workspace.

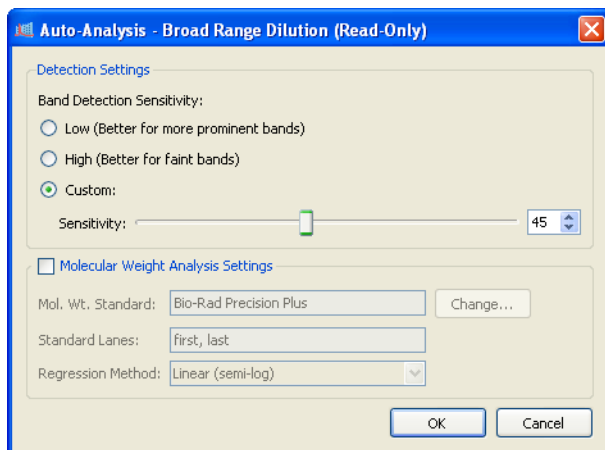
Auto Analysis Settings

Clicking Auto Analysis in the Analysis Tool Box enables you to do the following:

- Analyze images that were obtained with protocols that did not include steps for detection and analysis
- Change your analysis parameters to reanalyze your images

Note: If you change any settings for an analyzed gel, the initial analysis is overwritten. To preserve both analyses, save the image file with a different name.

DETECTION SETTINGS



Band detection options are as follows:

Low Band Detection Sensitivity— This sets detection at a low level, for images with prominent bands. Faint bands are not detected with this setting.

High Band Detection Sensitivity— This sets detection at a higher level for images that are faint. Extraneous bands can be removed using the Band Tools in the Analysis Tool Box. See Lane and Band Tools on page 68.

Custom— You can set a numerical value between 1 and 100 to select the best detection sensitivity for your sample.

When Low or High Band Detection Sensitivity is used, the following numerical values are set: low sensitivity = 25; high sensitivity = 75.

MOLECULAR WEIGHT ANALYSIS SETTINGS

Molecular Weight Standard—Choose any of the many Bio-Rad standards or other standards you have placed in your standards list. For more information, refer to Standards on page 40.

Standard Lanes—Choose or change the lanes in which the standards are placed.

Regression Method—Four regression methods are available. For more information, see Regression Methods on page 41.

Analysis Toolbox Tools

All Analysis Toolbox tools customize the *analyzed* data in image files. These tools are available only when an image file is open. Click a specific image to select among many windows that may be open in your workspace.

Note: Some tools delete the existing analysis.

To access a tool:

- Click any of the tool box buttons

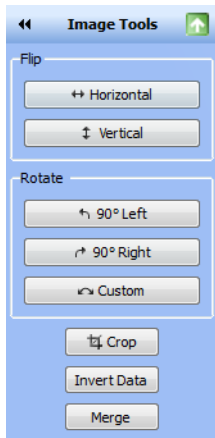
To return to the Analysis Tool Box menu:

- Click the green Up-arrow to the right of the tool name



Image Tools

To show the image tools menu, click Image Tools.



Click to return to the Analysis Toolbox menu

FLIP

You can flip the gel image horizontally or vertically.

ROTATE

You can rotate the gel image 90° to the left or right by clicking the Rotate buttons.

To correct a slanted gel:

1. Click Custom.
2. Rotate the red arrows that appear over the gel to any degree between 0–360° by dragging them.
3. Right-click the gel image and choose Rotate to set the gel in the new position.

CROP

You can save crop settings and use them to crop other images. This feature is useful when you want to crop the same area in several images.

To crop a gel image:

1. Click Crop. A red box outlines the image area.
2. Drag the red box to surround the image area you want to keep.
3. (Optional) Right-click the image to open the Crop menu and click Save Crop Settings.

4. (Optional) Type a name for the crop settings in the dialog box that appears and click OK.
5. Right-click and select Crop or Cancel. Selecting Crop crops the image to the area inside the red box.

To crop an image using saved crop settings:

1. Click Crop. A red box outlines the image area.
2. Right-click the image to open the Crop menu and click Load Crop Settings.
3. Select the saved crop settings in the dialog box that appears and click Load. The red box resizes and the crop specifications appear on the image.
4. Right-click and select Crop. The image is cropped to the area specified in the crop settings you selected.

To delete crop settings:

1. With an image open, click Crop.
2. Right-click the image inside the red box to open the Crop menu and click Delete Crop Settings.
3. Select the crop settings in the dialog box that appears and click Delete.

INVERT DATA

Invert Data is used for negative stains and zymograms. Intensity values of bands must be greater than background to perform analysis on the gel. View the gel as a 3-D projection to determine if the data must be inverted.

MERGE

Use this button to merge a chemiluminescent blot image with a colorimetric image of the same blot. If you have used colorimetric prestained standards for a chemiluminescent blot, you can acquire an epi-white light image of the blot to show the standards and a chemiluminescent image to show immunodetection. These two images can then be merged into a combined image with both signals.

Note: Merging images can have an adverse effect on quantitation. If accurate quantitation is required, perform analysis on the original, separate images. Only images of the same size can be merged.



Lane and Band Tools

Click Lane and Band Tools to select a tab—one to use the lane tools and one for band tools.

LANE TAB

You can choose how lanes are detected by selecting Automatic or Manual Lane Finder.



- Click Automatic if the gel image is fairly typical
- Click Manual to detect a specific number of lanes or if automatic lane detection did not find all the lanes. You can then drag the handles on the corners of the red square to resize the lane frame

ALL LANES

Resize—you can resize all lanes by dragging the handles on the corners of the red square to fit the gel image.

Adjust—if the gel image is irregular, you can adjust the orientation of all lanes by dragging a single corner of the lane frame. The Adjust tool does not resize the lane width.

You can also add more anchor points on the top or bottom borders of the rectangle by clicking the lane frame. Remove any unneeded anchor point by right-clicking it. By dragging these anchor points, you can adjust for so-called smiling gels.

Delete—you can delete all lanes.

SINGLE LANE

Add—you can add a single lane to a gel image. First click Add, and then click within the lane frame where you want to place the new lane. The lanes are renumbered.

Note: To add a lane outside the frame, add a lane inside the frame and click Move to expand the lane outside the frame's boundaries.

Delete—you can delete a single lane. First click Delete, and then click either the lane or its lane number. The lanes are renumbered.

Bend—you can bend a single lane to better fit the gel image. First click the Bend button and then drag one of the square anchors to fit the image.

You can add more anchor points within the lane by left-clicking it. Drag these anchor points to adjust the lane to fit the gel image. Remove anchor points by right-clicking them.

Move—you can move a single lane to a new position on a gel image. First click Move, and then click the lane you want to move. Drag it to a new location. The lanes are renumbered according to their new position.

Width—you can change the width of a single lane.

LANE BACKGROUND SUBTRACTION

Perform lane-based background subtraction by selecting Enable Subtraction in the Background Subtraction field. Use the Lane Profile view to see the subtracted lane background.

Rolling Disk—you can specify the size of a hypothetical rolling disk (between 1–99 mm) that removes background levels along the length of the lane. The size of the disk determines how much background is subtracted.

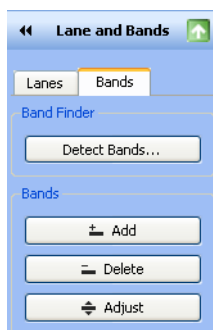
A large disk follows the profile trace less closely, touching fewer points along the trace and removing less background. A smaller disk more closely follows the profile trace, removing more background.

A disk radius that is too large will result in poor background removal. A disk radius that is too small may subtract actual data. For most samples, a size of ≤ 10 mm is usually appropriate. You can perform this task several times until you are satisfied with the amount of background removed. Use the Lane Profile tool to evaluate the appropriate disc size for background subtraction. See Lane Background Subtraction on page 69.

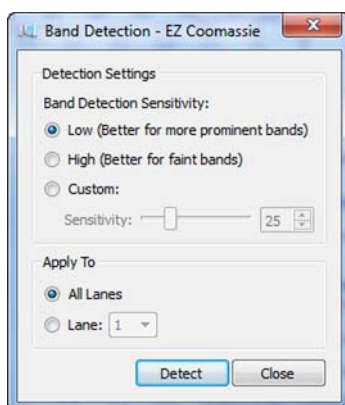
Apply to selected Lane—when you select this box, the level of background subtraction specified with the previously described buttons is applied only to the selected lane. This option enables you to set different background subtraction levels for each lane.

BANDS TAB

This tab enables you to detect bands or reset band detection settings. Click a button in the menu, and then click a lane in the image.



Detect Bands—this option opens a dialog box in which you can select band detection sensitivity and the lanes to which it applies.



Add—use this option to add faint bands. First click Add, and then add a new band by clicking anywhere inside the lane.

Note: You can darken your entire image to view faint bands more easily using the Gamma slider in the Image Transform dialog box. For instructions, see Image Transform on page 52.

Delete — You can delete bands that are not relevant to your analysis. First click Delete, and then click any bands you want to remove.

Adjust—you can adjust the height of a band. First click Adjust. Two boundary lines appear around each band. Move over a boundary line until you see a double-headed arrow. Move the boundary line up or down; the center recalculates; the band appears there.

Note: You can also adjust the band boundaries in the Lane Profile view.



Molecular Weight Analysis Tools

Molecular Weight Analysis Tools enable you to determine molecular weight (or base pairs, if using nucleic acid gels) by comparing a test sample with known standards.

You can view each band's molecular weight in the molecular weight column of the Lane and Band tab in the Analysis Table view or in the gel image by selecting Mol Wt. from the Band Attributes section of the Display Gel Options window:

Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		250.0	0.096	177,345	N/A	N/A	6.1	5.3
2		150.0	0.195	155,799	N/A	N/A	5.4	4.6
3		100.0	0.290	149,184	N/A	N/A	5.2	4.4
4		75.0	0.367	543,501	N/A	N/A	18.8	16.1
5		50.0	0.480	593,271	N/A	N/A	20.5	17.6
6		37.0	0.567	215,145	N/A	N/A	7.4	6.4
7		25.0	0.694	556,416	N/A	N/A	19.2	16.5
8		20.0	0.743	173,250	N/A	N/A	6.0	5.1
9		15.0	0.831	148,239	N/A	N/A	5.1	4.4
10		10.0	0.913	181,062	N/A	N/A	6.3	5.4

MOLECULAR WEIGHT STANDARD

You can change the standards that determine the molecular weight of test samples.

MW Analysis Tools

Standards

Bio-Rad Precision Plus

Change...

Standard Lanes

first, last

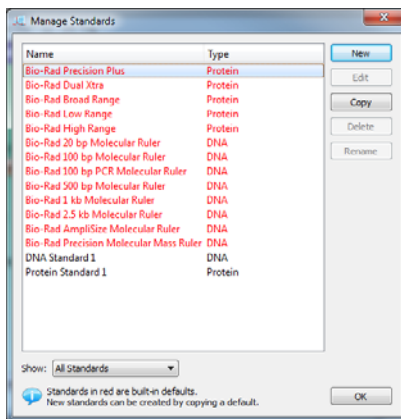
Select standard lanes by checking the box below the lanes

Regression

Method:

Linear (semi-log)

Click Change to access the Manage Standards dialog box, where you can choose another standard or add third-party standards.



STANDARD LANES

Standard samples are placed in the first and last lanes by default. You can specify other standard lanes by selecting the box below each lane or by entering the standard lane numbers separated by commas. Standard lanes are labeled Std below the lane.

REGRESSION METHODS

There are four regression methods. See Regression Methods on page 41.

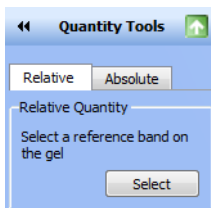


Quantity Tools

You can quantitate bands in test samples automatically using either the Relative or Absolute tabs under Quantity Tools.

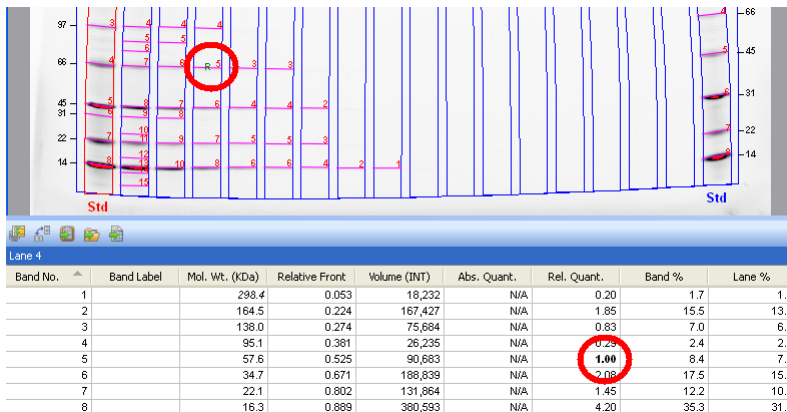
RELATIVE QUANTITY TAB

To compare the relative quantities of bands, first select Quantity Tools (Relative tab) as shown.



To select a reference band from an image and quantitate all other bands, relative to the reference band:

1. Click the Select button.
2. Click any band you want to use as a reference. A small R for Reference appears near the band you selected (see the red circle in the upper left).



To review the relative band quantities:

- Go to the Rel. Quant. column of the Analysis table (Lane and Band tab). The relative quantity is the ratio of the band volume divided by the reference band volume

All other bands now display numerical values that are relative to the reference band. Values above 1.00 indicate that the band quantity is greater than the reference band. Values below 1.00 indicate the band quantity is less than that of the reference band.

ABSOLUTE QUANTITY TAB

Absolute quantification is used to quantify bands based on known standard bands using a calibration curve. To determine the absolute quantities of bands, first select Quantity Tools (Absolute tab) as shown:

Quantity Tools

Relative Absolute

Absolute Quantity

Select Bands to add to calibration curve

Select

Standard Bands:

Ln	Bnd	Quantity
2	2	4.0
4	4	1.0

Delete

Units:

microgram

Regression

Method:

Linear

Force through origin

Click on band to select a standard band

To calculate the absolute quantities of the bands:

1. Click Select.
2. Select at least two standard (known) bands and assign quantity values. The values display in the Standard bands table. The greater the number of known bands and the wider the range of their values, the more accurate the absolute quantity calculation of the unknown bands.

Note: Any standard bands selections can be deleted. To do so, select the entry in the Standard Bands field by clicking it and then clicking Delete.

3. Select a unit of measure from the ten choices listed in the Units drop-down list.
4. Select a regression method from the three listed in the drop-down list.

Keep the following guidelines in mind while making your choice.

Linear—This method generates a straight line that is the best fit of the values you provided and is preferred in most cases.

Point-to-Point—This method generates a curve in which each data point is connected directly to the next, regardless of the shape of the resulting curve.

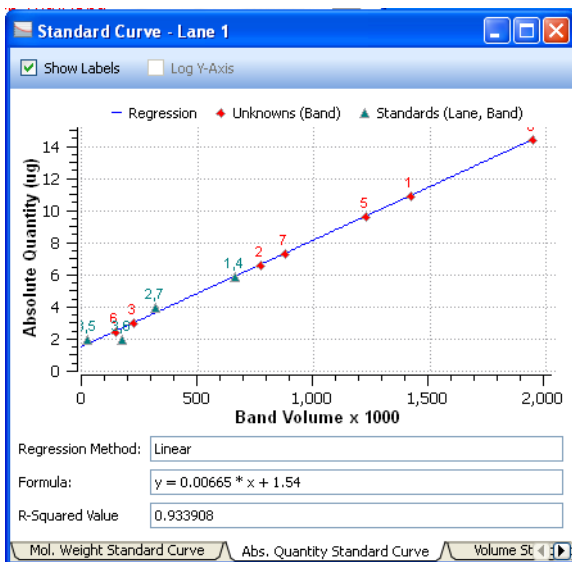
Cubic spline—This method generates a smooth curve that connects each data point. At least four standard points are required to use this method of least-squares polynomial fits.

Regression Method	Minimum Number of Standard Bands	Minimum Number with Force Through Origin Option
Linear	2	1
Point-to-point	2	1
Cubic spline	5	4

5. Click Standard Curve in the toolbar, select the Absolute Quantity Standard Curve tab, and a calibration curve displays. All standards are

represented by green triangles. Unknown values are represented by red triangles.

Note: Selecting the Force Through Origin checkbox always starts the standard curve graph at 0,0, regardless of the best curve fit.

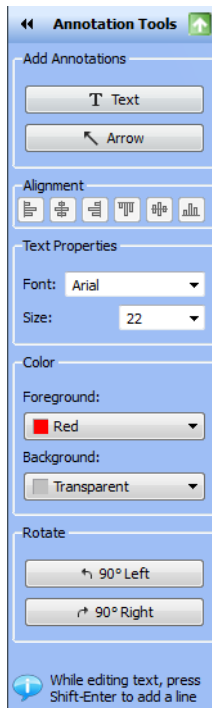


Note: Clicking the Standard Curve generates a crosshair tool that displays the numerical values associated with the placement of the cursor in the graph.



Annotation Tools

You can annotate results with text and arrows to draw attention to areas of interest.



ADD ANNOTATIONS

Text—you can add text annotations to gel images to draw attention to important details. First click Text, and then click an area you want to emphasize. A text box appears with a dotted-line border. Type your comment in the text box. Drag the box to change its position.

Note: While editing text, press SHIFT+ENTER to add text. This adds a new line in the annotation.

Arrow—to add an arrow, first click Arrow. Click the area where you want the arrow to start and then drag to stretch the arrow point to the location you want to emphasize. To move the arrow, click the middle and drag it. To change where the arrow points, click either end of the arrow. Square boxes appear; drag a box to change the length or orientation of the arrow.

ALIGNMENT

Alignment Icons—these buttons enable you to align multiple annotations, such as lane numbers, which you have manually added.

To select annotations—you can select several annotations by holding down the CTRL key (Command key on the Mac) and clicking each one or by dragging a selection box around them.

To copy annotations—you can copy annotations within or between images. First select the annotation you want to copy. Then press CTRL+C to copy and CTRL+V to paste the annotations.

TEXT PROPERTIES

You can change the size and type font of your text annotations. To select multiple items for change, hold down the CTRL key (Command key on the Mac) and click each item.

Font—first click the text box you want to change. Then open the drop-down Font list to show all fonts installed on your system. Click one of the font names to change the style of the text annotation.

Size—first click the text box you want to resize. Click the up and down Size arrows to increase or decrease the size of the text. You can set the font size between 6 and 72 points using the drop-down list.

COLOR

You can change the color of text annotations to make them visible with any color scheme and emphasize them further by adding a color to the annotation's background, which is invisible by default.

To change the color of multiple items, hold down the CTRL key and click each item.

Foreground—click a text annotation or arrow. This activates the Foreground field so you can use the drop-down list to choose a different color.

Background—click a text annotation. This also activates the Background field so you can use the drop-down list to choose a background color.

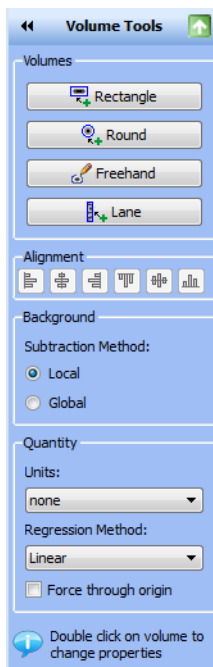
ROTATE

You can rotate text annotations 90° to the left or right by clicking the Rotate buttons.



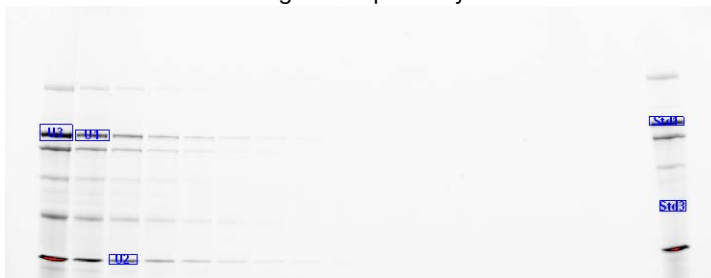
Volume Tools

Volume tools enable you to manually quantitate features on a sample image when automated lane and band analysis is not appropriate or possible, such as in dot blots.



You can use volume tools to quantitate the signal intensity of bands, spots, arrays, and other image data. You define an area of interest by surrounding it with a shape. You can choose a rectangle, circle, freehand shape, or lane by clicking the buttons under the Volumes field.

A default label appears within the shape drawn. The volume label can be one of three types—U for Unknown, Std for Standard, or B for Background—along with a number that is assigned sequentially.



Each new volume you create initially has a red border, which indicates that the volume is selected. When you click elsewhere on the image, the border changes to blue, indicating that the volume is no longer selected.

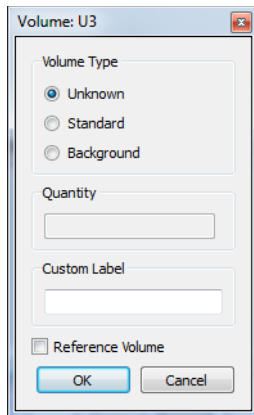
Note: Double-click a volume area to change its properties.

To review data for the volumes:

- Open the analysis table and select the Volume tab. Volumes are listed based on their number and/or the associated information per volume. See Volume Definitions on page 58

VOLUME TYPES

You can define the volume type (unknown, standard, or background), the quantity of standard volumes, or enter a custom name to replace the default label.



The image shows a dialog box titled "Volume: U3". Inside the dialog, there is a section labeled "Volume Type" with three radio buttons: "Unknown" (which is selected), "Standard", and "Background". Below this section are two text input fields: "Quantity" and "Custom Label". At the bottom of the dialog, there is a checkbox labeled "Reference Volume" which is currently unchecked, and two buttons: "OK" and "Cancel".

Unknown volumes are volumes you want to quantify.

Standard volumes are used for absolute quantities. See Absolute Volume Quantity on page 83.

Background volumes are used to remove the background from the calculation. The result of volume background subtraction appears in the Adjusted Volume column of the analysis table (Volume tab).

No.	Label	Type	Volume (Int)	Adj. Vol. (Int)	Mean Bkgd. (Int)	Abs. Quant.	Rel. Quant.
1	B1	Background	28,474,202	-44,446	1,805.7	N/A	N/A
2	Std1	Standard	44,462,671	265,608	1,909.8	100.0	1.00
3	Std2	Standard	29,249,190	695,097	2,071.2	150.0	2.62

VOLUME BACKGROUND SUBTRACTION

When you draw a volume, some non-data background pixels may be included inside the volume. These background pixels usually have an intensity value that you do not want to include in your volume quantitation. There are two ways of calculating this background intensity for your volumes: local and global.

Local—local background subtraction calculates a separate background intensity for each unknown and standard volume you create. For each volume, the intensities of the pixels in a 1-pixel border around the volume are added together and divided by the total number of border pixels. This gives an average intensity for the background around each, which is then subtracted from the intensity of each pixel inside the volume. If the background value is greater than the pixel value inside the volume, the background-adjusted quantity of the volume may be < 0 . In this case, redraw the border for this volume.

Global—global background subtraction calculates a single background intensity for the entire gel. This average background intensity is then subtracted from all the volumes in the gel. The average intensity of the pixels in the background volume is calculated and subtracted from each pixel in all standard and unknown volumes. Therefore, it is not necessary for the background volume area to be the same size as your unknown.

To calculate global background subtraction:

1. Create a volume using one of the volume tools in a representative background region of your image (that is, a non-data region similar to the background surrounding your data).
2. Double-click the volume. This opens the Volume Properties dialog box.
3. Select the Background option button.

Notes:

- If you select Global in the Volume toolbox but do not define a background volume as described, no background subtraction is performed

- If you create more than one background volume, all the pixels in those background volumes are used to calculate the average background. Your background volume(s) will have default names B1, B2, and so on based on the sequence in which they were created
- If the region you defined as background has a higher average intensity value than your data object, you obtain a negative value for your adjusted volume in the analysis table. If this happens, select a new background region with less intensity than your data object

RELATIVE VOLUME QUANTITY

You can choose any one volume as a Reference Volume by selecting the Reference Volume checkbox in the Volume Properties dialog box. The Reference Volume is indicated by an asterisk on the volume label, for example, U1*.

Relative quantities are displayed in the Relative Quantity column in the analysis table (Volume tab). The relative quantity is the ratio of the background-adjusted volume divided by the background-adjusted reference volume.

All other volumes now display numerical values relative to your reference volume. Values above 1.0 indicate that the volume is greater than the reference volume. Values below 1.0 indicate the volume is less than the reference volume.

ABSOLUTE VOLUME QUANTITY

If you have drawn your volume around an object of known quantity, you can use it to calculate the quantity of your unknown volumes. The quantities of your unknown volumes are calculated based on the standard volumes and the selected regression method.

To classify a particular volume as a standard:

1. Double-click the volume. This opens the Volume Properties dialog box.
2. Select the Standard option button and enter the quantity in the Quantity box.
3. Click OK to close the dialog box.

Standard volumes will have the default names S1, S2, and so on, based on the sequence of their creation.

To review the regression curve:

- Open the Standard Curve window and select the Volume Standard Curve tab

REGRESSION METHODS

Three regression methods are available to generate the volume quantitation curve used for absolute quantity: linear, point-to-point, and cubic spline. To display the standard curve, click the standard curve button in the toolbar and select the Volume Standard Curve tab in the Standard Curve dialog box. See Regression Calculation Methods to learn how each of these methods is calculated.

The data for volume standards is found in the Absolute Quantity column of the Volume Table.

Note: Selecting the Force Through Origin checkbox always starts the standard curve graph at 0,0, regardless of the best curve fit.

ALIGNMENT

Align volumes by using the appropriate alignment button. To select several volumes, CTRL+click each one or drag them to use a selection box.

Copy and paste selected volumes by pressing CTRL-C to copy and then CTRL+V to paste.

When you click Standard Curve in the toolbar, a chart displays all unknown and standard quantities.

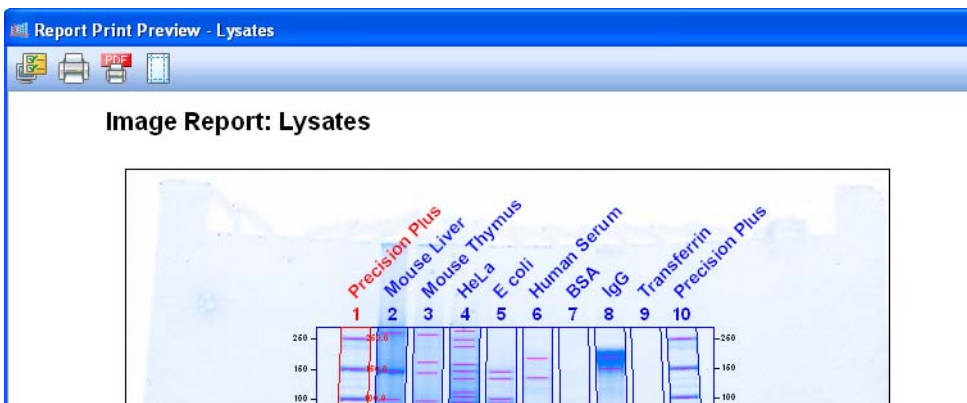
7 Generating Reports

After viewing results, you can generate a report that displays the analyzed gel images, all of the settings used in the protocol, and as much information about the data as you want to include.

The print report settings can be chosen with the Report Settings dialog box in the Edit menu or by clicking Report in the main toolbar.



Click Report to produce a Print Preview of your report.



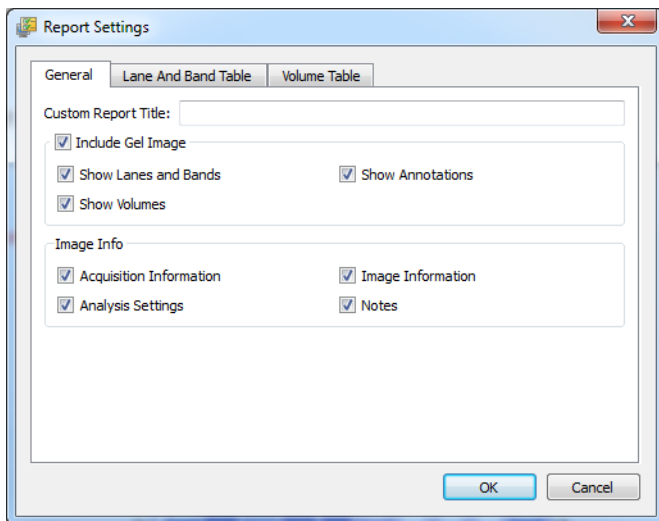
Clicking Print prints all of the above information to a printer.

Use the following dialog boxes to remove information from reports. Doing so does not delete the data from the analysis.

Display Report General Tab



The default setting is for all information to be included. These choices may have been made at other stages of the workflow, but you can customize reports here.



Clear any of the following checkboxes to exclude information from a report.

Custom Report Title—you can provide a custom report title for the report.

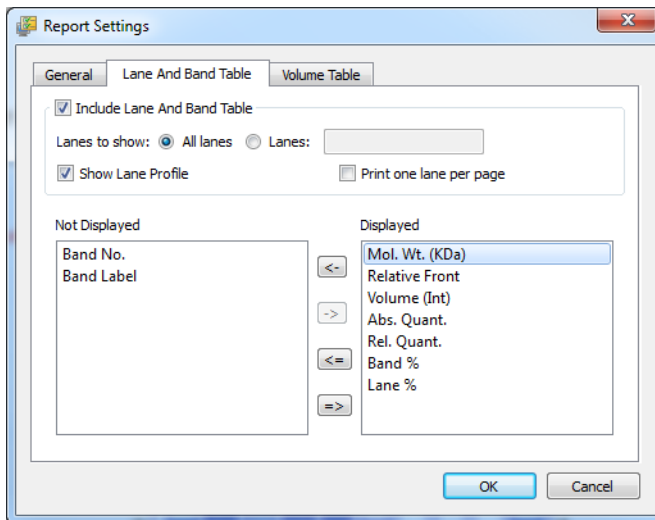
Include Gel Image—the following options determine which overlays display on the gel image.

- Show Lanes and Bands
- Show Volumes
- Show Annotations

Image Info

- Acquisition Information
- Analysis Settings
- Image Information
- Notes

Lane and Band Table Tab



Clear any of the following settings to exclude information from the report.

Include Lane and Band Table

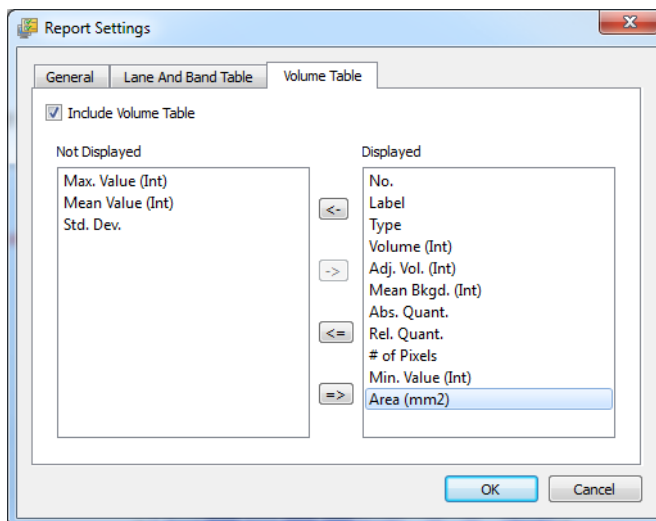
Lanes to show:

- All lanes or just user-defined lanes
- All lanes on one page, or one lane per page (add page break after each lane)

Show Lane Profile—includes the lane profile for each lane.

Move labels to the left—if you do not want these columns to be included in the report.

Volume Table Tab



Include Volume Table—clear to exclude this information from your report.

Move volume labels to the left—if you want to exclude their columns from the report.

Print Report

Click Print Report to print your report.

PRINT REPORT TO .PDF FILE

Print Report to .pdf File brings up a Save dialog box so your .pdf file can be saved on your system.

ADJUST PRINTER SETTINGS

Printer Settings accesses options for paper size, orientation, and page margins.

8 Exporting Results

The most convenient way to archive complete information about experiments is to produce reports. However, you may want to export only gel images or analysis table data for analysis in different programs such as Quantity One[®], FPQuest[™], or InfoQuest[™]FP software. Or you may need exported files for presentation or publication.

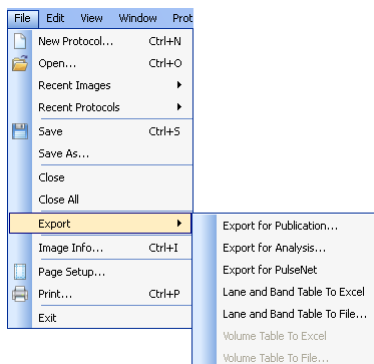
Exporting Gel Images

Image Lab[™] software includes features for exporting gel images several ways.

- You can export displayed image data to a publication (choose Export for Publication)
- You can export raw image data as a 16-bit TIFF file (choose Export for Analysis)
- You can export image data to PulseNet; doing so reduces the image to an 8-bit TIFF file, limits its resolution, and restricts its file size to 300 Kb
- You can export lane and band tables as well as volume tables to a spreadsheet program or to a file

The options to export gel images are available using the Export option in the File menu.

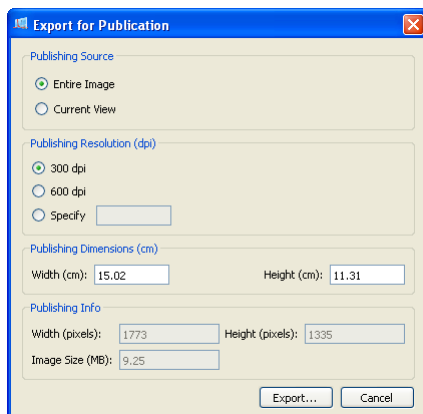
The available export options are described in the following sections.



EXPORTING GEL IMAGES FOR PUBLICATION


Use this format only to export visual information to presentation or word processing software, such as PowerPoint or Word.

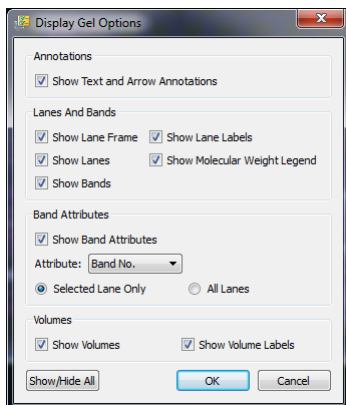
When you select File > Export > Export for Publication, you can export a displayed image to a file. You can select from .bmp, .png, .jpg, and TIFF formats. The gel displays with any lanes, bands, and annotations that appear on the screen.



Note that you can select the entire image or the current view, select the resolution or specify a custom resolution, specify the publishing dimensions, and view the resulting published image size and dimensions.

You can zoom in on an area in a current view to export only that area or you can export the entire image. You can exclude annotations or overlays by

clicking Display Gel Options  in the toolbar to access the appropriate settings.



EXPORTING GEL IMAGES FOR ANALYSIS

When you select File > Export > Export for Analysis, you can export full image data (raw data only) for analysis as a 16-bit TIFF file.

Note: 16-bit TIFF images are not compatible with all image viewers.

The image may require contrast adjustment when it is imported into analysis software. This option creates a file that can be analyzed in other programs such as Quantity One, FPQuest, or InfoQuestFP software.

EXPORTING GEL IMAGES TO PULSENET INTERNATIONAL

When you select File > Export > Export for PulseNet, Image Lab software reduces the image to an 8-bit TIFF image file. Resolution is limited and file size is restricted to 300 Kb.

Exporting Lane and Band Tables to Excel

If you have Excel (or Numbers, on a Mac) installed on your computer, choosing File > Export > Lane and Band Table to Excel opens a table directly in Excel. You can then use the Save As option to produce other formats.

Exporting Volume Tables to File

Choosing Export > Volume Table to File exports as a CSV (comma-separated values) file so the data file can be opened in a database application.

Screenshot Tool Export

Use the Screenshot tool, available in the toolbar, to capture a displayed image to the clipboard or to save it to a file (.bmp, .gif, or .png).

Analysis Table Export

You can export table analysis data from the File menu or by using the export buttons at the top of the Analysis Table window.

The Analysis Table window has several buttons to export data to different formats, depending on how the data are to be presented.

COPY ANALYSIS TABLE TO THE CLIPBOARD



Click this button to copy the analysis table to the clipboard and to paste the analysis table into word processing or presentation applications. It is best to use the vertical table orientation when copying to an 8-1/2 x 11-inch page to accommodate the columns.

EXPORTING ANALYSIS TABLE TO A FILE



This button exports an analysis table as a CSV (comma-separated values) file so your data file can be opened in a database application.

EXPORT ANALYSIS TABLE TO A SPREADSHEET



This button enables you to use Excel's sort and formula functions with your data. If you have Excel (or Numbers, on a Mac) installed on your computer, the data open in Excel.

9 Maintenance and Specifications

Cleaning the Sample Trays

Clean the sample trays with standard laboratory detergents or mild solvents such as EtOH or MeOH. Use lint-free tissue to wipe them dry. Dust particles or lint on a sample tray can glow under UV illumination.

UV-B Fluorescent Lamp Replacement

The UV-B lamps provide service under normal usage for 4–5 years. In case of a lamp failure, one of the following error messages appears:

- Fault in UV bulbs Bank 1&2
- Fault in UV bulbs Bank 1
- Fault in UV bulbs Bank 2

To replace the lamps:

1. Turn off the instrument and unplug the AC cord.
2. Remove and save the seven screws around the outside edge of the imager back. Do not remove the screw at the bottom left outside corner of the imager back.
3. Carefully slide the top cover toward the back of the instrument until it is all the way off.

4. On the left side of the instrument a single screw holds the lamp assembly in place. Remove and save this screw.
5. Carefully slide out the lamp assembly. Although only one lamp bulb may be out, it is recommended that all lamps be replaced to ensure even illumination and to reduce the need to access the lamps again.



6. Replace all lamps with UV-B fluorescent lamps (Bio-Rad part catalog #900-0217). Make sure each lamp is properly seated in its holder.
7. After replacing all the lamps, carefully slide the lamp assembly back into the instrument. Press on the side of the lamp assembly to the right of the screw hold-down tab, to make sure it is fully seated into the instrument. If the lamp assembly is not fully seated, the following error message appears the next time it communicates with Image Lab software: Light tray not detected.

Error Messages

Sample tray not detected

The sample tray has a magnet that senses when the sample tray is inserted. If you attempt to image and this message appears, the sample tray may not be pushed in all the way. Press the tray in until the magnet grabs the tray.

Light tray not detected

This error message appears if the lamp assembly is not fully seated into the imager. Reseat the lamp assembly as described on the previous page.

Door was opened during imaging

To prevent UV radiation exposure, the UV-B lamps do not turn on unless the front sample tray door is fully closed. This message also appears when the instrument cover has been removed.

Technical Specifications

Hardware Specifications	
Image area	15 x 11.2 cm
Excitation source	Trans UV B (302 nm peak)
Detector	Charge-coupled device
Image pixel size	107.8 x 107.8 μm (in microns)
Dynamic range	3.0 orders of magnitude
Pixel density	4,096 gray levels
Flat fielding	Yes
Instrument size	43 x 28 x 38 cm
Instrument weight	7.3 kg
Connector descriptions	USB-A connector is used for communication with a PC USB-B connector is used for the instrument interface
Software Specifications	
Operating system compatibility	Windows XP SP3 or higher Windows 7 (32- and 64-bit)
	Mac OS 10.5 or 10.6
Image file size	Approximately 2.5 MB
Computer interface	USB 2.0
Operating Ranges	
Operating voltage	110/115/230/240 VAC Nominal
Operating temperature	10–28°C (21°C recommended)
Operating humidity	< 70% noncondensing
Equipment Ratings	
Input voltage range	100–240 VAC
Input frequency range	50–60 Hz
Power	40 W
The Gel Doc EZ imager is for indoor laboratory use only.	
Automation Capabilities	
Workflow automated execution	Controlled by a protocol via setup for gel activation, image capture, analysis, and reporting
Workflow reproducibility	100% repeatability via recallable protocols, from gel activation and image capture to quantitative analysis and reports
Autoexposure	2 user-defined modes (intense or faint bands)

A. Using the Criterion Stain Free™ System

The Criterion Stain Free system comprises the Gel Doc™ EZ imager, Image Lab™ software, and three types of precast gels:

- Criterion™ TGX Stain-Free™
- Criterion Stain Free
- Mini-PROTEAN® TGX Stain-Free™

The Stain Free system eliminates the time-consuming staining and destaining steps required by other protein detection methods. Stain Free gels include unique trihalo compounds that allow rapid fluorescent detection of proteins with the Gel Doc EZ imager—without staining.

The trihalo compounds in the gels react with tryptophan residues in a UV light-induced reaction to produce fluorescence, which can be easily detected by the Gel Doc EZ imager within gels or on low-fluorescence PVDF membranes. Activation of the trihalo compounds in the gels adds 58 Da moieties to available tryptophan residues and is required for protein visualization. Proteins that do not contain tryptophan residues cannot be detected using this system. The sensitivity of the Stain Free system is comparable to staining with Coomassie Brilliant Blue for proteins with a tryptophan content >1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content >3%.

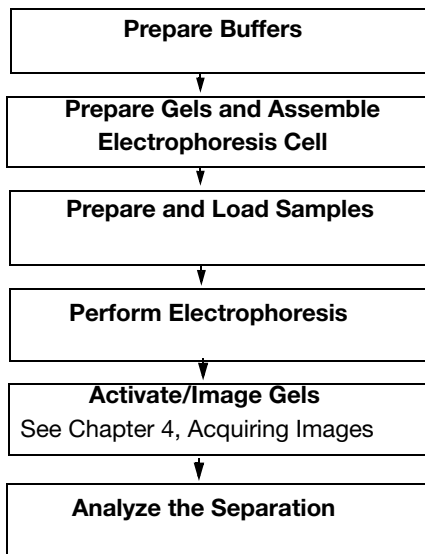
Benefits of the Stain Free system include:

- Elimination of staining and destaining steps for faster time to results
- No background variability within a gel or between gels (as is often seen with standard Coomassie staining)

- Elimination of the need for acetic acid and methanol in staining and destaining, reducing organic waste
- Visualization of transferred or blotted proteins on low-fluorescence PVDF membranes

Stain Free Workflow

For detailed information about workflow steps, refer to the Criterion™ Precast Gels Instruction Manual and Application Guide or to the Mini-PROTEAN® Precast Gels Instruction Manual and Application Guide except where otherwise noted.



Electrophoresis with Stain-Free Gels

Stain Free gels are made and packaged without SDS so they can be used for both SDS and native PAGE applications.

To perform electrophoresis with these gels, prepare the sample and running buffers, set up the electrophoresis cell, and perform the run.

Imaging Gels

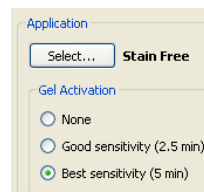
Use unstained standards with Stain Free gels, as some prestained standards are not detected by the Stain Free system. To monitor electrophoresis, use a 1:1 mixture of unstained and prestained standards.

Setting up a protocol for Stain Free gels is in many ways the same as setting up protocols for other applications. Follow the instructions in Setting Up a Default Protocol, on page 33 but after step 3, set the activation options for the gel as follows.

Set the activation options: Visualizing proteins requires activation of the gel.

Choose an activation time option based on your sample and the purpose of your experiment:

- Use 2.5 min activation when samples are abundant and when a fully optimized signal-to-noise ratio is not necessary
- Use 5.0 min activation for detection of proteins that are in low concentration and for the best quantification of the maximum number of bands. Because the reaction is near completion after 5 min, this method offers an optimal signal-to-noise ratio



Note: If the gel has already been activated for 2.5 min, activating it for another 2.5 min may improve it; but activating an image for more than 5 min will not.

Continue setting up the protocol at step 4, Set the image exposure, on page 36.

Imaging Blots

To blot Stain Free gels, use standard blotting procedures as described in the instruction manual you are using. Use only PDVF membranes with low background fluorescence, as membranes other than low-fluorescence PDVF can result in high background or low sensitivity with the Gel Doc EZ imager.

To assess transfer efficiency, be sure to activate and visualize the gel using the Gel Doc EZ imager before transfer.

B. Regression Calculation Methods

Each regression method calculates a standard curve. Some of the methods provide the formula for the standard curve. In this case, the molecular weight can be calculated by:

x = relative front of the band of interest

y = molecular weight of the band of interest

Linear (semilog): The linear equation is $y = a + bx$, where a is the intercept and b is the slope of the line.

NOTE: The linear equation is calculated on the **log** of the molecular weight values.

The R^2 value may be used to determine the overall quality of the linear fit. A linear regression with an R^2 value of >0.99 is considered a very good fit. The primary advantage of this method is that it is extremely simple. The primary disadvantage is that it will deliver incorrect results if the data are not very linear.

Point-to-Point (semilog): No single equation is available for the point-to-point method. The slope of each segment of the curve between data points is calculated independently.

NOTE: The log of the molecular weight values is used to calculate the slope for each segment of the curve.

Logistic: The Logistic-4PL equation is

$$y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^b}$$

where:

x = mobility

y = molecular weight

a = estimated molecular weight at infinity

b = slope of the tangent at midpoint

c = midpoint

d = estimated molecular weight at zero mobility

Since the curve generated by the logistic-4PL regression method represents a perfectly shaped S, it might not fit the data very well in all cases.

Cubic spline: Cubic spline curves are smooth curves that go through every data point. The model is a cubic polynomial on each interval between data points. In some cases, a spline curve can work well as a standard curve for interpolation. However, because the curve is calculated individually for every pair of points, it does not correspond to any single equation.

C. Troubleshooting

Problem	Possible Cause	Solution
Green LED on front panel is off	AC power cord is not connected	Connect AC power cord to the imager and an appropriate wall socket
	Power is off	Turn the power switch on
Front panel LEDs remain flashing	Firmware is not loading	Call Bio-Rad Technical Support for help
Image is not visible on the monitor	No sample on sample tray	Place a sample on the tray and run the protocol again
Image is not bright enough	Underdeveloped gel	If initially stopped after 2 min, allow the gel to develop for 2–3 more min
Red LED flashing on front panel	Sample tray not inserted all the way	Push the sample tray into the imager until the magnet pulls the sample tray
	Door interlock broken	Close the door
	Bulb fault	Replace all the lamps according to the instructions in Appendix A
	Communication interrupted	Make sure imager power is on and the USB cable is connected to the PC. Restart the imager
	Imaging button is pressed on front panel while software is not running	The red light stops flashing when the software is opened. Press the imaging button again to initiate imaging

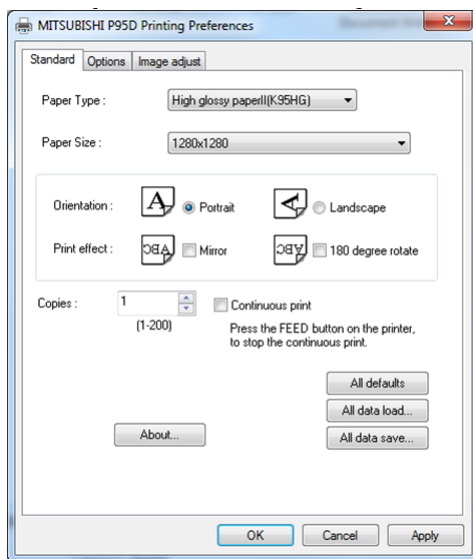
D. Mitsubishi P93/P95 Thermal Printer Settings

Windows

The printer driver can be found on the Image Lab™ software installation CD in the Misc directory.

To set up a thermal printer on a Windows system:

1. Install the printer driver.
2. Open the printer section in Control Panel.
3. Click the thermal printer icon and select Printing Preferences.



4. Configure the correct paper size: select 1280 x 1280.
5. Click OK to apply your changes.

Mac

The printer driver can be found on the Image Lab software installation CD in the Misc directory.

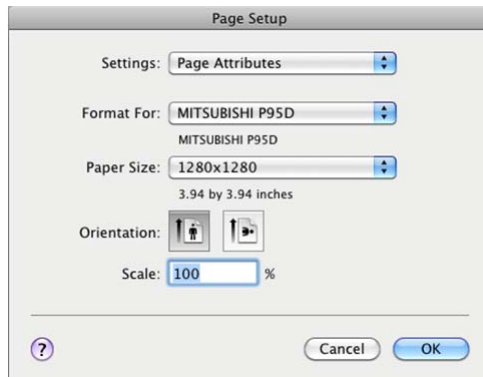
To set up a thermal printer on a Mac system:

1. Install the printer driver.
2. Connect the printer to the computer.

To configure the correct paper size:

1. Start Image Lab software.
2. Select File > Page Setup.

Thermal Printer Settings



3. In the Settings drop-down list, select Page Attributes.
4. In the Format For drop-down list, select the Mitsubishi printer.
5. In the Paper Size drop-down list, select 1280 x 1280.
6. In the Settings drop-down list, select Save as Default.
7. Click OK to save the settings.

Glossary

CCD: Charge-coupled device. A light-sensitive silicon chip used as a photodetector in digital camera systems.

Colormaps: Different color representations of a gel image.

Electrophoresis: A technique for separating molecules based on the differential movement of charged particles through a matrix when subjected to an electric field.

EtOH: Ethanol.

Example precision: The number of decimal places chosen for a measurement.

Flat fielding: An average intensity computation that compensates for nonuniformities generated by an instrument.

Histogram: Representation of the brightness, or gray value, of an image as a graph.

MeOH: Methanol.

Quantitative imaging: Determines the quantity of a protein's components through analysis of the pixel values in a digital image of the sample.

Relative quantity: A measurement of a specific band's relative quantity to the total of all identified bands within a given lane.

SDS: Sodium dodecyl sulfate (sodium lauryl sulfate); a denaturant that provides a negative charge.

SDS-PAGE: Polyacrylamide gel electrophoresis performed with the denaturant sodium dodecyl sulfate.

Tris: Tris(hydroxymethyl)aminomethane; a buffer component.

Tryptophan: An essential amino acid that is a precursor of important biochemical molecules indoleacetic acid, serotonin, and nicotinic acid.

UV-B: The range of ultraviolet light used by the Gel Doc™ EZ system.

UV transilluminator: The part of the Gel Doc EZ imager that transmits UV light through a sample.

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Czech Republic 420 241 430 532 **Denmark** 44 52 10 00 **Finland** 09 804 22 00 **France** 01 47 95 69 65
Germany 089 31 884 0 **Greece** 30 210 777 4396 **Hong Kong** 852 2789 3300 **Hungary** 36 1 459 6100
India 91 124 4029300 **Israel** 03 963 6050 **Italy** 39 02 216091 **Japan** 03 6361 7000 **Korea** 82 2 3473 4460
Malaysia 60 3 2117 5260 **Mexico** 52 555 488 7670 **The Netherlands** 0318 540666
New Zealand 64 9 415 2280 **Norway** 23 38 41 30 **Poland** 48 22 331 99 99 **Portugal** 351 21 472 7700
Russia 7 495 721 14 04 **Singapore** 65 6415 3170 **South Africa** 27 861 246 723 **Spain** 34 91 590 5200
Sweden 08 555 12700 **Switzerland** 061 717 95 55 **Taiwan** 886 2 2578 7189 **Thailand** 66 2 6518311
United Kingdom 020 8328 2000