Criterion Stain Free™ Imaging System

Instruction Manual

BIO-RAD

Criterion Stain Free™ System

Instruction Manual

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NOTICE

The Criterion Stain Free™ 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 the user's 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

Your Criterion Stain Free 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 to keep our system and peripheral 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 would be pleased to answer any questions you may have.

Bio-Rad Resources and References

Bio-Rad Laboratories provides many resources for scientists. The following web sites 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 phone numbers:

Toll free: 1-800-4BIORAD or 1-800-424-6723
Fax:1-510-741-5802
e-mail: lsg.techserv.us@bio-rad.com

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

Safety and Regulatory Compliance

The Criterion Stain Free™ Imaging System 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 the user's accidental exposure to UV radiation.

If the Criterion Stain Free Imager is used in a manner not specified by Bio-Rad Laboratories, the protection provided by the Criterion Stain Free 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 Criterion Stain Free 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

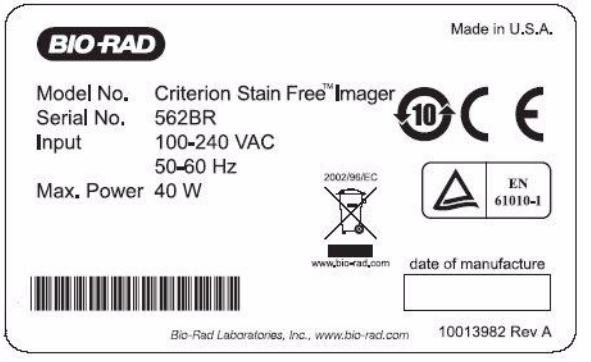


Figure 1. Stain Free Imager Certification Label

For easy customer access, the serial number information is 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.

Table 1. 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” in capital letters.
	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 “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 are located in the main text, are preceded by “Warning” and are accompanied by the Warning Symbol in the left margin.

The technical specifications of the Criterion Stain Free Imager are in the table below:

Table 2. Technical Specifications

Hardware Specifications	
Image Area	13.9 x 9.4 cm
Excitation Source	Trans UV 302
Detector	CCD
Image Resolution (Horizontal x Vertical)	1392 x 940 pixels
Image Pixel Size (in Microns)	100 x 100 um
Dynamic Range	3.0 Orders
Pixel Density (gray levels)	4096
Flat Fielding	Yes
Instrument Size	43 x 28 x 38 cm
Instrument Weight	7.3 kg
Software Specifications	
Operating System Compatibility	Windows XP SP2 or higher or Windows Vista
	Mac OS 10.5 or higher
Image File Size (MB)	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% non-condensing

Table 3. Equipment Ratings

Input voltage range	100-240 VAC
Input frequency range	50-60 Hz.
Power	40 W
The Criterion Stain Free Imager is for indoor laboratory use only.	

Connector Descriptions

USB-A connector is used for communication with a PC.

USB-B connector is used for the instrument interface.

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.

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 Criterion Stain Free Imager. 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 Criterion Stain Free 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.

Table of Contents

1 Criterion Stain Free System Overview

This manual describes the Criterion Stain Free™ System, which consists of the Criterion Stain Free™ Imager, Criterion Stain Free™ Gels, and Image Lab™ software.

The Criterion Stain Free System is a reproducible and fast label-free SDS-PAGE/Native PAGE system that eliminates time-consuming staining and destaining steps. It has been developed for researchers who are running electrophoresis gels as a detection method for protein characterization, purity assessment, profiling, and quantitation of proteins separated by SDS-PAGE or Native PAGE.

Exposure of the Stain Free gel proteins to ultraviolet light causes a Stain Free gel component to react with the gel proteins, allowing the proteins to be visualized.

The Image Lab image acquisition and analysis software works with the Criterion Stain Free Imager and Criterion Stain Free Gels to create an automated and time-saving system to image and analyze electrophoresis gels. The data can be viewed, modified, and reported using Image Lab.

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

Researchers can run a default protocol or easily design their own. Image Lab software allows you to view your analyzed data, adjust the analysis, and produce customized reports that show precisely what settings have been applied, in order to assure repeatable results.

Applications

Image Lab software has been designed to perform automated protocols for routine gel imaging documentation and analysis.

Gel Documentation

Gel electrophoresis is a common way to separate, identify, and purify proteins. The Criterion Stain Free System allows users to image and print gels for documentation in laboratory notebooks, or to export images for publication or presentations.

Purity Assessment

The relative quantity of a protein band in a given sample is used to assess purity. This is expressed as the percent of total signal in defined bands, in any given lane.

Molecular Weight Assessment

SDS-Polyacrylamide gel electrophoresis is used to separate proteins according to their size. Protein molecular weight standards provide a reference for estimating the molecular weight of the proteins being tested.

Overview of the Imaging Process

There are two ways to image your gel on the Criterion Stain Free Imager. Image Lab must be open on your PC, and the Imager must be on.

You can run the *default* protocol included with Image Lab, which activates and images the gel with one touch of a button, or *customize* a protocol based upon your exact requirements.

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 you feel the magnet grab the tray.
3. Press the green Run button on the front panel of the Imager. Alternatively, you can press the Run Default Protocol button in the Quick Start Guide. The default protocol will run automatically. See “Running the Default Protocol” on page 33 for full information.

To run a custom 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.
3. Select “Create Protocol” from the Image Lab Quick Start button, or “File > New Protocol”. Select the parameters for your protocol. Refer to “Creating and Running Custom Protocols” on page 34 for full information.

4. Click the green Run Protocol button on the Protocol Steps screen to run the protocol.

5. Depending upon the protocol you have specified, the software will control the imager to activate the sample, analyze an image, then generate and print a report.

Criterion Stain Free Imaging System

The Criterion Stain Free 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 sample tray and a one-touch button to image your gel using either a default or custom protocol.

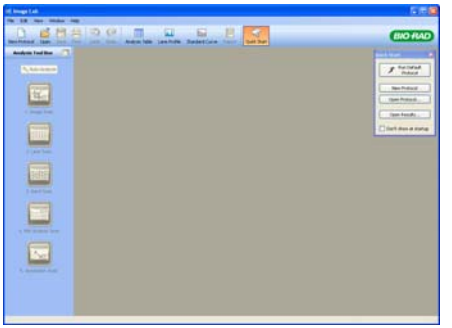
Criterion Stain Free Imaging System, catalog # 170-8160

Criterion Stain Free Gels

These patent-pending gels contain a chemical, which in combination with UV light exposure from a Criterion Stain Free Imager, allows detection of proteins containing tryptophan. These gels eliminate the time-consuming staining and destaining steps required by other protein detection methods.

This manual provides instructions for using the Criterion Stain Free Gels. See Chapter 3, “Criterion Stain Free Gels” for more information.

Image Lab Software



Minimum configurations for Image Lab software:

- Windows XP or Vista operating system with Pentium-4 or later processor and 1 GB RAM
- Macintosh OSX 10.5 or later operating system, Intel processor and 1 GB RAM.

Refer to Chapters 4 through 9 for instructions on using Image Lab software.

Setting Up the System

Installing the Image Lab Software

MACINTOSH

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 Image Lab.dmg file.
4. Drag the Image Lab application icon into the Applications folder.
5. Double-click the Image Lab icon from your Applications folder, to open the application.

WINDOWS XP OR VISTA

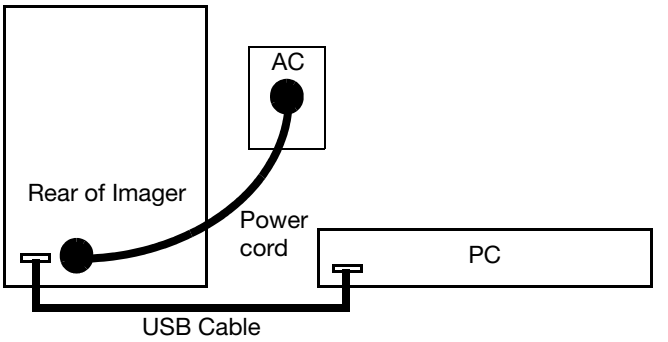
1. Place the Image Lab software CD in your CD-ROM drive. The installation should start automatically. If not, open “My Computer” and click your CD drive icon. Double-click *setup.exe* and execute.

2. When the Image Lab software icon appears on your desktop, double-click to open the software.

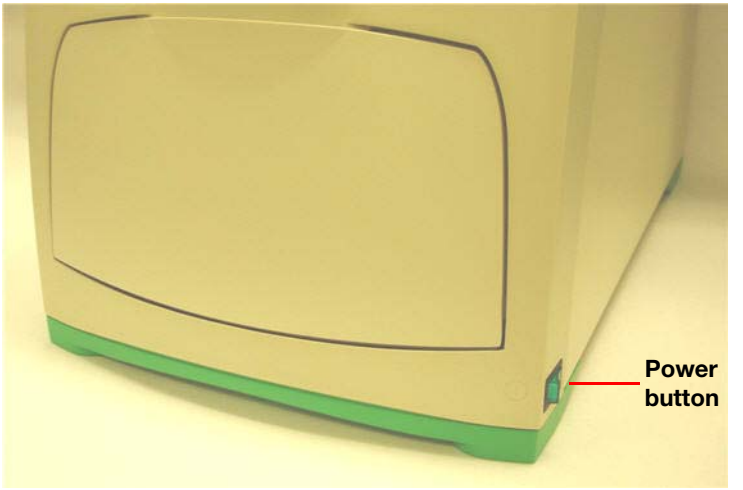
Connecting the Imager to the Computer

1. Install the Image Lab software before connecting the Criterion Stain Free Imager to a PC computer.
2. Connect the Imager to the PC, using the USB cable provided with the system.
3. Connect the Criterion Stain Free Imager to AC power, using the provided power cord.

See diagram below:



4. Turn on power to the Imager using the switch on the side of the instrument. See the picture below:

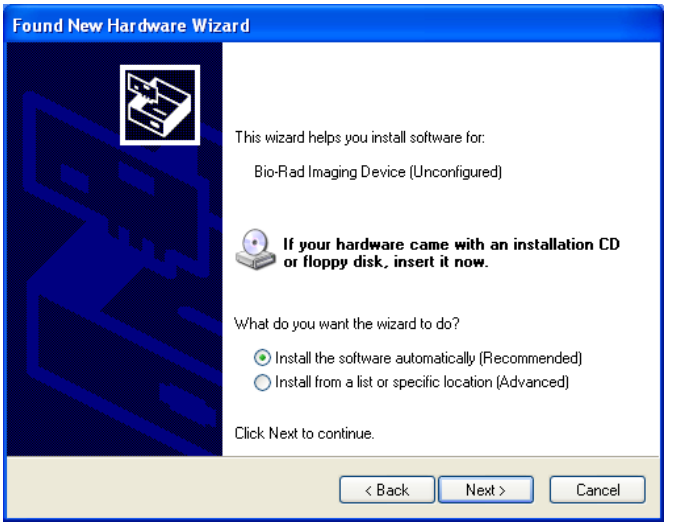


IF YOUR COMPUTER IS RUNNING WINDOWS XP:

After the power is turned on, the PC will automatically recognize the Imager, and the software will prompt you to install the drivers. The screen below appears:

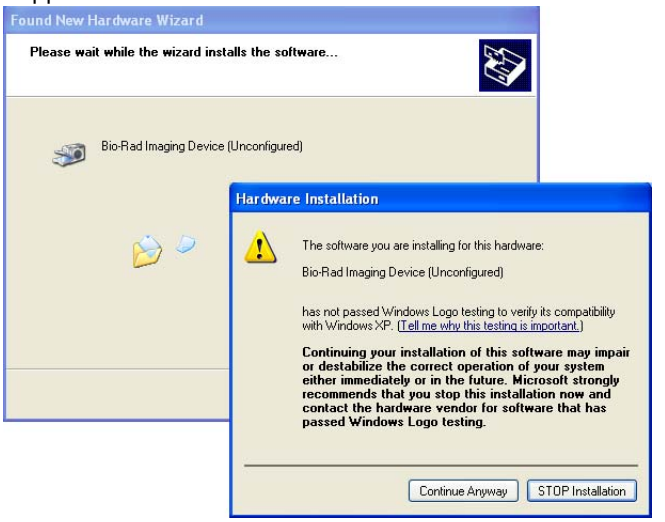
5. Select “No, not this time”, and then, “Next”.

The screen below appears:



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

The screen below appears:



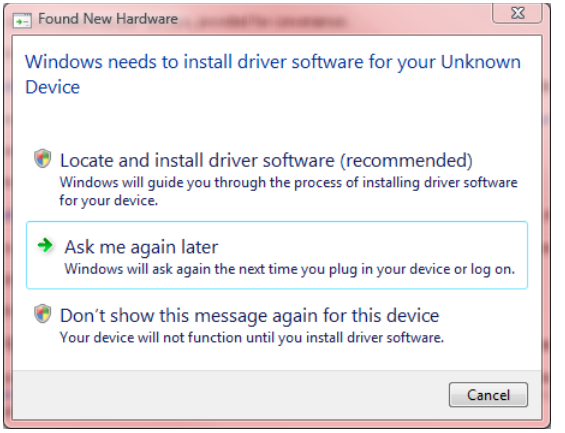
7. Click on “Continue Anyway”. Windows will install the driver.

8. Click on the “Finish” button when it appears. A message appears briefly on the lower right corner of the screen indicating “Your new hardware is ready to use”.

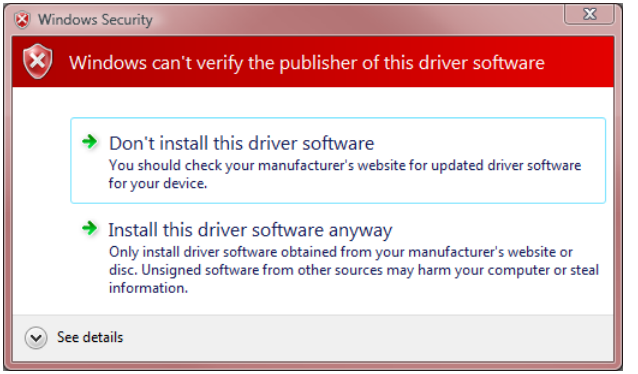
   NOTE: Windows XP may prompt you to go through the driver installation a second time. If this happens, repeat steps 5 through 8, until you receive the “Your new hardware is ready to use” message.

IF YOUR COMPUTER IS RUNNING VISTA:

After the power is turned on, the PC will automatically recognize the Imager, and the software will prompt you to install the drivers. This screen appears:



1. Click “Locate and install driver software”. Vista finds the drivers automatically.
2. You may see this screen. If you do, click “Install this driver software anyway”. The drivers will be installed.



3. Click on the “Finish” button when it appears. A message appears briefly on the lower right corner of the screen indicating “Your new hardware is ready to use”.

   NOTE: Windows Vista may prompt you to go through the driver installation a second time. If this happens, repeat steps 1 through 3, until you receive the “Your new hardware is ready to use” message.

2 Criterion Stain Free Imager

The Criterion Stain Free™ Imager is a quantitative imaging system for capturing high resolution digital images of Bio-Rad Criterion Stain Free™ gels. Using low noise CCD technology, exceptionally uniform UV-B excitation, and a highly efficient optical design, the Criterion Stain Free Imager offers researchers the sensitivity, uniformity, flexibility, and dynamic range required for the analysis of electrophoretically separated proteins.

With unique Criterion Stain Free Gels, samples are activated and imaged using automated protocols, increasing laboratory throughput, decreasing workflow time, and eliminating generation of hazardous waste solutions.

Flexibility and Ease of Use

The Criterion Stain Free™ System has a very flexible design. The Criterion Stain Free Imager offers one-button gel activation using a default protocol that a user can run right after installation.

The Image Lab™ software allows substantial flexibility in the presentation of captured images, and provides many data analysis tools. These include automated lane and band finding, molecular weight determination, and relative quantity analysis.

To get started, simply connect the instrument to a PC computer where the software has been installed, open the Image Lab software, load the sample, and press the green Run button on the front of the imager to run the default protocol.

You can watch the first image of your gel progress through the development process, obtaining usable data within 2.5 minutes. You can choose to develop the gel for up to 5 minutes, to ensure your sample is fully developed. The level of gel development is user controllable, allowing for data that suits your needs.

Imager Description

The Criterion Stain Free Imager combines several key components into a unique, powerful, and fully integrated image analysis system:

CCD Technology

The Criterion Stain Free Imager uses CCD technology for image capture and improves image quality by reducing background noise, and enhancing the signal-to-noise ratio.

Unique Transillumination Source

The Criterion Stain Free Imager system incorporates unique UV transillumination, which serves the dual functions of developing the sample and exciting the fluorescent protein product.

Removable Sample Tray

The Criterion Stain Free Imager has a fully removable sample tray that incorporates a sample platen made of UV-B filter glass. Because it is removable, you can take it to your lab bench to place the gel on the sample tray.

USB Interface

The Criterion Stain Free Imager connects with the PC computer using a USB 2.0 interface.

Mechanical Description

The Criterion Stain Free Imager consists of the following main hardware components:

- Light-tight enclosure, which contains a UV transilluminator
- CCD camera
- Low distortion lens
- Removable sample tray with UV-B filter glass
- Electronics for controlling the UV lights and camera

	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.

Maintenance

Cleaning the Sample Tray

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

UV-B Fluorescent Lamp Replacement

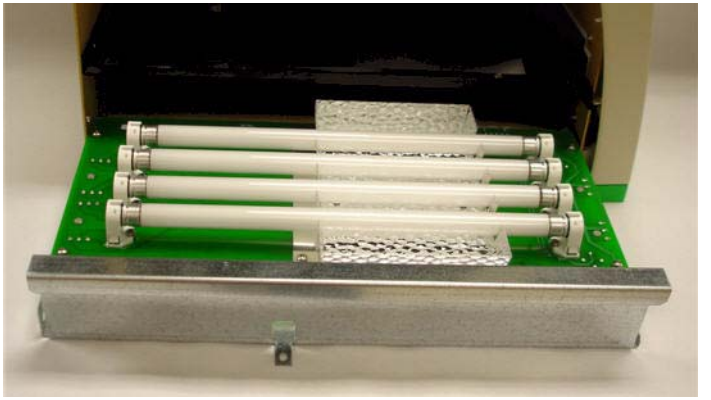
The UV-B lamps will provide service under normal usage for 4-5 years. In case of a lamp failure, you will see one of the following error messages:

- *“Fault in UV bulbs Bank 1&2.”*
- *“Fault in UV bulbs Bank 1”*
- *“Fault in UV bulbs Bank 2”*

Follow these instructions to replace the lamps.

1. Turn off the instrument and unplug the AC cord.
2. Remove and save the screws holding the top cover at the back of the instrument.
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 there is a single screw holding 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 reduce the need to access the lamps again.



6. Replace all lamps with UV-B fluorescent lamps (Bio-Rad PN 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, you may get the following error message: *"Light tray not detected"*

Other error messages you may see are explained below:

"Sample tray not detected"

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

"Light tray not detected"

This error message will appear if the lamp assembly is not fully seated into the imager. You will need to replace the lamp assembly as described above.

"Door was opened during imaging"

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

3 Criterion Stain Free Gels

Because of Criterion Stain Free™ Gels' unique composition, allowing gel imaging without staining, you must use them with the Criterion Stain Free™ Imager. The following table details Bio-Rad Stain Free Gels.

Available Criterion Stain Free Gels

Formulation	Comb	Catalog #
10% Tris-HCl	12-well	345-1012
10% Tris-HCl	18-well	345-1018
4-20% Tris-HCl	12-well	345-0412
4-20% Tris-HCl	18-well	345-0418
4-20% Tris-HCl	26-well	345-0426
8-16% Tris-HCl	IPG+ 1 well	345-8161

Criterion Comb Configurations

Comb	Load Volume	Comments
12+2 well	45 µl with two 15 µl reference wells	Multichannel pipet compatible
18-well	30 µl	
26-well	15 µl	Multichannel pipet compatible
IPG+1 well	11 cm ReadyStrip™ IPG strip with one 15 µl reference well	

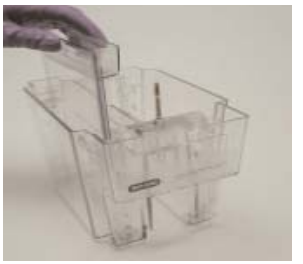
Gel Specifications

Gel material	Polyacrylamide
Gel dimensions	8.7 x 13.3 cm
Gel thickness	1.0 mm
Resolving gel height	6.5 cm
Cassette dimensions	10.6 x 15.0 cm
Cassette material	Styrene copolymer
Comb material	Polycarbonate
Storage tray material	PET
Upper running buffer volume	60 ml
Lower running buffer volume	800 ml
Storage conditions	Store flat at 4°C; DO NOT FREEZE

Setup and Basic Operation

Setting Up and Running Criterion Gels

1. Each Criterion gel is packaged individually in a plastic storage tray. Remove the cover by gently pulling the square corner tab up and diagonally across the package. Remove the gel from the package.
2. Remove the comb and gently rinse the wells with double distilled H_2O or running buffer.
3. Remove the tape from the bottom of the cassette by pulling the tab across the gel.



4. Insert the Criterion gel into one of the slots in the Criterion cell tank. Ensure that each integral buffer chamber faces the center of the cell.

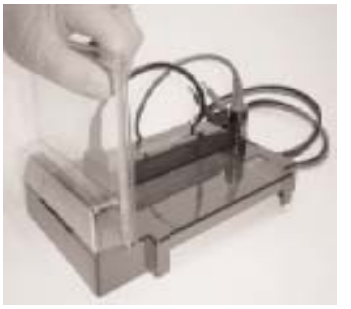
5. Fill each integral buffer chamber with 60 ml running buffer.

6. Load samples using a Hamilton syringe or a pipet with gel loading tips. A sample loading guide can be placed on the outer edge of the cassette, to aid in aligning pipet tips with the wells. This is especially useful with multichannel pipets.

7. Fill each half of the lower buffer tank with 400 ml of running buffer to the marked fill line.
8. Place the lid on the tank, aligning the color-coded banana plugs and jacks. See “Running Conditions” (page 21) for power conditions.

Opening Criterion Cassettes and Removing the Gel

1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the lid from the tank, and remove the Criterion gel from the cell. Pour off and discard the upper running buffer.
3. Invert the cassette and place the integral buffer chamber over the cassette-opening tool built into the Criterion cell lid.



4. Firmly press down on the cassette to crack the cassette welds on both sides of the cassette. The cassette will split open approximately 1/3 of the way. Alternatively, the gel cassette can be opened by sliding the tapered back of the comb into the slits on either side of the cassette.
5. Pull the two halves of the cassette apart to completely expose the gel.
6. Carefully lift the gel from the cassette.

Placing the Gel on the Sample Tray

Place the gel onto the sample tray immediately after removal from the Criterion cassette.

1. Place a few milliliters of water onto the sample tray, so that the gel can slide easily. Don’t use so much water that it may spill inside the instrument.
2. Place the gel on the sample tray, taking care to square the gel edges and eliminate any bubbles which can distort the gel image.

SDS-PAGE

Criterion Tris-HCl gels provide a versatile system for the separation of proteins by molecular weight (SDS-PAGE conditions) or charge-to-mass ratio (Native conditions). (See page 20 for Native PAGE applications and protocols.) This versatility is possible because Criterion Tris-HCl gels are made without SDS, allowing the sample buffer and running buffer to determine the separation mechanism. Historically, SDS-PAGE systems contained SDS in both the gel and running buffer. Reproducible SDS-PAGE separations are performed in gels lacking SDS, provided the sample buffer and running buffers contain sufficient SDS to saturate the proteins during electrophoresis. The recommended concentration of SDS is 2% in sample buffer and 0.1% in running buffers.

SDS-PAGE uses discontinuous chloride and glycine ion fronts to form moving boundaries that stack and then separate SDS coated polypeptides by molecular weight. Protein samples are prepared in a reducing denaturing sample buffer containing either 2-mercaptoethanol or dithiothreitol (DTT) as the reducing reagent, and heat and SDS are used to denature the proteins. 2-mercaptoethanol and dithiothreitol eliminate protein secondary structure by reducing disulfide bonds. SDS minimizes charge variability among proteins, giving them the same charge-to-mass ratio and forcing them into rod-like shapes. This effectively eliminates the effects of protein conformation and native charge density on the electrophoretic migration distance. Molecular weight determinations are obtained by plotting the logarithm of protein molecular mass vs. the relative mobility (Rf = distance migrated by protein/ distance migrated by dye front).

Criterion Tris-HCl Composition

Gel buffer	0.375 M Tris-HCl, pH 8.6
Cross linker	2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.6
Stacking gel	4% T, 2.6% C
Shelf life	~12 weeks; individual expiration date is printed on each cassette

Criterion Tris-HCl Gel Separation Guide

Tris-HCl gels are available in a wide selection of single percentages and gradients for the separation of proteins by SDS-PAGE.

Tris-HCl Gels	Optimal Separation
10%	30-150 kD
4-20% gradient	10-200 kD

SDS-PAGE Buffers

Running buffer, 1x	25 mM tris
	192 mM glycine
	0.1 SDS
	DO NOT ADJUST pH
Sample buffer	62.5 mM Tris-HCl, pH 6.8
	2% SDS
	25% glycerol
	0.01% Bromophenol Blue
	5% 2-mercaptoethanol or 350 mM DTT (added fresh)

Sample Preparation

Determine the appropriate protein concentration of your sample based on the detection method and load volume used. Dilute 1 part sample with 1 part sample buffer and heat at 95ºC for 5 min.

Running Conditions

Power conditions	200 V constant
Starting current	90-120 mA/gel
Final current	35-55 mA/gel
Run time	55 minutes

Native PAGE

Criterion Tris-HCl gels are made without SDS, allowing separation of proteins in their native conformation. The nonreducing and nondenaturing environment of Native PAGE allows the detection of biological activity and can improve antibody detection. Native PAGE can also be used to resolve multiple protein bands where molecular mass separation by SDS-PAGE would reveal only one.

Native PAGE uses the same discontinuous chloride and glycine ion fronts as SDS-PAGE to form moving boundaries that stack and then separate polypeptides by charge-to-mass ratio. Proteins are prepared in a nonreducing, nondenaturing sample buffer that maintains the proteins' secondary structure and native charge density. Native PAGE is not suitable for accurate molecular weight determination due to the variability of charge-to-mass ratio among different proteins.

Criterion Tris-HCl Composition

Gel buffer	0.375 M Tris-HCl, pH 8.6
Cross linker	2.6% C
Storage buffer	0.375 M Tris-HCl, pH 8.6
Stacking gel	4% T, 2.6% C
Shelf life	~12 weeks; individual expiration date is printed on each cassette

Criterion Tris-HCl Gel Separation Guide

Native PAGE separates by charge-to-mass ratio, making individual protein migration protein dependent. Optimal Tris-HCl gel percentages will have to be determined experimentally.

Native PAGE Buffers

Running buffer, 1x	25 mM Tris
	192 mM glycine
	DO NOT ADJUST pH
Sample buffer	62.5 mM Tris-HCl, pH 6.8
	25% glycerol
	0.01% Bromophenol Blue

Sample Preparation

Determine the desired protein concentration and load volume of your sample based on the detection method used. Sample preparation for Native PAGE applications requires special consideration. In the absence of SDS, the net charge of a polypeptide will be determined by the pH of the sample buffer.

Only polypeptides with a net negative charge will migrate into a native PAGE Criterion Tris-HCl gel. Most polypeptides have an acidic or slightly basic pI (~3–8). These proteins can be separated using a standard protocol by diluting 1 part sample with 1 part native sample buffer (DO NOT HEAT SAMPLES).

Strongly basic peptides (pI >9) will have a net positive charge in a Native PAGE Criterion Tris-HCl gel. In order for polypeptides with a net positive charge to migrate into a Native PAGE Criterion Tris-HCl gel, the polarity of the electrodes must be changed by reversing the color-coded jacks when connecting to the power supply.

Running Conditions

Power conditions	200 V constant
Starting current-	90-120 mA/gel
Final current-	35-55 mA/gel
Run time	55 minutes

Electrophoresis Troubleshooting

Improper storage of Criterion gels can produce numerous artifacts. Criterion gels should be stored flat at 4ºC.

Avoid freezing or prolonged storage above 4ºC. If you suspect your gels have been stored improperly, DO NOT USE THEM.

Problem	Possible Cause	Solution
Samples do not migrate into gel	Tape at the bottom of the cassette not removed	Remove tape
	Insufficient buffer in integral buffer chamber	Fill integral buffer chamber with 60 ml running buffer
	Insufficient lower electrode buffer	Fill both halves of the lower buffer tank with 400 ml running buffer when running two gels
	Electrical disconnection	Check electrodes and connections
Bands “smile” across gel, band pattern curves upward at both sides of the gel	Excess heating of gel	Check buffer composition
		Completely fill both halves of the lower buffer tank with 400 ml running buffer when running two gels
		Do not exceed recommended running conditions
Skewed or distorted bands, lateral band spreading	Excess salt in samples	Remove salts from sample by dialysis or desalting column prior to sample preparation
	Insufficient sample buffer or wrong formulation	Check buffer composition and dilution instructions
Vertical streaking	Overloaded samples	Dilute sample
		Selectively remove predominant protein in the sample
	Sample precipitation	Centrifuge samples to remove particulates prior to sample loading
Gels run too fast, provide poor resolution, and gel temperature is too high	Running buffer is too concentrated	Check buffer composition
Artifact bands at 60-70 kD	Possible skin keratin contamination	Clean all dishware and wear gloves while handling and loading gel
		Filter all solutions through nitrocellulose
		Use 10% iodoacetamide to eliminate keratin bands

Electrophoresis Supplies

Criterion Gel Accessories

Accessories	Catalog #
Criterion Sample Loading Guide, 12+2 well, 1	165-6006
Criterion Sample Loading Guide, 18-well, 1	165-6007
Criterion Sample Loading Guide, 26-well, 1	165-6008

Buffers

Premixed Running Buffers	Catalog #
10x Tris/Glycine/SDS, 1 L	161-0732
10x Tris/Glycine/SDS, 5 L	161-0772
10x Tris/Glycine, 1 L	161-0734
10x Tris/Glycine, 5 L	161-0771

Premixed Sample Buffers	Catalog #
Laemmli Sample Buffer, 30 ml*	161-0737
Native Sample Buffer, 30 ml	161-0738

* Requires addition of 2-mercaptoethanol or DTT

Individual Reagents

Tris, 1 kg	161-0719
Glycine, 1 kg	161-0718
SDS, 100 g	161-0301
2-Mercaptoethanol, 25 ml	161-0710
Dithiothreitol (DTT) , 5 g	161-0611
Bromophenol Blue, 10 g	161-0404

Protein Standards

Bio-Rad Precision Protein Plus Unstained Standards, 1ml	161-0363
Bio-Rad SDS-PAGE Standards, broad range, 200 µl	161-0317
Bio-Rad SDS-Page Standards, low range, 200 µl	161-0304
Bio-Rad SDS-PAGE Standards, high range, 200 µl	161-0303

Equipment

Criterion Cell, includes tank, lid with power cables, three sample loading guides	165-6001
Criterion Blotter with Plate Electrodes	170-4070
Criterion Blotter with Wire Electrodes	170-4071

4 Image Lab Software Overview

Image Lab™ image acquisition and analysis software works with the Criterion Stain Free™ Imager and Criterion Stain Free™ Gels to create a reproducible, automated and time-saving system for running protocols on electrophoresis gels, without time-consuming staining and destaining steps.

Researchers can run a default protocol or easily design their own. The data can be viewed, modified, and reported using Image Lab.

Image Lab software allows you to view analyzed data, edit the analysis, and produce customized reports that show precisely what settings have been applied, in order to ensure repeatable results.

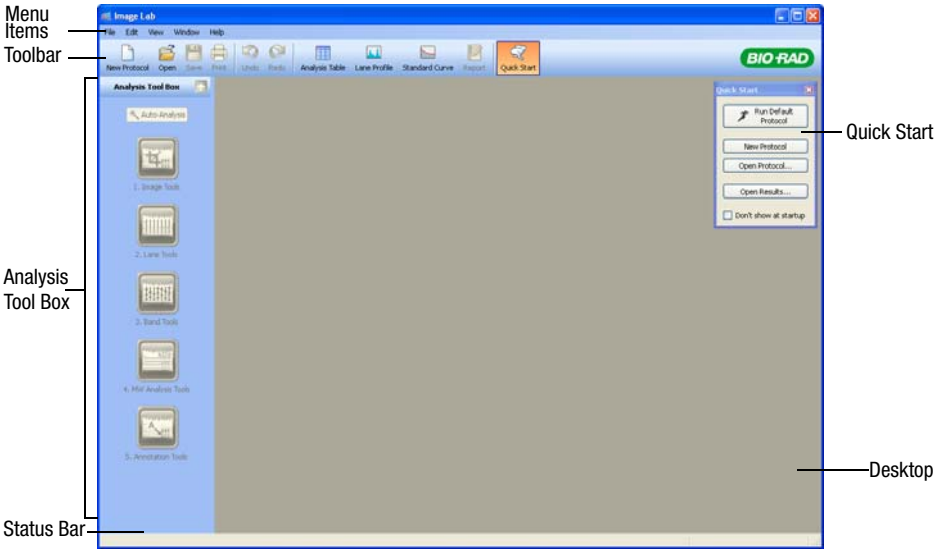
There are two main types of files generated by Image Lab:

- *Protocol* files, which describe the parameters for activating, imaging, and analyzing your gel images. Protocol files are saved with a .ptl extension
- *Results* files, which contain the imaged gel, lanes, annotations, and analysis performed on the gel. Results files are saved with a .scn extension

An imaged gel, run according to a *Protocol File*, generates a *Results File*.

Software Overview

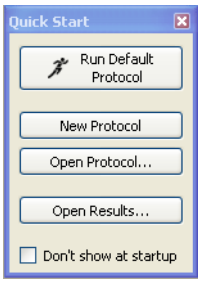
The following illustration shows the Image Lab main window. The paragraphs below describe the major software elements.



Main Window

There is one main window used in Image Lab. Results Files, protocols, and dialog boxes presenting choices all open on the Desktop.

Quick Start



The Quick Start guides users through creating, opening, and viewing protocols and results. It also allows one-button operation for running a default protocol.

Menu Items

The following section describes all of the choices under the Menu Items; File, Edit, View, Window, and Help. Many of these functions are also available in other locations, such as the Toolbar or the Quick Start Guide.

FILE

File > **New Protocol** allows the user to Create a new Protocol through one wizard which contains all of the necessary steps and choices. See Chapter 5, “Designing Protocols” for detailed instructions. The Protocol can also be altered, and stored for reuse.

File > **Open** browses the user’s file system to retrieve a previously-saved Protocol File or Results File.

File > **Save As** allows the user to name and store a Protocol or Results File in their file system. Protocols are stored with a .ptl suffix. Results Files are stored with a .scn suffix.

File > **Default Protocol** gives the user a choice of Opening or Running the Default Protocol.

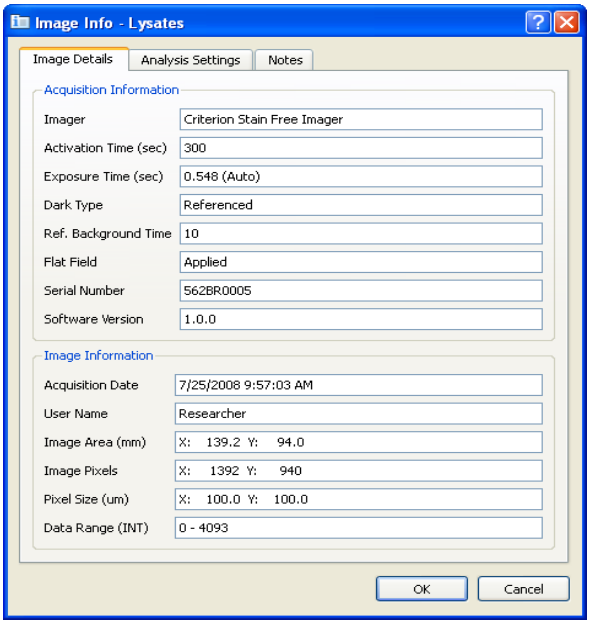
File > **Close** gives the user a choice of closing one desktop window, or closing all of the windows.

File > **Export** allows the user to export gel images or Analysis Tables. There are four export choices:

- **Raw Image** - Uses a .tif when you want all gel image data to be retained. This option creates a large file, but allows you to analyze the image in other software such as InfoQuest FP or Quantity One®
- **Displayed Image** - You can choose between .bmp, .png, and .jpg formats when exporting a gel image. The gel will display with any lanes, bands, and annotations, but cannot be analyzed in other software. Exporting as a .jpg produces the smallest file, but the conversion to .jpg loses some data. This format should only be used for export of visual information for presentation software, such as Power Point or Word.
- **Analysis Table to Excel** - If you have this program installed on your customer, Excel will open with your Analysis Table displayed as a spreadsheet.
- **Analysis Table to File**- This choice exports as a CSV (comma-separated values) file, so your Analysis Table can be opened in a database application.

See Chapter 9 for complete information about exporting your files.

File > **Image Info** shows information about the individual gels, such as acquisition date, the data range, and acquisition details, such as the activation time used. There are three tabs (Image Details, Analysis Settings, and Notes) which reveal specific information. The user can enter findings into the Notes tab.



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

File > **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/time it was printed. The usual Windows Print screen is available as well, which lets you select a printer, and the number of copies.

EDIT

Edit > **Undo** undoes the last user action.

Edit > **Redo** restores the last user action, after an Undo.

Edit > **Preferences** contains two tabs: Protocol Settings and Colors.

- The Protocol Settings tab contains some presets for naming your Results Files. You can choose to include a designated Prefix, User Name, Date and/or Time in the name of your Results files.

- The Colors tab allows you to choose colors for the graphic elements in your gels, such as Lane Fame, Lane, Band, Band Attribute and MW Legend. This functionality ensures that these elements will be visible, whatever colors your gels are.

VIEW

View > **Image Overview** opens a small window which shows your entire gel image, and a red rectangle outlining the area visible in the larger main window. This is useful when you are zoomed into a small section of your image.

View > I**mage Transform** opens a window showing a histogram, where you can adjust the light and dark values of your gel image.

View > **Operations History** opens a window showing sequential actions performed both by the user and the software.

WINDOW

The Window controls allow you to show and hide multiple open results files on your desktop.

Window > **Tile** aligns all open results files so that they are all visible at once.

Window > **Cascade** stacks all open results files and protocols with overlapping title bars, so that each one can be easily chosen for view.

Window > **Next** cycles through all open results files from oldest to newest.

Window > **Previous** cycles through all open results files from newest to oldest.

HELP

Help > **Image Lab Help** displays built-in help which runs on both Windows and Macintosh systems. You can find answers quickly using the task-based clickable interface.

Help > **User Guide** displays this User Guide, available in .pdf form for printing.

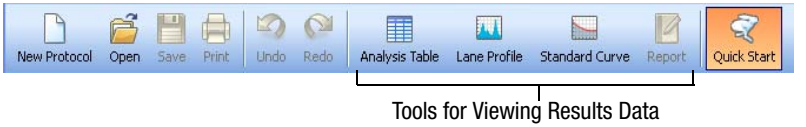
Help > **About** offers Image Lab software version information and release date.

Desktop

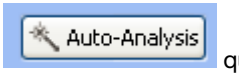
The desktop is the gray area in the main window where protocols and results files are displayed. You can choose between many open Results files and/or Protocols by clicking on the dark blue bar at the top of the selected Window. The Tool Box, Analysis Table Lane Profile and Standard Curve screens always show analysis from your selected Results File or Protocol.

Toolbar

Many of the tools available elsewhere in the software can be chosen directly by clicking on these icons. The tools for Viewing Results Data are shown below.These tools are described in detail in Chapter 6, “Viewing Results” on page 43.



Analysis Tool Box

The Auto Analysis button 

quickly analyzes images which have not been analyzed previously.

The rest of these tools customize the *analyzed* data.

NOTE: A Results File must be active (that is, the title bar is dark blue) in order for any of these analysis tools to be available for use on your Results Files.

1. **Image Tools** allows the user to flip and rotate images, crop, transform, and change the image colors of the results files. All analysis will be deleted.

2. **Lane Tools** control the lane detection function, allowing the user to resize, adjust, and bend the lanes.

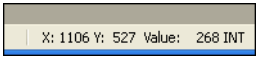
3. **Band Tools** detect, adjust, add or delete the bands.

4. **Molecular Weight Tools** allow the user to choose standard samples, assign standard lanes, and choose a regression method.

5. **Annotation Tools** are useful for drawing attention to any area of a gel that is of interest to the researcher.

These tools are all described in detail in Chapter 6, “Viewing Results” (page 43).

Status Bar



The status bar in the lower right-hand corner of the main window shows X and Y values for the cursor position on a results file. The INT (intensity) values show a numerical value for the image intensity at the mouse position. The maximum data range is 0 and 4095, but will vary according to the values contained within your image.

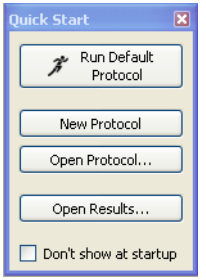
See “Generating Reports” on page 65 for information about the options available when you generate your report.

5 Designing Protocols

Image Lab™ software can be used to run a simple default protocol, or to design custom protocols with a wide variety of settings.

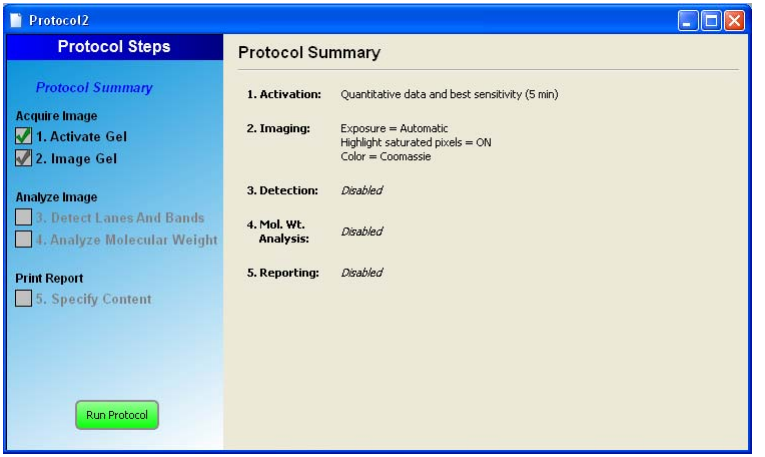
Running the Default Protocol

Run the Default Protocol simply by clicking the Run Default Protocol button on the Quick Start Guide, or by choosing File > Default Protocol > Run from the menu bar. A third option is pressing the green Run button on the Imager.



The original Default Protocol has preset Activation and Imaging settings, and does not include any Band or Lane Detection, Molecular Weight Analysis or Reporting functions.

To change the settings of the default protocol, use File > Default Protocol > Open Default Protocol from the menu bar. Make and save your changes without renaming the Protocol. This customized file is now your Default Protocol.



Creating and Running Custom Protocols

You start your new protocol by choosing the New Protocol box on the Quick Start Guide, or by choosing New Protocol from the toolbar or menu bar. Custom Protocols involve choosing many parameter settings.

Protocol Summary

The Protocol Summary screen (shown above) displays first, showing the current parameters.

Protocol Processes

Protocols involve choices under the three major processes; Acquiring an Image, Analyzing the Image Gel and Printing a Report.

Protocol Steps

There are steps under each process where parameters are set. The choices available under each step appear as each Protocol Step is chosen.

To make choices under a protocol step, click on its description, such as “3. Detect Lanes and Bands”.

To enable or disable any step, click on its checkbox. The exception to this is “2. Image Gel”, because every protocol will image a gel.

Acquire Image

STEP 1. ACTIVATE GEL

ACTIVATION OPTIONS

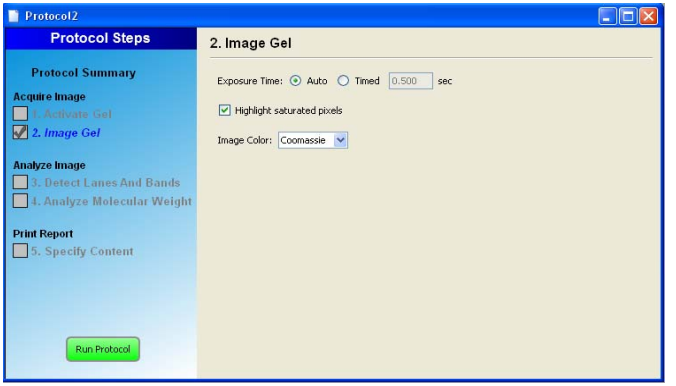
Visualizing the proteins requires activation of the gel. Exposure of the Stain Free gel proteins to ultraviolet light causes a Stain Free gel component to react with the gel proteins.

There are two activation time options, one that runs for 2.5 minutes and one that runs for 5 minutes. This choice is based upon your sample and the purpose of your experiment:

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

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

Step 2. Image Gel



Exposure Time

Image Lab will acquire an image using the optimal exposure time, if you select the Auto button. This option will ensure the best use of the dynamic range.

Use a longer exposure time to visualize very faint bands. That may cause saturation (overexposure) of more prominent bands, so they cannot be quantified. Exposure time can range from 0.001 to 10 seconds. Select the Timed exposure to override automated imaging, if you want to see a stronger signal from very faint bands. Whatever time is chosen, you can view the actual exposure used in the Image Info window later.

Highlight saturated pixels

Check this box if you would like to see any saturated pixels in red, in order to show how much of the gel image is saturated. This option can be changed later on by selecting View > Image Transform.

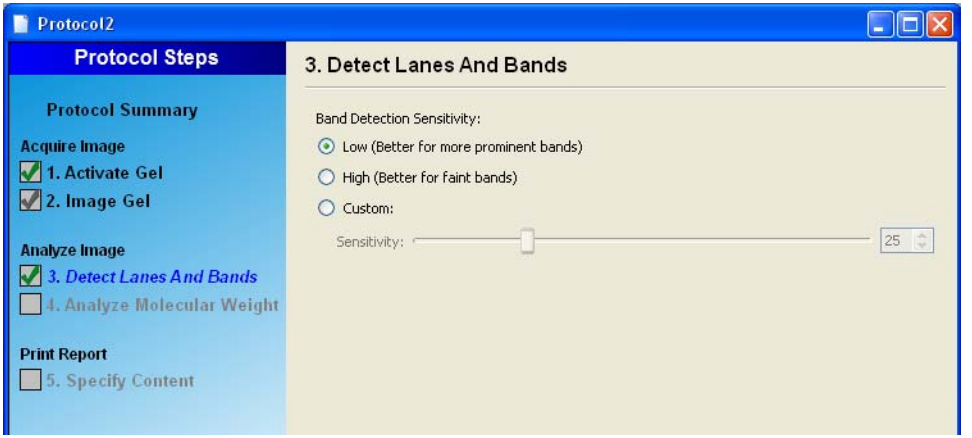
Image Color

You have seven color choices. The first three are explained below. The remaining choices supply enough color variation to highlight small differences in the image data. See page 47 to see the color choices dialog box.

- Gray - gray scale data representation, good for black/white printing
- Stain Free - true color representation of the fluorescent signal
- Coomassie - mimics the color of a Coomassie-stained gel
- False Color
- Spectrum
- Gold-Silver
- Pseudo

Analyze Image

Step 3. Detect Lanes And Bands



To perform any analysis on the gel, Image Lab needs to detect lanes and bands on the gel image. The lanes are detected automatically.

You have the following choices to customize band detection:

Low Band Detection Sensitivity

This setting will set detection at a low level, for images with more prominent bands. Faint bands will not be detected with this setting.

High Band Detection Sensitivity

This setting will set detection at a higher level, for images that are more faint. Extraneous bands can be removed later, using the Band Tools in the Analysis Tool Box. See “3. Band Tools” on page 60.

Custom

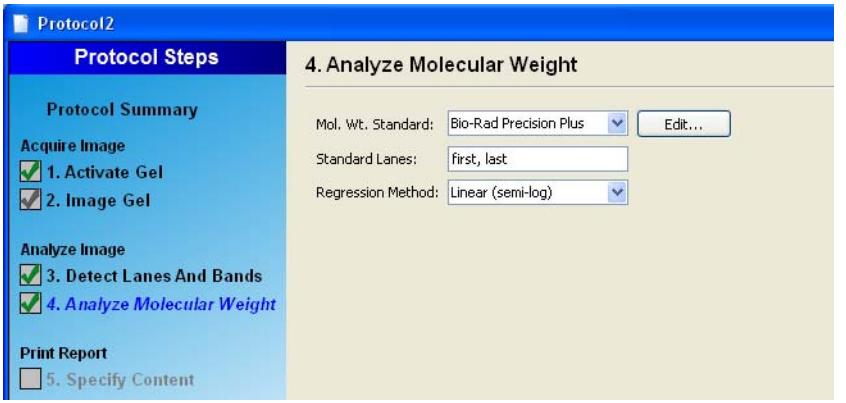
You can set a numerical value between 1-100, in order to select the best detection sensitivity for your sample.

NOTE: When Low or High Band Detection Sensitivity is used, these are the numerical values that are set:

Low sensitivity = 25

High sensitivity = 75

STEP 4. ANALYZE MOLECULAR WEIGHT



If you want to estimate the molecular weight of the bands in your gel, use this step to enter the standards you are using, and also to specify the lane(s) in which the standards are placed.

You analyze molecular weight automatically by clicking Protocol Step 4. “Analyze Molecular Weight” shown above. If this box is checked, your standards will be labeled with their molecular weights, for easy comparison with your test samples.

MOLECULAR WEIGHT STANDARDS

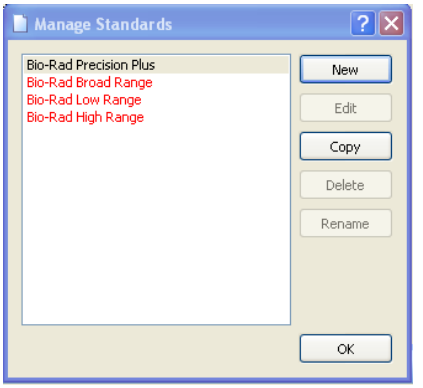
Determining molecular weight depends upon the selection of the proper standards. Four are available from Bio-Rad. See “Protein Standards” on page 24 for catalog numbers.

- Bio-Rad Precision Plus Protein
- Bio-Rad Broad Range
- Bio-Rad Low Range
- Bio-Rad High Range

NOTE: Colored (or pre-stained) standards cannot be used with this system, because they will not be detected or seen.

Edit Button

The Edit button opens the Manage Standards dialog box, which allows you to add other third-party standard samples, and to edit your list.



Standard Lanes

You can choose which lane will display your Standards, by typing lane numbers, or the words First and Last, in the Standard Lanes field. The format is xx, 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

To calculate the molecular weight of the unknown bands, a regression model is used. The software will use the relative front and molecular weight values of the standard bands to calculate the standard curve. This 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. You have a choice of four regression methods.

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

Regression Method	Minimum number of standard bands
Linear (semi-log)	2
Point-to-Point (semi-log)	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 will not be calculated.

Which method to select depends on the gel type:

- **4-20% gradient gels:** The Linear (semi-log) regression method works well for these gels, since the mobility of the bands is linear to the log of their molecular weight. As an alternative, the Point-to-Point (semi-log) method can be used, if the r^2 value is not good enough.
- **10% gels:** These gels have a non-linear relationship between the mobility and the molecular weight. Good choices for regression methods are Logistic or Cubic Spline.

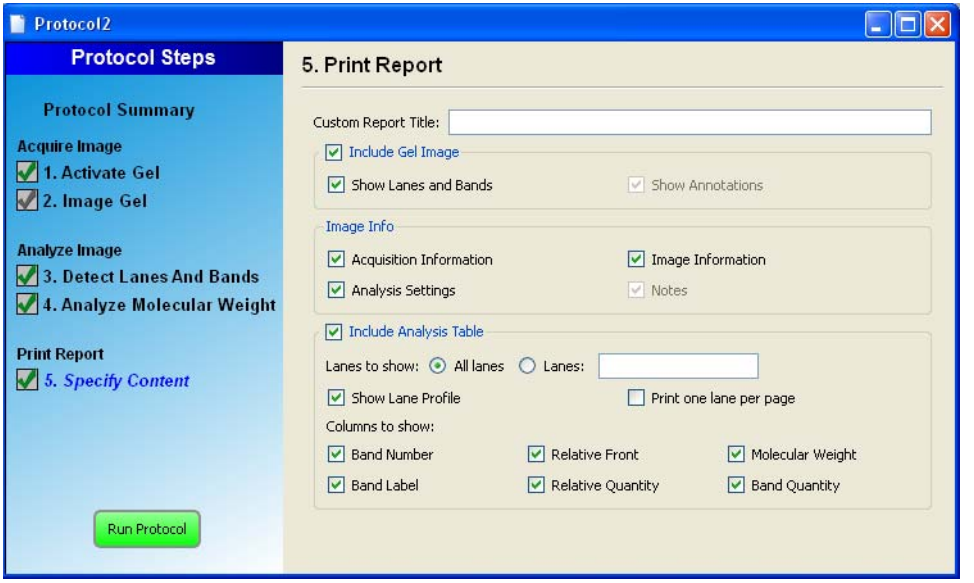
You can check how well each regression method fits the data in the standard curve window (for more information, see “Standard Curve” on page 54.) The Linear (semi-log) regression method provides a measurement which describes how well the standard curve fits the data, r^2 value. The closer the r^2 value is to 1.0, the better the data fits the standard curve.

For information about the calculations behind the regression methods, see the Glossary.

Configure and Print Report

This menu presents choices about what you want included in an image report. For more complete information, see Chapter 8, “Generating Reports”.

Step 5. Print Report



Custom Report Title

You can provide a custom report title for your Report.

Include Gel Image

You can choose whether to include your gel image in the report, and whether lanes appear over the bands. You can also include text annotations and arrows over the gel image, to point out areas of interest.

Image Info

This part of the report describes how the imaging was performed; the Acquisition Information, Analysis Settings, Image Information, and any Notes which the researcher has included.

Include Analysis Table

This dialog allows you to choose the parts of the analysis table that are included in your image report.

Under Lanes to Show, you can choose all or just some lanes.

The Lane Profile can be included in your report, or omitted.

Under Columns to Show, you can include the following information. Each type of information is defined below:

- **Band Number -** Each band is numbered from top to bottom, within each lane frame.
- **Band Label -** You can enter a name for specific samples in the Analysis Table Band Label column.
- **Relative Front -** This is the relative distance the protein has traveled through the gel, measured from the top of the lane frame. It ranges from 0 on the top through 1 on the bottom of the gel.
- **Relative Quantity -** This is a measurement of a specific band's relative quantity to the total of all identified bands within a given lane.
- **Molecular Weight -** This measurement is an absolute value, based on an interpolation of a given band between known standard values.
- **Band Quantity -** This absolute value is the sum of all intensities within the band boundaries.

Opening and Editing Protocols

Existing *Custom* protocols can be changed and renamed, using the tools in Image Lab.

The original default protocol can be edited according to your requirements. It need not be renamed, but becomes your customized *Default* protocol.

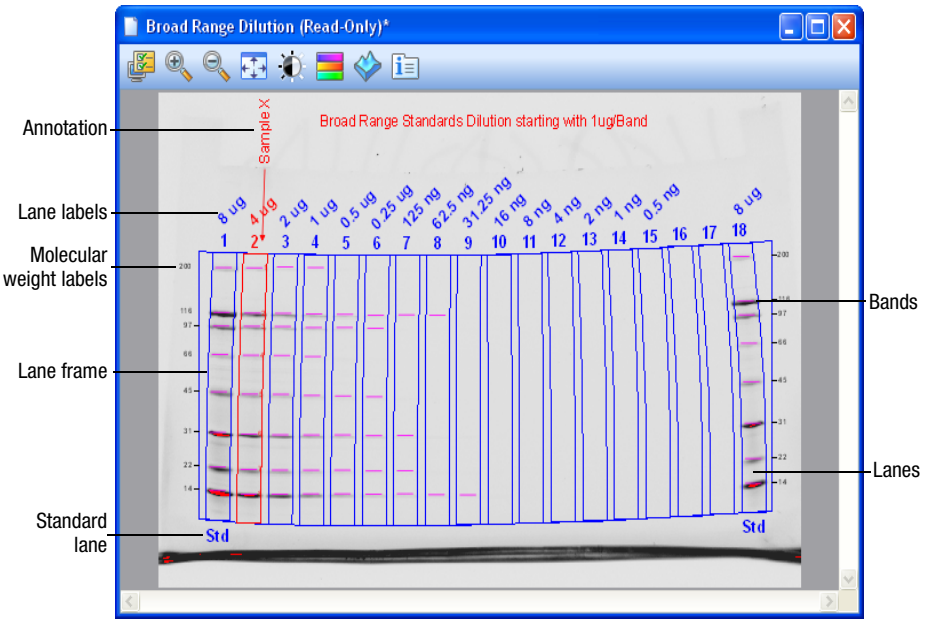
Open any protocol you have saved in the past by choosing File > Open. To edit the protocol, you will be presented with the same set of menus and choices described in the previous section, "Creating and Running Custom Protocols".

6 Viewing Results

Once your gel has been imaged, the results file appears on the desktop. There are many controls to optimize viewing your results files. You can make use of as many views as you need, and then print your image report.

See “Generating Reports” on page 65 to choose which information to include in your Image Reports.

Results File Overview



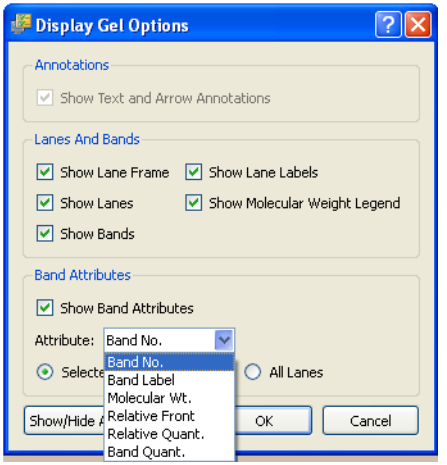
The above results file shows an activated gel with band and lane detection. The callouts in the screenshot above show elements you can turn on or off in your Results Files.

There are many ways to view the numerical data associated with your gel images. See “Displaying Results Data” on page 50.

Displaying Gel Images

Notice the display tool bar icons above the gel image. Each of these tools are described below.

Display Gel Options 



Annotations

You have a choice whether or not to show text and arrow annotations.

Lanes and Bands

You can turn lane frames, lanes, bands, lane labels and molecular weight legends on or off.

Band Attributes

You can show the following attributes for selected lanes, or all lanes. For definitions of these attributes, see “Include Analysis Table” on page 42.

- Band number
- Band label
- Molecular weight
- Relative front
- Relative quantity
- Band quantity

Zoom Tools 

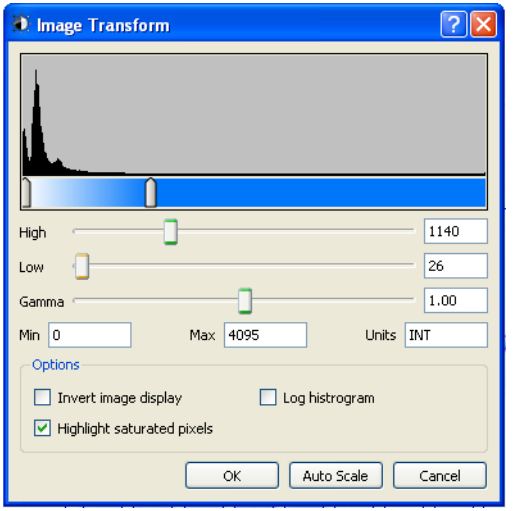
The zoom tools resize your gel image. Click on the magnifying glass with the plus to make your image larger; the minus to make your image smaller.

You can also zoom with the right mouse button. Click and drag the right mouse button to marquee an area you want magnified. Click the right mouse button again to return to the original view.

Fit in Window 

Whatever the size of your results file, clicking this button will fit it into the window.

Image Transform



Use the Image Transform dialog box to adjust image brightness and contrast, optimizing the image display. The minimum to maximum range will vary, according to the light and dark values present in your image.

> NOTE: These adjustments do not change the data, only the way the data is displayed. The human eye cannot see as much range as is contained in the image.

The Frequency Distribution Histogram shows the total data range in the image and the amount of data at each point in the range.

The Auto Scale button will determine an optimal setting for your particular image automatically. The lightest part of the image is set to the minimum intensity, and the darkest is set to the maximum.

- Use the High slider to determine the darkest value that will appear in your gel image
- Use the Low slider to determine the lightest value to appear in your image
- The Gamma slider lightens or darkens your image uniformly

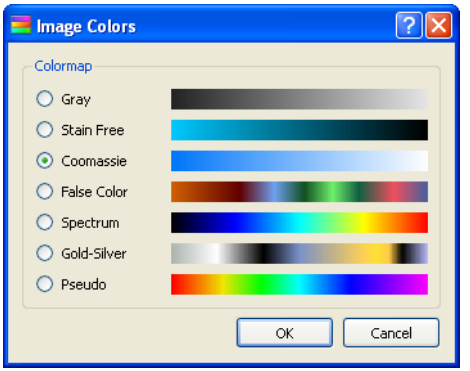
You can also type numerical values in the text boxes next to the sliders. Clicking anywhere on the slider bars will move the sliders incrementally.

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 are highlighted in red
- **Linear or Logarithmic histogram** - click the log checkbox if your image data is in a narrow range, to allow for finer adjustments

Image Colors

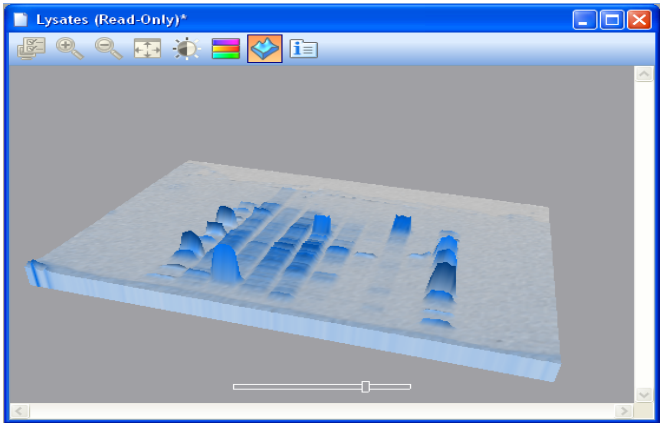


This dialog allows you to choose a colormap for your image results file. Its can be useful to view the image with a different color scheme, making it easier to see all of the elements in the image.

You have seven color choices. The first three imitate the colors of stained gels. The remaining choices supply enough color variations to highlight small differences in the image data.

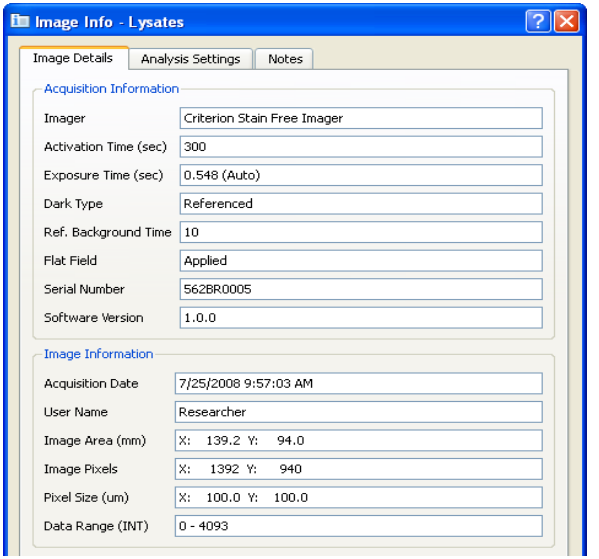
- Gray
- Stain Free
- Coomassie
- False Color
- Spectrum
- Gold-Silver
- Pseudo

3-D Projection



Clicking the 3-D icon transforms your gel image to a solid dimensional model spinning in space with x, y and z dimensions. Relative heights of data points can be accentuated or diminished by pulling the slider to the right or left.

Image Info



The Image Info dialog box displays three information tabs about your image.

Image Details

Acquisition and image Information is displayed in this dialog box.

Analysis Settings

The settings that were used when the gel was analyzed (Band Detection and whether Molecular Weight Analysis was performed) are displayed here.

Notes

You can add notes, make custom labels for each of the lanes, point out the type of samples used, or anything else you want to point out about the results.

Exporting Gel Images

There are two ways to export gel images, depending upon how the image will be used. If you want to export all of the results file data to a different analysis program, you should export as a .tif file, because this preserves all of the data.

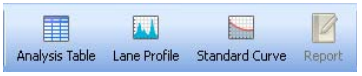
If you want to export a displayed image which can be used in a word processing or presentation program, you can save the file as a. .png, .bmp, or .jpg file. See Chapter 9 for complete information on exporting files.

Exporting Analysis Table Data

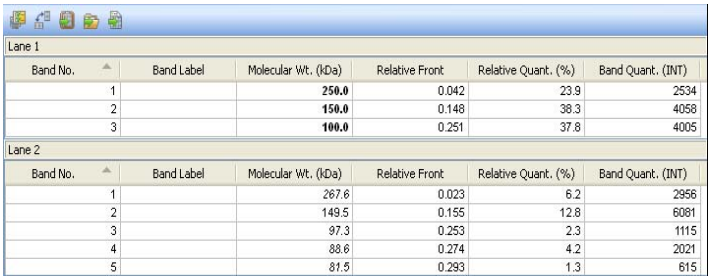
There are two ways to export table Analysis data. One is to use the File menu, and the other is to use the export buttons found on top of the Analysis Table window. See Chapter 9 for complete information about exporting your files.

Displaying Results Data

Results data associated with the gel images can be visualized several different ways; as an Analysis Table, a Lane Profile, a Standard Curve, or in a Report. Buttons to toggle these views on and off are located in the main toolbar. All of them can be viewed at one time. Each are described below.



Analysis Table  Options



Lane 1

Band No.	Band Label	Molecular Wt. (kDa)	Relative Front	Relative Quant. (%)	Band Quant. (INT)
1		**250.0**	0.042	23.9	2534
2		**150.0**	0.148	38.3	4058
3		**100.0**	0.251	37.8	4005

Lane 2

Band No.	Band Label	Molecular Wt. (kDa)	Relative Front	Relative Quant. (%)	Band Quant. (INT)
1		*267.6*	0.023	6.2	2956
2		149.5	0.155	12.8	6081
3		97.3	0.253	2.3	1115
4		*88.6*	0.274	4.2	2021
5		*81.5*	0.293	1.3	615

Clicking on the Analysis Table button opens a tabular display of the data extracted from the Results File. The icons above the table offer several ways of displaying and exporting Analysis Table data.

Set Window Size

To change the size of your Analysis Table window, move your mouse 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 your data.

NOTE: Resizing the Analysis Table window is restricted while a Protocol window is open.

Display Data Options 

There are three tabs in this dialog box: Measurements, Display and Export.

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

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

Per Measurement Precision - You can set the precision (decimal places) for four types of information:

- Molecular weight (kDa)
- Relative front
- Relative quantity %
- Set all columns

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

Export formatting - This field offers two checkboxes to include lane headers and/or column headers in your exported file.

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

- Comma delimited 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 beside each other, so the user can scroll through the table from left to right.

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

Exporting an Analysis Table

The next three buttons offer several ways of exporting an Analysis Table, depending on how the table will be presented. See Chapter 9 for complete information on exporting your files.

Copy Analysis Table to the Clipboard 

Click this button to copy the Analysis Table to your clipboard, in order to copy your Analysis Table to word processing or presentation applications. It is best to use the Vertical table orientation when copying to an 8-1/2x11" page, to allow the columns enough room to display.

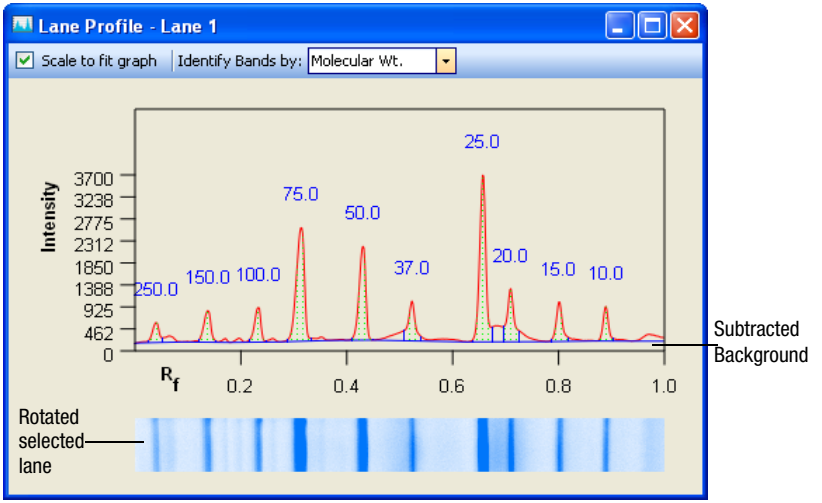
Export Analysis Table to a File 

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

Export Analysis Table to a Spreadsheet 

This function allows you to use Excel's sorting and formula functions to manipulate your data. If you have this program installed on your customer, Excel will open with your spreadsheet displayed.

Lane Profile 



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

The Lane Profile shows the subtracted background under the blue line, and the area used for quantification in faint green dots under the red line.

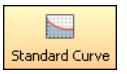
You can change how the bands are identified by choosing from the options in the drop down list. By default, the bands are labeled with the band number.

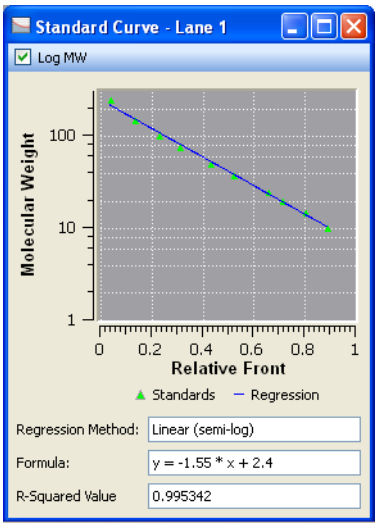
Scale to Fit Graph

You can choose the highest point of the display to define the range of the graph, to provide the best view of one gel image.

You may want to uncheck the Scale to Fit Graph checkbox, to display the entire range of possible intensity values (0-4095) in your graph. This allows valid comparisons between gel images.

Standard Curve





This dialog displays the best fit curve for your defined standards and the bands relative to this curve, for the lane you have selected in the image. Standards are displayed in green, and unknown bands are in red. You can change the molecular weight display on the Y axis between linear and log scale, by clicking the Log MW box at the upper left. The regression method you have chosen in Molecular Weight Analysis Tools is displayed, as well as the line formula (if applicable) and R-squared value for that lane's data.

Report

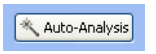
See Chapter 8 "Generating Reports" on page 65 to learn about all the choices you have when deciding what to include in your report.

7 Editing Results

The Analysis Tool Box tools are enabled once a results 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 allows you to edit one of many open results files on your desktop.

Analysis Toolbox

Auto Analysis

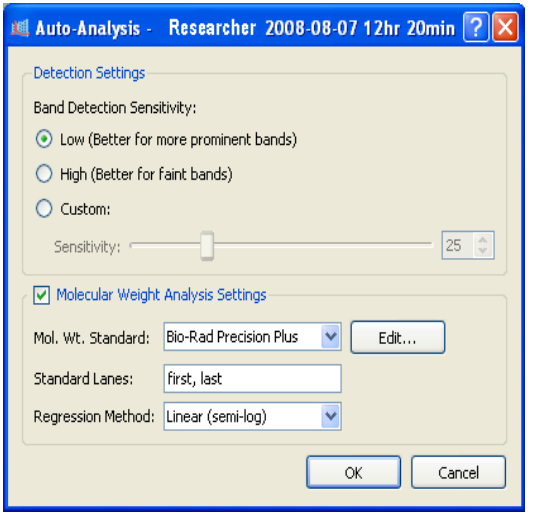


Pressing the Auto Analysis button in the Analysis Tool Box allows you to do the following:

- Analyze images which have been run without detection and analysis
- Re-analyze gels when their settings have been changed

  NOTE: The existing analysis is deleted, if you change any settings to an analyzed gel. The gel will be re-analyzed based on the new settings.

DETECTION SETTINGS



You have the following choices for band detection:

Low Band Detection Sensitivity - This setting will set detection at a low level, for images with more prominent bands. Faint bands will not be detected with this setting.

High Band Detection Sensitivity - This setting will set detection at a higher level, for images that are more faint. Extraneous bands can be removed later, using the Band Tools in the Analysis Tool Box. See “3. Band Tools” on page 60.

Custom - You can set a numerical value between 1-100, in order to select the best detection sensitivity for your sample.

NOTE: When Low or High Band Detection Sensitivity is used, these are the numerical values that are set:

Low sensitivity = 25

High sensitivity = 75

Molecular Weight Analysis Settings

Molecular Weight Standard - Run any of the four Bio-Rad standards, or third-party standards, which you have placed in your standards list. See “Molecular Weight Standards” on page 38 for full information.

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

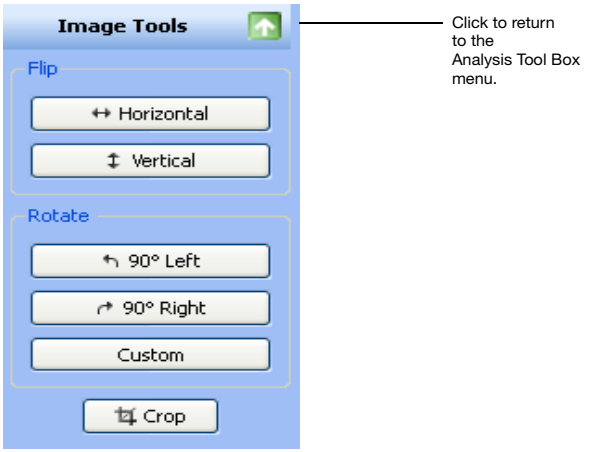
Regression Method - You have a choice of seven regression methods. For complete information, see “Designing Protocols” on page 33.

All of the following tools customize the *analyzed* data in your Results Files. These tools are only available when there is an open Results File. Click on a specific Results File to choose among many which may be open on your desktop. The menu bar becomes a darker blue than other windows.

Click on any of the Tool Box icons to access tools. Click the green upward-pointing arrow button to the right of the tool name, to return to the Analysis Tool Box menu.

Some of these tools will delete the existing analysis. You need to analyze the gel again, based on the new view.

1. Image Tools



Flip

You can flip your imaged gel horizontally or vertically.

Rotate

You can rotate your gel image 90°, using the Left or Right buttons. To correct a slanted gel, click the Custom button and do the following:

1. Rotate the red arrows which appear over the gel to any degree between 0-360°, by clicking and dragging with your mouse.
2. Right click your mouse on the gel image and choose Rotate, to set your gel in the new position. You will be prompted to Cancel or Continue, in case you want to reset the rotation.

Crop

If you want to analyze only a portion of the gel image, follow these instructions to crop the image:

1. Click on the Crop button.
2. Using the square handles, drag the red box to include the area you want to analyze.
3. Right-click your mouse within the red box to remove the rest of the image. You will be prompted to Cancel or Continue, in case you want to reset the area.

2. Lane Tools

Lane Finder

You can choose how your lanes are detected, using the Automatic or Manual buttons. Either will delete previous lane detection.

- Click the Automatic button if your gel image is fairly typical
- Click the Manual button if you only want to detect a specific number of lanes, or if the automatic lane detection fails. 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 of the lanes by dragging the handles on the corners of the red square to fit your gel image.

Adjust - If your gel image is somewhat irregular, you can adjust the orientation of all lanes by dragging a single corner of the lane frame.

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

Delete - You can delete all lanes.

Single Lane

Add - You can add a single lane to your gel image. First click the Add button, then click within the lane frame where you want the new lane. The lanes will renumber.

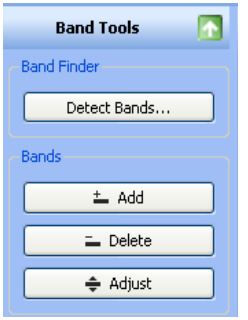
Delete - You can delete a single lane. First click the Delete button, then click either on the lane or its lane number. The lanes renumber.

Move - You can move a single lane to a new position on your gel image. First click the Move button, then click on the lane you want to move. Drag it to a new location. The lanes will be renumbered, according to their new position.

Bend - You can bend a single lane to better fit your gel image. First click the Bend button, then click and drag one of the square anchors to fit your image.

You can add additional anchor points within the lane, by left-clicking your mouse. Drag these anchor points to adjust the lane to fit the gel image. Remove anchor points by right-clicking them with your mouse.

3. Band Tools



Band Finder

This tool allows you to detect bands or reset band detection settings. See “Step 3. Detect Lanes And Bands” on page 37 for a discussion of this tool.

Band Tools

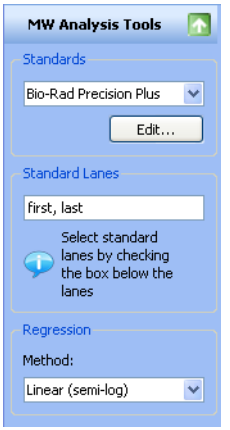
Add - Use this option to add faint detected bands. First click the Add button, then add a new band by clicking anywhere inside of a lane.

> NOTE: You can darken your entire image to view detected bands more easily, by using the Gamma slider in the Image Transform dialog. For complete instructions, see “Image Transform” on page 46.

Delete - You can delete bands which are not relevant to your analysis. First click the Delete button, then click any bands you want to remove.

Adjust - You can adjust the height of a band. First click the Adjust button. Two boundary lines appear around each band. Move your mouse over a boundary line until you see a double-headed arrow. Move the boundary line up or down, the center is recalculated, and the band appears there.

4. Molecular Weight Analysis Tools



Molecular Weight Standard

You can change the standards used to determine the molecular weight of your test samples. Click the pulldown menu to choose another standard, or click the Edit button to access the Manage Standards dialog, where you can 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 checking the box below each lane, or by entering the standard lane numbers separated by commas. The Standard lanes are shown with the label “Std” below the lane.

Regression Methods

There are four regression methods. Read about which to use in “Regression Methods” on page 39.

5. Annotation Tools

Add Annotations

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

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

Text Properties

You can change the size and type font of your text annotations. To select multiple items for change, hold down the keyboard Control button and left-click each item with your mouse.

Font - First click on the text box you want to change. Click on the pulldown Font menu to show all fonts installed in your system. Click on one of them to change the style of your text annotation.

Size - First click on the text box you want to resize. Click on the up and down Size arrows to increase or decrease the size of your text. You can make text as small as 6 point and as large as 24 point.

Color

You can change the color of your 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 keyboard Control button and left-click each item with your mouse.

Foreground - Click on your text annotation or arrow. This enables the Foreground field, so you can use the pulldown menu to choose a different color.

Background - Click on your text annotation. This also enables the Background field, so you can use the pulldown menu to choose a background color.

Rotate

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

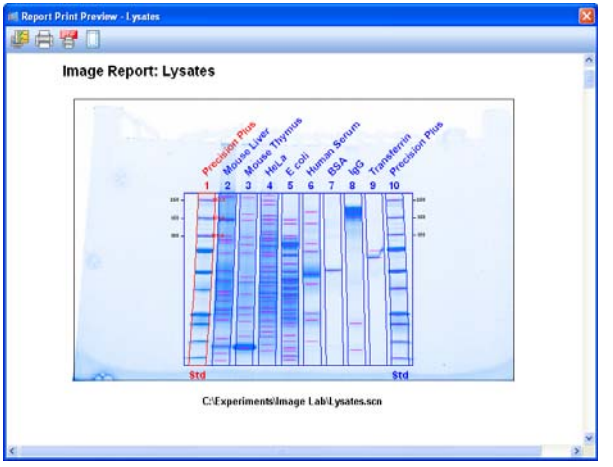
8 Generating Reports

After viewing your Results Files, you can print the gel images with analysis, and include information about the protocol used and all other settings that were applied.

The print report settings can be chosen initially when setting up your protocol (Step 5, Print Report), or after analyzing and viewing your Results Files.

Report 

You simply click the Report icon to produce a Print Preview of your report.

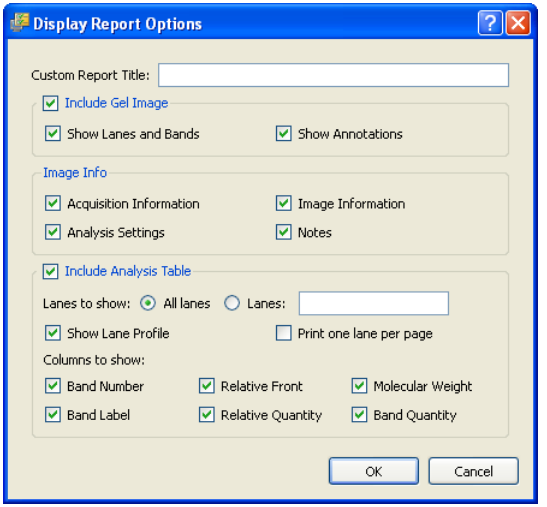


Clicking the Print icon allows printing of all of the above information to a printer or .pdf file.

The Report screen includes the following icons:

Display Report Options

Shows options of what to include in your report; simply uncheck a checkbox to remove an item.



This dialog shows all information included as a default. These choices may have been made at other stages of your workflow, but you can customize your reports here, based on the information you want to include.

Custom Report Title

You can provide a custom report title for your Report.

Include Gel Image

- Show Lanes and Bands
- Show Annotations

Image Info

- Acquisition Information
- Analysis Settings
- Image Information
- Notes

Include Analysis Table

Lanes to show:

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

Show Lane Profile

Print one Lane per Page

Columns to Show:

- Band Number
- Band Label
- Relative Front
- Relative Quantity
- Molecular Weight
- Band Quantity

Print Report 

You can select or add printers, and set the number of copies you want here.

Print Report to .pdf File 

Brings up a Save dialog so your .pdf file can be saved somewhere on your system.

Adjust the Printer Settings 

Gives choices for paper size, orientation, and page margins.

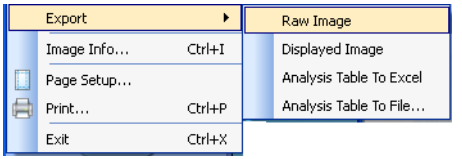
9 Exporting Results

The most convenient way to keep complete information about your experiments is by producing Reports, which is discussed in Chapter 8.

But you may want to export just your gel images or Analysis Table data for analysis in different programs, such as InfoQuest FP or Quantity One®. Or you may need exported files for presentation or publication. This chapter describes all the ways Image Lab can export your results.

File Menu Export

File > **Export** allows you to export gel images and Analysis Table data. There are four export choices under the File menu:



Follow these steps to export from the file menu:

1. Choose File > Export >and one of the choices shown above.
2. A browse window opens, where you can choose the type of image, the file format, and where to save the file.
3. Click the Save button.

See below for which file format to use for each type of export:

- **Raw Image** - Use a .tif when you want all data to be retained. This option creates a larger file, but allows you to analyze the image in other software such as InfoQuest FP or Quantity One®
- **Displayed Image** - You can choose between .bmp, .png, and .jpg formats, when exporting a displayed image. The gel will display with any lanes, bands, and annotations.

  Exporting as a .jpg produces a smaller file, but the conversion to .jpg loses some data. This format should only be used for export of visual information to presentation or word processing software, such as Power Point or Word.
- **Analysis Table to Excel** - If you have Excel installed on your computer, choosing File > Export > Analysis Table to Excel opens your Analysis Table in Excel directly. You can then use Excel's Save As function to produce several other useful formats.
- **Analysis Table to File** -Exports as a CSV file, so your data file can be opened in a database application.

Analysis Table Export

The Analysis Table window has several buttons to export your data to different formats, depending upon how the data is to be presented.

Copy Analysis Table to the Clipboard 

Click this button to copy the Analysis Table to your clipboard, to paste your Analysis Table into word processing or presentation applications. It is best to use the Vertical table orientation when copying to an 8-1/2x11" page, to allow the columns enough room to display.

Export Analysis Table to a File 

This exports 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 allows you to use Excel's sort and formula functions on your data. If you have Excel installed on your computer, your data opens in Excel.

Appendix A: Troubleshooting

Follow the recommendations below to troubleshoot problems with the Imager.

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 protocol again
Image is not bright enough	Underdeveloped gel	If initially stopped after 2 minutes, allow the gel to develop for 2-3 more minutes
Red LED flashing on front panel	Sample tray not inserted all the way	Push sample tray into the imager until you feel the magnet pull the sample tray
	Door interlock broken	Please close the door
	Bulb fault	Replace all the lamps according to the instructions in Chapter 2
	Communication interrupted	Make sure Imager power is on the USB cable is connected to the PC. Restart the imager
	Imaging button is pressed on front panel while software is not yet open	Red light will stop flashing when the software is opened. Press the imaging button again to initiate imaging

Glossary

The following glossary defines words used in this Criterion Stain Free System manual.

The equations used to calculate regression methods are also in this appendix, see “Regression Method Calculations” (page 75).

2-Mercaptoethanol (BME): A protein denaturant which breaks disulfide bonds.

CCD: (Charge-Coupled Device) A light-sensitive silicon chip used as a photodetector in digital camera systems.

Charge-to-Mass Ratio: The value measured by electrophoresis.

Colormaps: Different color representations of a gel image; Stain Free provides Gray, Stain Free, Coomassie, False Color, Spectrum, Gold-Silver, Pseudo

Dithiotreitol (DTT): A reducing agent

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 which compensates for non-uniformities generated by an instrument.

histogram: A graphed representation of the brightness, or gray value, of an image.

MeOH: Methanol.

Native-PAGE: Polyacrylamide gel electrophoresis performed without the use of SDS.

Native charge density: The inherent electrical charge of a protein without the addition of SDS.

PAGE: Polyacrylamide gel electrophoresis; an electrophoretic method in which molecules migrate through a molecular lattice created by polymerized, cross-linked polyacrylamide.

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.

Relative Quantity: This is 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 which 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 which is a precursor of important biochemical molecules indoleacetic acid, serotonin, and nicotinic acid.

UV-B: The range of ultraviolet light used by the Stain Free system.

UV transilluminator: The part of the Stain Free Imager that transmits UV light through a sample.

Regression Method Calculations

Each regression method will calculate 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 (semi-log): 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-square value may be used to determine the overall quality of the linear fit. A linear regression with an R-squared value of greater than 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 wrong results if the data is not very linear.

Point-to-Point (semi-log): 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 is the mobility,

y is the molecular weight,

a is the estimated molecular weight at infinity,

b is the slope of the tangent at midpoint,

c is the midpoint, and

d is the 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.

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