Gel Doc™ EZ Imaging System with Image Lab™ Software

Instrument Guide

Version 6.0

BIO·RAD

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Bio-Rad Technical Support Department

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1. Image Lab software is based in part on the work of the Qwt project (http://qwt.sf.net).

2. Image Lab software is based in part on the work of the CImg project (http://cimg.sourceforge.net/). See license for details at: http://www.cecill.info/licences/Licence_CeCILL-C_V1-en.html

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Safety and Regulatory Compliance

The Gel Doc™ EZ imaging system is intended for laboratory use only. 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

Fig. 1. Gel Doc EZ imager certification label.
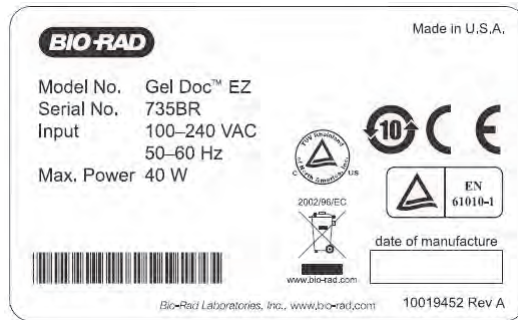
For easy customer access, the serial number appears in two locations on your instrument: on the back panel and inside the front door.

Alert Icons

Alert icons call attention to caution and warning paragraphs. The icon indicates the type of hazard addressed.

Table 1. How alert icons are used in this user guide

Icon	Explanation
	General Indicates a potential hazard requiring special attention. This icon is used when the hazard or condition is of a general nature.
	Electrical hazard Indicates a potential hazard requiring special attention when you are working with electricity or electrical equipment.
	Extreme heat and flammable materials Indicates a potential hazard requiring special attention when you are working with extreme heat and flammable materials.
	Radiation hazard Indicates a potential hazard requiring special attention when you are working with UV radiation.

Cautions

A caution alerts you to take or avoid a specific action that could result in loss of data or damage to the instrument. A caution can also indicate that, if the precaution against a potential hazard is not taken, minor or moderate injury might occur.

Example

Caution: With the exception of cleaning or replacing light bulbs, refer all servicing to qualified Bio-Rad personnel or their agents.

Warnings

A warning precedes an action that, if not followed correctly, could cause serious injury or death to the operator, serious or total loss of data, or serious damage to the instrument.

Example

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

Instrument Safety Warnings

Before you operate the instrument, carefully read the contents of Table 2.

Table 2. Safety cautions and warnings for the instrument

Icon	Meaning
	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.
	Caution: If the case interlock is defeated, there is a possibility of UV-B radiation hazard due to UV-B light exposure. Exercise caution when servicing the instrument.
	Caution: Disconnect the AC power cord before removing the instrument cover.
	Warning! This instrument must be connected to an appropriate AC voltage outlet that is properly grounded.

Notice

The Gel Doc EZ imaging system is meant for use by specialized personnel who know the health risks associated with reagents normally used in electrophoresis. 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. Alteration voids the manufacturer's warranty and might create a potential safety hazard for the user.

Warranty

Each Gel Doc EZ imager 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. 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 might have.

Safety and Regulatory Compliance

1   Introduction

The Gel Doc™ EZ imaging system consists of the Gel Doc EZ imager and Image Lab™ software. The imager connects to a separate computer running Image Lab. This combination creates an automated system for imaging and analyzing gels and blots. Data can be viewed, modified, and reported using Image Lab software.

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

Image Lab protocols automate sample image acquisition, analysis, and report generation with the push of a button on the imager.

For complete information about Image Lab software, see the Image Lab Software User Guide.

Gel Doc EZ Imaging System

The Gel Doc EZ imager combines a low-noise detection camera with a UV transilluminator-based system. Place a sample on one of four application-specific trays, then push the green Run button on the front of the imager to start the default protocol and image the sample using Image Lab.

Four sample trays are available for the Gel Doc EZ imager. Each tray supports applications requiring a different set of stains and detection reagents, as shown in Table 3.

Table 3. Sample Tray Types and Detection Reagents

UV Tray Catalog #1708271	White Light Tray Catalog #1708272	Blue Tray Catalog #1708273	Stain-Free Tray Catalog #1708274
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			

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
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)

Image Lab Software Capabilities

Image Lab software runs customizable, automated protocols on the Gel Doc EZ system for routine gel and blot imaging 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 and blot images for documentation in laboratory notebooks and to export images for publication or presentation.

Molecular Weight Assessment

Sodium dodecyl sulfate 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.

Quantitation

The components in a sample (bands) are quantitated 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 quantitation 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 signals in the sample lane (lane%). Determining expression levels of a protein or nucleic acid requires quantitation 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.

For More Information

For detailed information about Image Lab software, see the Image Lab Software User Guide.

2 Setting Up Gel Doc EZ

Configuring the Imager

Configure the Gel Doc EZ imager before using it with Image Lab^{TM} software. To do so, you must initialize the sample trays and correct the dark image.

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.

To configure the imager

1. Verify that the Gel Doc EZ is connected to the computer running Image Lab software.

2. If a tray is in the imager, remove it and close the door.

3. Turn on the imager and start Image Lab software.

   The setup wizard appears.

4. Complete the steps in the wizard.

Note: You must complete all the steps in the setup wizard or the imager will not be usable. However, if you are not going to use a certain tray type, you can skip configuring that tray type and go to the next screen.

The first page of the setup wizard indicates that a new instrument has been found and displays the instrument serial number.
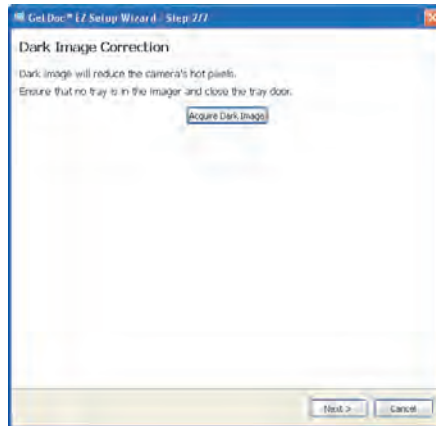
1 Click Next.

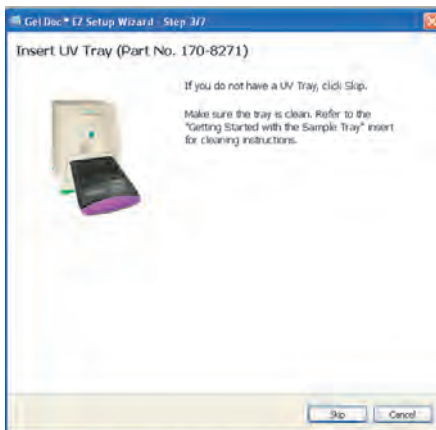The Dark Image Correction page appears.

2 Click Acquire Dark Image.

A progress bar monitors the acquisition of the dark image.

When the dark image has been acquired, the wizard prompts you to initialize the UV tray. If you do not have a UV tray, click Skip and go to Step 7.

3 Make sure the UV tray is clean. Then insert the tray into the imager and close the door.

4 Click Next to initialize the UV tray.

A progress bar monitors the initialization of the tray.

When the tray has been initialized, the next screen confirms that the tray has been found and initialized.

5 Remove the UV tray from the imager.

6 Click Next.

The wizard prompts you to initialize the white tray. If you do not have a white tray, click Next and go to Step 11.

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

8 Click Next to initialize the white tray.

A progress bar monitors the initialization of the tray.

When the tray has been initialized, the next screen lists the applications you can run with the tray.

9 Remove the white tray from the imager.

10 Click Next.

White Tray Found and Initialized

You can run the following applications with this tray:

- Fast Blast
- Coomassie Blue
- Copper Stain
- Zinc Stain
- Silver Stain

The wizard prompts you to initialize the blue tray. If you do not have a blue tray, click Skip and go to Step 15.

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

Insert Blue Tray (Part No. 170-8273)

If you do not have a Blue Tray, click Skip.

Make sure the tray is clean. Refer to the "Getting Started with the Sample Tray" insert for cleaning instructions.

12 Click Next to initialize the blue tray.

A progress bar monitors the initialization.

When the tray has been initialized, the next screen confirms that the tray has been initialized.

13 Remove the blue tray from the imager.

14 Click Next.

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 18.

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

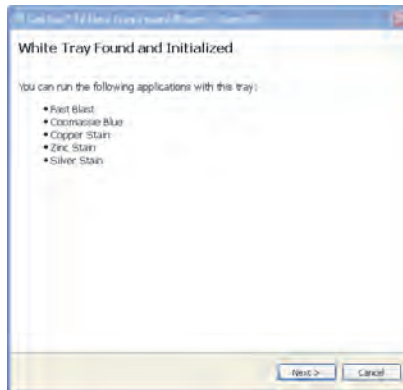**16** Click Next to initialize the stain-free tray.

A progress bar monitors the initialization.

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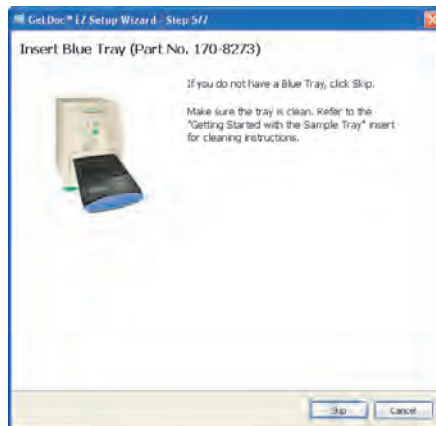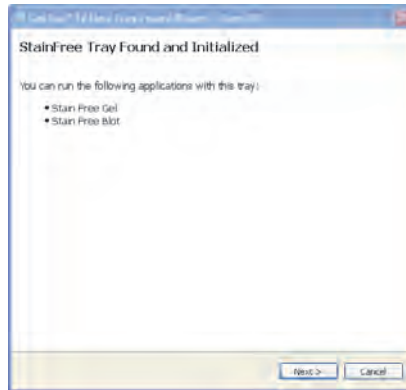
17 Click Next.

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

18 Do one of the following:

- Click Finish to leave the wizard and display the Default Protocol Setup screen, where you can start creating a default protocol for the new tray. For more information, see Creating a Protocol on page 26.

- Clear the checkbox at the bottom of the wizard page to skip setting up a default protocol.

19 Click Finish to leave the wizard.
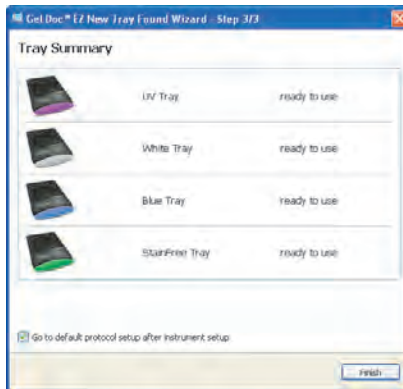
To use the green button on the front of the imager to run default protocols, you must set up at least one default protocol. However, you can set it up later. See Chapter 3, Acquiring Images, for information about creating a default protocol for each type of sample tray.

Initializing an Additional Tray

After you configure the imager, you can add another tray to the system at any time.
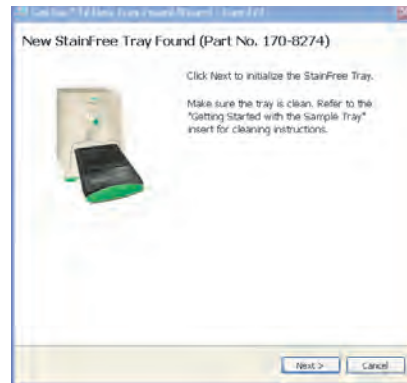
To initialize an additional tray

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

2. Complete the steps in the wizard.

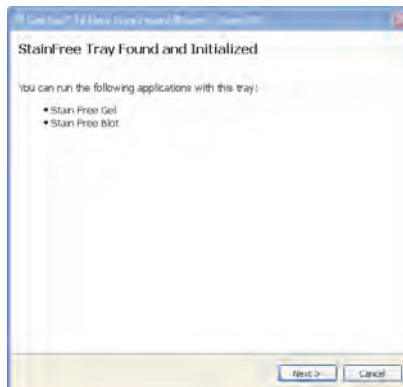The wizard identifies the new tray and asks whether you want to initialize it.

1 Click Next.

The wizard begins initializing the tray.
A progress bar monitors the initialization.

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 trays.

3 Do one of the following:

- Click Finish to leave the wizard and display the Default Protocol Setup screen where you can start creating a default protocol for the new tray. For more information, see Creating a Protocol on page 26.

- Clear the checkbox at the bottom of the wizard page to skip setting up a default protocol. Then click Finish to leave the wizard.
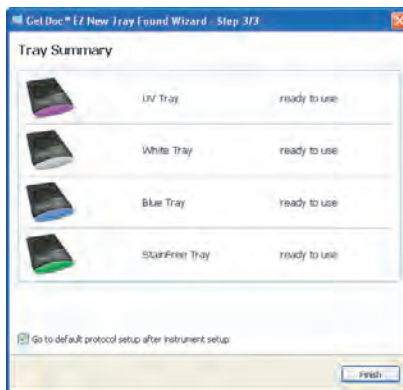
Recalibrating Flat Field and Dark Image

The Instrument Setup dialog box displays the following information about the instrument:

- Instrument name and serial number

- Camera serial number

- Firmware version

- Available sample trays with the names of associated default protocols

- Flat field type and creation date

In this dialog box you can recalibrate flat field or dark image correction or both.

To access Instrument Setup

▶   Select Edit > Instrument Setup.

To recalibrate Flat Field

▶   Insert a white tray, click Reset under Instrument Calibration and click OK.

To recalibrate Dark Image

▶   Insert any type of tray, click Reset under Dark Image Correction and click OK.

3　Acquiring Images

Image Lab™ software runs configurable application-based protocols for sample imaging. In a single acquisition, a protocol runs a combination of settings for acquiring an image, analyzing it, and creating a customized report. An Image Lab protocol defines an application, tray type, and configurable settings. A protocol can also include analysis and output settings. Protocols can be retrieved, revised, and reused for repeatable results.

You can create a default protocol for each tray type with a set of acquisition settings that you use regularly. You can run a default protocol by inserting a tray and pressing the green Run button on the imager. The imager acquires the image using the default protocol application and settings. You can also create protocols with different settings and run them in Image Lab.

This chapter explains how to create and run Image Lab protocols. For more information about protocols, see the Image Lab Software User Guide.

Selecting a Sample Tray

When you select an application type in Image Lab, a dropdown menu displays applications predefined for use with that application type. When you select an application, the screen displays the tray designed to work best with the application. See Gel Doc EZ Imaging System on page 11 for a table of tray types and the applications they support.

If the stain you want to use is not on the application list and you are not sure which tray to use, contact Bio-Rad Technical Support.

Note: Use the stain-free tray for stain-free gels and blots.

Creating a Protocol

Important: You must initialize each tray type before you can select it to create a protocol. See Initializing an Additional Tray on page 22 to learn how to initialize trays.

Creating a protocol consists of configuring settings in Protocol Setup screens. The title bar displays the type of protocol, the imager name, and tray type.
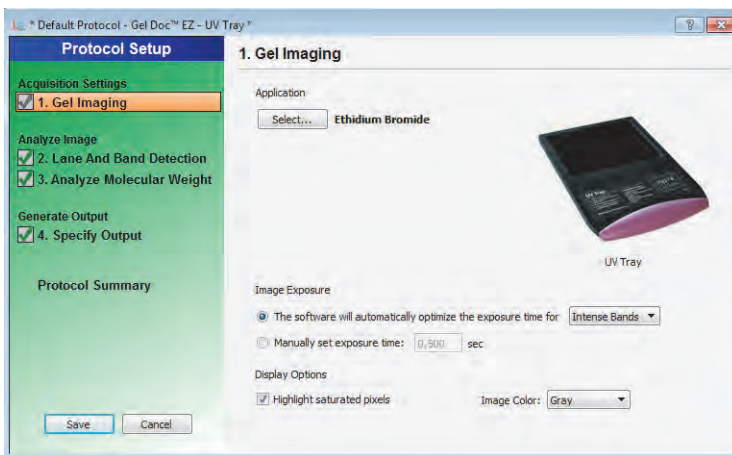
Main steps appear as headings in the left pane of each screen. Numbered steps appear under these headings. To choose options for a protocol step, select the checkbox for the numbered step.
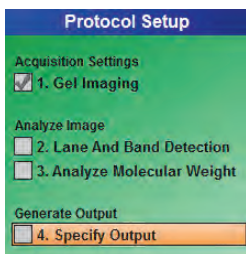
To disable a numbered step, clear its checkbox.

Options for the selected step appear on the right side of the screen.

You can create one default protocol for each computer user account. Multiple scientists can share the same user account, but only one default protocol can be created for that user account.

To set up a default protocol

1. Verify that Gel Doc EZ appears on the status bar with a green check mark.
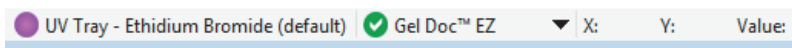
The green check mark indicates that the imager is connected to the computer running Image Lab software and is communicating with Image Lab.

2. Click Default Protocols on the toolbar.

The Default Protocol Setup screen displays each sample tray type.

3. Click Create next to the sample tray type for which you want to create a protocol.

Tip: Create changes to View/Edit after you create a default protocol for that tray type.

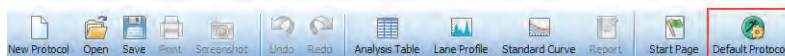The Protocol Setup Gel Imaging screen appears.
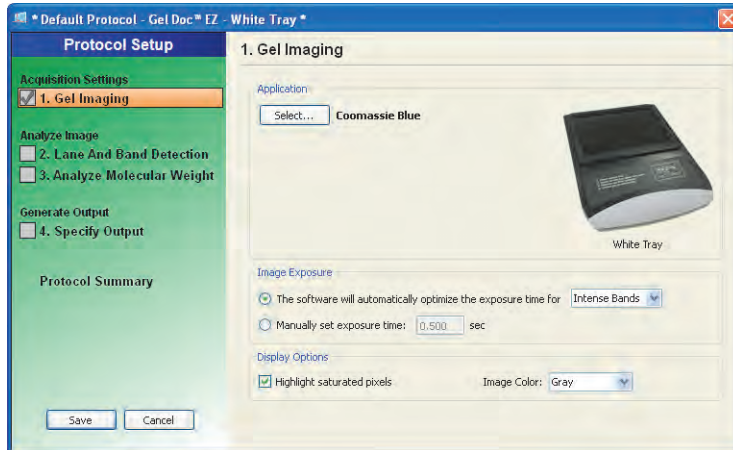
To set up any other protocol

1. Verify that Gel Doc EZ appears on the status bar with a green check mark.
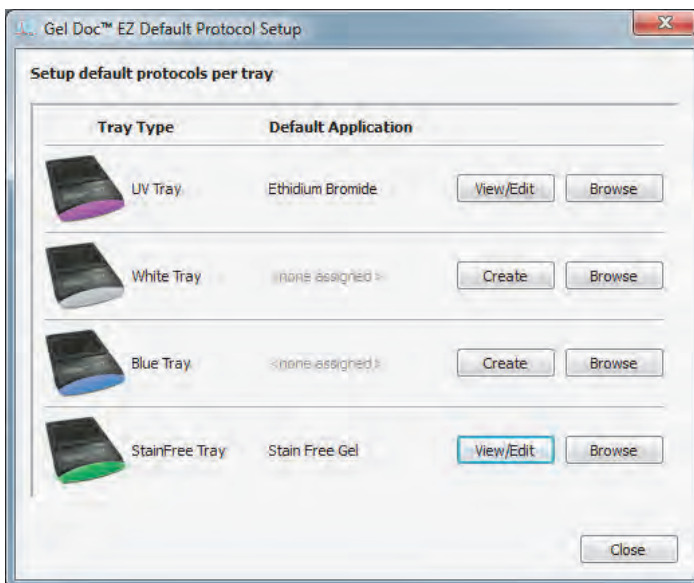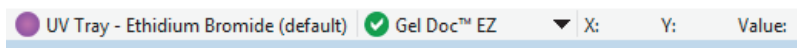

The green check mark indicates that the instrument is connected to the computer running Image Lab software and is communicating with Image Lab.

2. Click New Protocol on the toolbar.
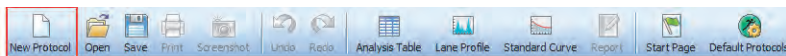
The Protocol Setup screen appears with Gel Imaging selected in the left pane.

The title bar displays the protocol name. You can change this name when you save the protocol.

Note: The left pane of the default protocol is green. The left pane of all other protocols is blue.

Configuring Acquisition Settings

The term *application* refers to sample type. The following sample types are supported:

■ Nucleic acid gel, protein gel, or blot

■ Detection reagent — dye or stain

On the Gel Imaging Protocol Setup screen, you can choose from a list of predefined applications for common sample types. These applications have predefined image acquisition settings optimal for each sample type.

To use a dye or stain not listed in the Application dropdown menus, see Creating a Custom Application.

Note: When you first configure default protocols, custom applications do not appear on the Application > Custom dropdown menu. You must first create a custom application in another protocol. Custom applications then populate the Application > Custom dropdown menu for default protocols.

When you select the Stain Free application, you can choose the gel activation time. See Appendix D, Using Bio-Rad Stain-Free Technology.

To configure acquisition settings for any protocol

1. In the Gel Imaging pane, click Select and choose an application that matches the sample type.
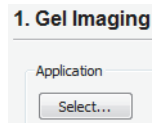
2. (Optional) Choose Custom when you want to do one of the following:

   ■ To create an application that uses a dye or stain not listed on the Application dropdown menus

   ■ To choose a custom application

   ■ To edit or rename a custom application

3. Under Image Exposure, select one of the following options:
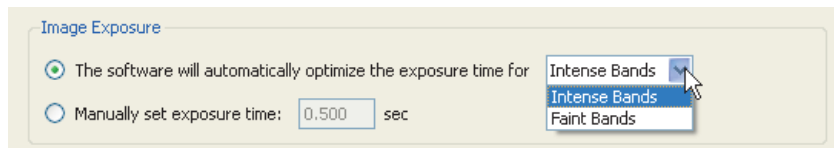
   ■ **Auto Exposure** — estimates an optimal exposure time and ensures the best use of the dynamic range.

   ☐ **Intense Bands** — optimizes exposure for all bands

☐   **Faint Bands** — uses a longer exposure time, making faint bands more visible, but more prominent bands might be overexposed

Tip: After imaging a sample optimized for automatic exposure, the previous exposure time appears. You can use it as a reference point when you set a manual exposure time.
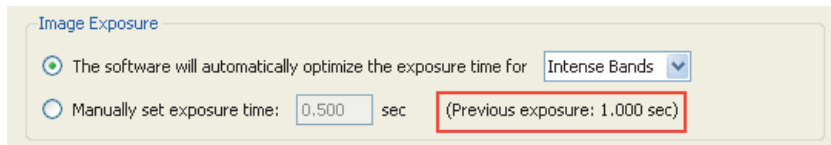
■   **Manual Exposure** — overrides the automated option. Exposure time can range from 0.001–10 sec.

Note: You can view the image exposure time in the Image Info box, which you can access in the Display Toolbox above the on-screen image.

4. Set the Display Options.

■   **Highlight saturated pixels** — displays saturated pixels in red, which indicates how much of the sample image is saturated. Saturated pixels are beyond the maximum quantifiable range of the imaging system. You can change this setting later in the Image Transform dialog box, which you can access in the Display Toolbox.

■   **Image Color** — select a color for the sample image. Viewing the image with a different color scheme can make it easier to see all of its elements. For more information about color choices, see the Image Lab Software User Guide.

5. Do one of the following:

■   Save the protocol and run it with the options you selected.

■   Go to the analysis or output settings.

Configuring Analysis Settings

To analyze the gel or blot automatically, configure the following analysis settings:

- Detect Lanes and Bands

- Analyze Molecular Weight

For information about these settings, see Protocols in the Image Lab Software User Guide.

Configuring Output Settings

You can view or print a single image or report. Image Lab prints to the default printer unless you select another one. For information about customizing reporting options, see Generating Reports in the Image Lab Software User Guide.

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

When you display a report, a scrollable report screen opens in which you can view the image, acquisition settings, and analysis data.

To specify protocol output

1. Select Specify Output in the left pane of the Protocol Setup screen. Output options appear in the right pane.
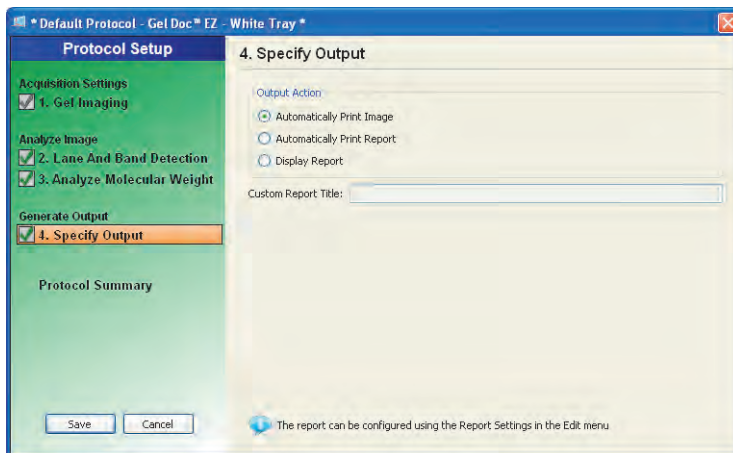
2. In the right pane, choose one of the following:

    ■   Automatically print the image

    ■   Automatically print a report

    ■   Display the report

3. Click Save and close the Default Protocol Setup screen.

Running a Default Protocol

Save the default protocols and close the setup screen before you start the next procedure.

To run a default protocol for one sample

1. Place a sample on the appropriate tray and insert it 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 screen opens and the default protocol runs automatically.

   After the protocol runs, the acquired image appears with the protocol window 0open behind it. The application name appears in the status bar below the gel preview window.

To run a default protocol for several samples

1. Place a sample on the appropriate tray and insert it 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 screen opens and the default protocol runs automatically.

    After Image Lab acquires the image, the Protocol Summary screen opens.

Run button

4. Remove the sample tray and insert a tray with the new sample.

    Important: Ensure that the sample imaging application and tray type match the Protocol acquisition settings.

5. Close the door.

6. On the Protocol Summary screen, click the Run button.

7. Repeat steps 4–6 to run each additional sample.
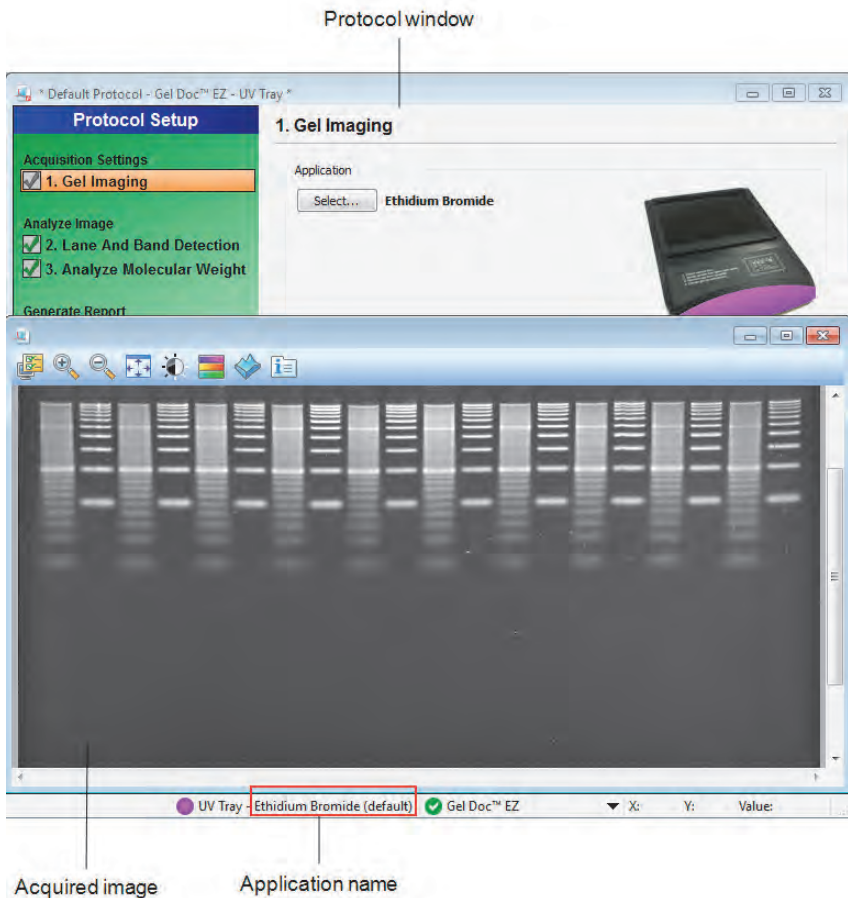
Running a Protocol

In Image Lab software, run a protocol.

To run a protocol

1. Place the sample on the appropriate sample tray and insert it into the imager until the magnet grabs the tray.

2. Close the door.

3. On the toolbar, click Open.

4. Browse to the protocol, select it, and click Open.

   The Protocol Summary screen opens.

   Important: Ensure that the sample imaging application and tray type match the Protocol acquisition settings.

5. Click Run Protocol in the left pane of the Protocol Summary screen. The protocol runs automatically.

After the protocol runs, the acquired image appears with the Protocol screen open behind it.

Protocol window

Acquired image
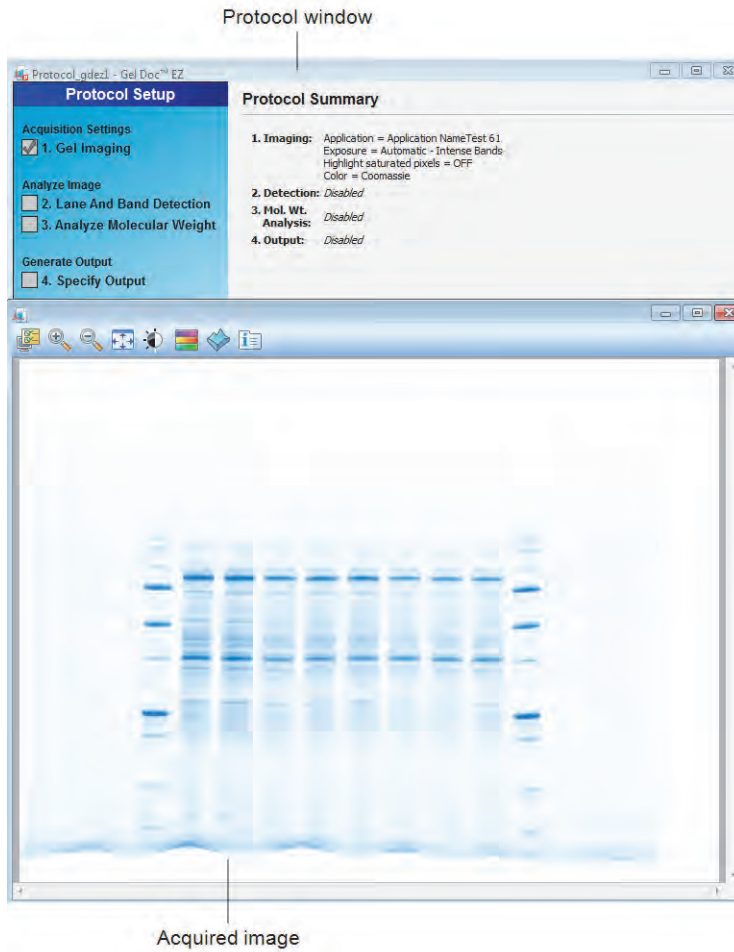
Creating a Custom Application

You can create a custom application using a dye or stain different from those listed in the Application dropdown menus.

Note: When you first configure default protocols, custom applications do not appear on the Application > Custom dropdown menu. You must first create a custom application in another protocol. Custom applications then populate the Application > Custom dropdown menu for default protocols.

To create a custom application

1. On the Protocol Setup screen, select Gel Imaging.

2. In the right pane under Application, click Select and choose Custom on the dropdown menu that appears.

   The Manage Custom Applications dialog box appears.

3. Click New.

   The Create Custom Application dialog box appears.
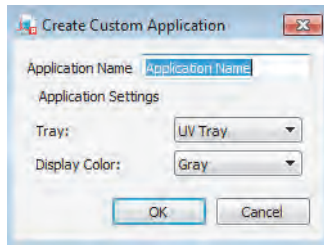
4. Enter a unique application name.

5. Select a tray type.

   Note: Selecting the tray type conveys important information to the instrument about the dye or stain, such as the recommended excitation wavelength. This ensures proper image acquisition.

6. Select a display color.

    Tip: Viewing the image with a different color scheme can make all elements more visible.
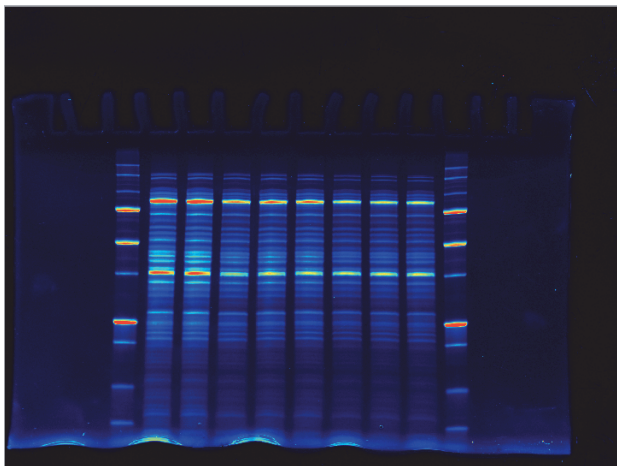
    For more information about color choices, see the Image Lab Software User Guide.

7. Click OK.

Note: If you are not sure how to configure the custom application for a dye or stain, contact Bio-Rad Technical Support.

Editing a Protocol

You can open a protocol, change its settings, and save the protocol with another name. You can also disable a protocol step.

Note: After you edit and save a default protocol, it is no longer considered a default protocol.

A   Maintenance and Specifications

Cleaning the Sample Trays

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

UV-B Fluorescent Lamp Replacement

The UV lamps provide service under normal usage for 4–5 years. When a lamp fails, 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. Leave in place 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 completely removed.

4. On the left side of the instrument locate the single screw that holds the lamp assembly in place. Remove and save this screw.

5. Carefully slide out the lamp assembly.

Although only one lamp bulb might have failed, Bio-Rad recommends replacing all lamps to ensure even illumination and to reduce the need to access the lamps again.
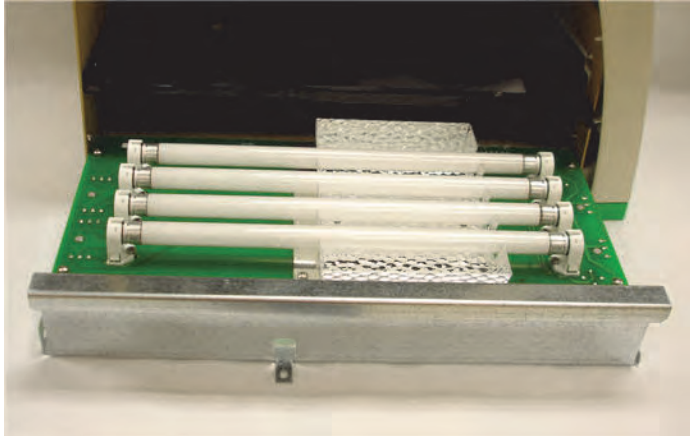
6. Replace all lamps with Standard 302 nm UV lamps (catalog #1708097). Make sure each lamp is properly seated in its holder.

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 ensure that it is fully seated in the instrument.

   If the lamp assembly is not fully seated, the following error message appears the next time it communicates with Image Lab^{TM} 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 this message appears when you attempt to image, the sample tray might 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 when the lamp assembly is not fully seated in the imager. Reseat the lamp assembly as described in UV-B Fluorescent Lamp Replacement.

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. Ensure that the door is fully closed. This message also appears when the instrument cover has been removed.

B   Troubleshooting

Problem	Possible Cause	Solution
Green LED on front panel is off	The AC power cord is not connected.	Connect an AC power cord to the imager and an appropriate wall socket.
	The power is off.	Turn the power switch on.
Front panel LEDs remain flashing	The firmware is not loading.	Call Bio-Rad Technical Support for help.
Image is not visible on the monitor	The sample tray does not contain a sample.	Place a sample on the tray and run the protocol again.
Image is not bright enough	The gel is underdeveloped.	If initially stopped after 2 min, allow the gel to develop for 2–3 more min.
Red LED flashing on front panel	The sample tray not inserted all the way.	Push the sample tray into the imager until the magnet pulls the sample tray.
	The door interlock is broken	Close the door.
	A bulb has failed.	Replace all the lamps according to the instructions in UV-B Fluorescent Lamp Replacement on page 41.
	The communication has been interrupted.	Make sure imager power is on and the USB cable is connected to the PC. Restart the imager.
	The green Run button on the imager front cover was pressed when Image Lab™ software was not running.	The red light stops flashing when Image Lab software starts. Press the green Run button again to initiate imaging.

B | Troubleshooting

C Accessories

Ordering Information

The following table lists catalog numbers and descriptions for all parts available for the Gel Doc™ EZ imaging system, plus all optional accessories and replacement parts. For more information, see the Bio-Rad catalog.

Table 4. Ordering information

Catalog #	Description
1708270	Gel Doc EZ imaging system with Image Lab^{TM} software, compatible with PC or Mac, includes darkroom, camera, cables, Image Lab software; stain-free sample tray # 170-8274; other sample trays available separately
Image Lab Software	
1709690	Image Lab software, Windows/Mac
Optional Accessories	
1708271	UV sample tray
1708272	White light sample tray
1708273	Blue sample tray
1708274	Stain-Free sample tray
1708276	Sample tray holder
	Lamp Kit
1708097	302 nm lamp kit, (6 lamps)

D Using Bio-Rad Stain-Free Technology

Bio-Rad stain-free gels eliminate 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.

When using Image Lab™ software, the Gel Doc EZ imager is stain-free enabled to image the following gels:

- Criterion™ TGX Stain-Free™ precast gels

- Criterion Stain Free™ precast gels

- Mini-PROTEAN® TGX Stain-Free™ precast gels

- TGX Stain-Free™ FastCast™ acrylamide solutions for handcast gels

When trihalo compounds in the gels encounter tryptophan residues, a UV light-induced reaction produces fluorescence, which can be easily detected by the imager in gels or on low fluorescence polyvinyl difluoride (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 technology. The sensitivity of stain-free gels 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%.
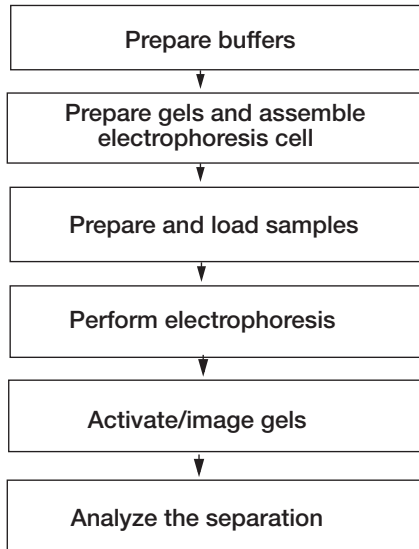
The benefits of stain-free technology include

- 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, which reduces organic waste

■ Visualization of transferred or blotted proteins on low fluorescence PVDF membranes

Stain-Free Workflow

For detailed information about the Activate/image gels step, refer to Chapter 3, Acquiring Images. For all other workflow steps, refer to the Criterion™ Precast Gels Instruction Manual and Application Guide (bulletin #4110001) or to the Mini-PROTEAN® Precast Gels Instruction Manual and Application Guide (bulletin #1658100).

```
┌─────────────────────────────┐
│      Prepare buffers        │
└─────────────────────────────┘
              ↓
┌─────────────────────────────┐
│  Prepare gels and assemble  │
│     electrophoresis cell    │
└─────────────────────────────┘
              ↓
┌─────────────────────────────┐
│   Prepare and load samples  │
└─────────────────────────────┘
              ↓
┌─────────────────────────────┐
│   Perform electrophoresis   │
└─────────────────────────────┘
              ↓
┌─────────────────────────────┐
│     Activate/image gels     │
└─────────────────────────────┘
              ↓
┌─────────────────────────────┐
│    Analyze the separation   │
└─────────────────────────────┘
```

Electrophoresis with Stain-Free Gels

Stain-free gels are made and packaged without sodium dodecyl sulfate (SDS), allowing them to be used for both SDS and native polyacrylamide gel electrophoresis (PAGE) applications.

To perform electrophoresis with stain-free gels

1. Prepare the sample and running buffers.

2. Set up the electrophoresis cell.

3. Perform the run.

Imaging Gels

Use unstained standards with stain-free gels, as some prestained standards are not compatible with stain-free technology. To monitor electrophoresis, use a 1:1 mixture of unstained and prestained standards.

Setting up a protocol for stain-free gels is similar to setting up protocols for other applications. Follow the instructions in Creating a Protocol on page 26. Choose one of the following activation times based on the sample and the purpose of your experiment:

- **Gels used in blotting** — use 1 min activation for optimal results when performing western blotting followed by immunodetection.

- **Good sensitivity** — use 2.5 min activation when samples are abundant and when a fully optimized signal-to-noise ratio is not necessary.

- **Best sensitivity** — use 5.0 min activation for detection of proteins that are in low concentration and for the best quantitation 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 been activated for 2.5 min, activating it for another 2.5 min might improve it. But activating an image for more than 5 min will not.

Imaging Blots

To blot stain-free gels, use standard blotting procedures as described in the instruction manual you are using. Use only PVDF membranes with low background fluorescence, as membranes other than low fluorescence PVDF can result in high background or low sensitivity with the imager.

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

Glossary

Aspect ratio	The ratio of the width to the height of an image.
CCD	(Charge-coupled device) A light-sensitive silicon chip used as a photodetector in Gel Doc^{TM} EZ 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.
Example precision	The number of decimal places chosen for displaying a measurement.
Flat fielding	An average intensity computation that compensates for nonuniformities generated by an instrument.
Histogram	A graphed representation of the brightness, or gray value, of an image.
Imager	The instrument without Image Lab software.
Imaging system	The instrument connected to a computer running Image Lab™ software.
Native charge density	The inherent electrical charge of a protein without the addition of SDS.
pI	Isoelectric point; the pH at which a protein molecule carries no net charge.
Quantitative imaging	Determines the quantity of a protein's components through analysis of the pixel values in a digital image of the sample.
UV-B	The range of ultraviolet light used by the system.
UV transilluminator	The part of the imager that transmits UV light through a sample.

Glossary

BIO·RAD

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