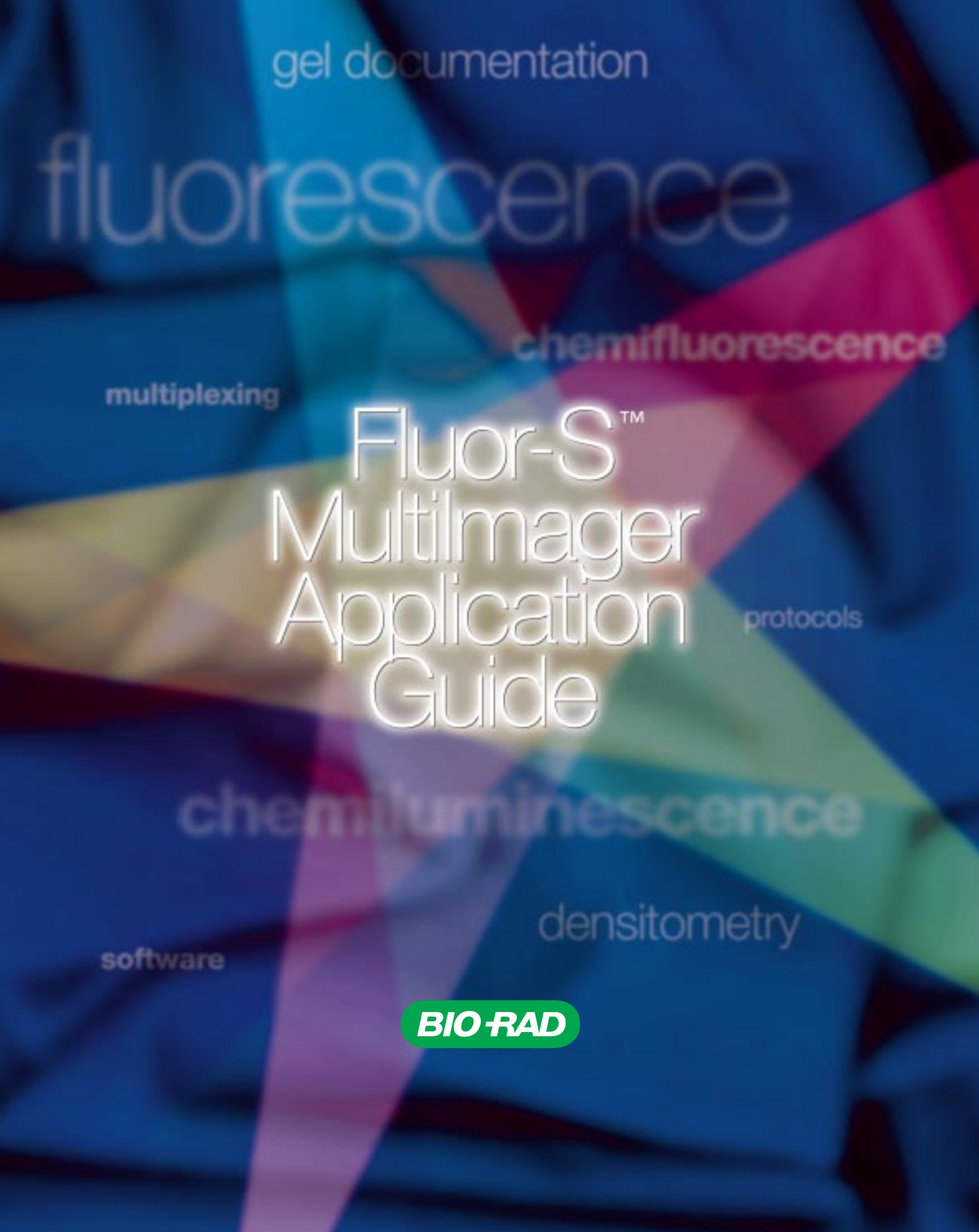

Fluor-S™ MultiImager Application Guide

BIO-RAD

Introduction

The Fluor-S MultiImager system combines several different imaging capabilities into one instrument. The capabilities of this unique imaging system include:

- Single-color fluorescence detection
- Multiple-color fluorescence detection
- Chemiluminescence detection
- Chemifluorescence detection
- Colorimetric detection
- Densitometry
- Digital documentation

This Application Guide provides information on many of the applications supported by the new Fluor-S MultiImager system. These applications are presented in an easy-to-use format, and include suggestions on how to obtain the best results with the Fluor-S MultiImager system. Sample images are presented to demonstrate the capabilities of the system.

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Fluor-S MultiImager System

The Fluor-S MultiImager system combines several key elements in a unique, powerful, and integrated fashion.

Cooled CCD Technology

The Fluor-S MultiImager system uses advanced cooled CCD technology for image capture. Cooling of the CCD chip improves image quality by improving the signal-to-noise ratio. This is particularly important for low light applications such as fluorescence and chemiluminescence. The CCD imaging chip is a rectangular array of 1,340 x 1,037 pixels. This provides high resolution imaging, double that of the typical CCD-based imaging system. The dynamic range of the Fluor-S MultiImager system is also dramatically improved over that of the typical CCD-based system. Instead of the standard 8-bit gray scale, with 256 levels of gray, the Fluor-S MultiImager system has a dynamic range of 12-bits, *i.e.* 4,096 levels of gray. This allows accurate quantitation up to three orders of magnitude (Figure 1).

Interchangeable Lenses

The Fluor-S MultiImager system comes with a 20–40 mm, f2.8–3.5 zoom lens. This lens permits flexible imaging of samples from 30 x 25 cm down to 15 x 12.5 cm. Optional F-mount lenses can be interchanged with the standard lens to support imaging of smaller or larger samples.

Automated Image Capture

Image capture on the Fluor-S MultiImager system is simple because the system has many automated features. A Position/Focus mode allows you to position the sample and focus the lens with almost instantaneous feedback. An Automatic Integration mode lets the Fluor-S MultiImager system determine the optimal integration time.

Unique Scanning Illumination Source

The Fluor-S MultiImager system has a unique scanning system for both UV and white light illumination. The scanning design provides much more uniform illumination than the fixed transilluminator bulbs found in other instruments. Epi-illumination light sources illuminate samples on opaque materials such as membranes and blots. The standard white light and broadband UV bulbs can easily be exchanged with bulbs of different wavelengths when your applications require other illumination wavelengths.

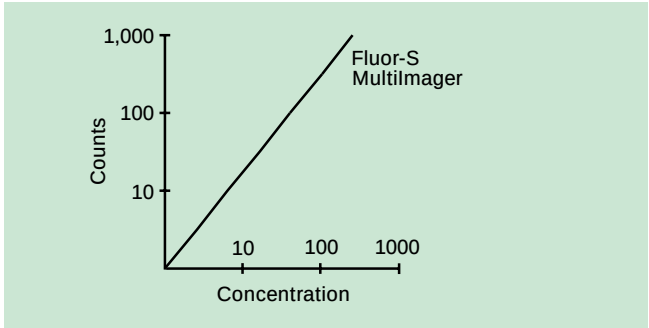


Fig. 1. Dynamic range.

Single and Multi-Color Detection

The Fluor-S MultiImager system provides high sensitivity imaging of single-color and multi-color fluorescent samples. Broadband UV excitation allows detection of many dyes, compared to a small number of dyes which can be excited with a single narrow band laser (Figure 2). Eight filter positions, four with standard filters and four for optional filters, permit both multi-color discrimination and the detection of many different fluorescent dyes. One standard broadband filter is optimized for single color detection of various stains and labels including ethidium bromide, SYBR Green I stain, Radiant Red stain, SYPRO Orange stain, SYPRO Red stain, Texas Red label, many Cy dyes, and most fluorescein and rhodamine derivatives. Two standard filters are for the independent detection of fluorescein and Texas Red label, thus allowing multiplexing of samples for higher throughput and more accurate molecular weight determination. One filter is clear for maximum light collection from both chemiluminescent samples and white light samples.

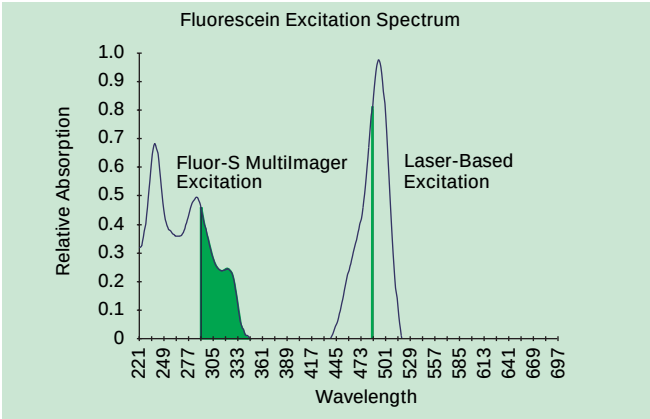


Fig. 2. Broadband UV excitation.

Two Detection Modes

In High Resolution mode, each pixel of the CCD chip provides a data point for the image, thus providing the highest resolution possible. This is the most useful mode for images containing many samples. In High Sensitivity mode, four CCD pixels are combined to form each data point, resulting in an improved signal-to-noise ratio. This is the most useful mode for low-light applications.

New Multi-Analyst® Software

The new Multi-Analyst software features automated single and multi-color image acquisition. Image and data quality are maximized by several approaches to background correction. Dark current and bias corrections remove electronic noise accumulated during long integration times, resulting in a dramatic improvement in image quality. The Multi-Analyst software allows substantial flexibility in the presentation of the image and also provides many tools for data analysis, including automated molecular weight determination, automated lane and peak finding, accurate concentration analysis, and much more. The Multi-Analyst instruction manual provides a full description of this new software package.

Certified Reagents

Bio-Rad offers a number of certified reagents and technical support for most of the applications described in this guide.

Selection of Fluorescent Dyes

This is a brief review of basic fluorescence principles to help you obtain the best results with the Fluor-S MultiImager system.

Excitation and Emission Spectra

Fluorescent molecules absorb energy at particular wavelengths and emit this energy at longer wavelengths. A spectrum for fluorescence excitation illustrates the wavelengths and the strength of this excitation. For example, fluorescein is excited by light in the visible region of the spectrum, with peak excitation at ~500 nm. Fluorescein also has secondary excitation peaks in the UV region (Figure 2), thus permitting broadband UV excitation of the dye. The stronger the excitation peaks in the UV region of the spectrum between 290 and 365 nm, the better the dye will perform on the Fluor-S MultiImager system.

Fluorescent molecules also have characteristic emission spectra. Filters are used to shield the detector from the excitation wavelengths and to select the dye emission wavelengths which pass to the detector. With the standard filters, the Fluor-S MultiImager system can detect dyes which emit from the green (520 nm), up to the near infrared (~660 nm). Custom filters can also be used to detect dyes which emit deeper in the blue such as coumarin, DAPI and Hoechst (all with peaks at 440–460 nm). Figure 3 shows a compilation of emission spectra from several fluorescent dyes and the best choice of the standard Fluor-S MultiImager filters to visualize these dyes.

For multi-color detection, ideally you should choose dyes with similar excitation profiles and with emission spectra that do not overlap. For two-color detection an excellent combination of labels is fluorescein and Texas Red.

The "Brightness" of a Dye

The efficiency with which light is absorbed by a molecule is one determinant of the "brightness" of its fluorescence. This efficiency is reported as the molar extinction coefficient (Em) at the excitation peak. Em can vary over several orders of magnitude among different dyes. Note that the extinction coefficient is reported for a wavelength and in a specific buffer, since absorbance can vary significantly in magnitude and wavelength under different buffer conditions.

The efficiency of conversion of the absorbed photons into emitted light is termed the quantum yield. The quantum yield ranges from 0 to 1, with 1 representing complete conversion of the excitation energy into emitted light.

Fluorescent dyes can also vary in their fluorescence enhancement upon binding to the target molecule. For example, the fluorescence of SYPRO Orange stain increases more than 500 fold when bound to a protein-SDS complex. Dyes with a higher fluorescence enhancement will perform better since the background fluorescence from unbound dye will be lower. However, excessive bound dye can result in quenching and a lower signal.

Dyes also vary in their susceptibility to photobleaching, the destruction of the molecule's ability to absorb photons. Select dyes with high extinction coefficients, large quantum yields, high fluorescence enhancement upon binding, and the lowest susceptibility to photobleaching.

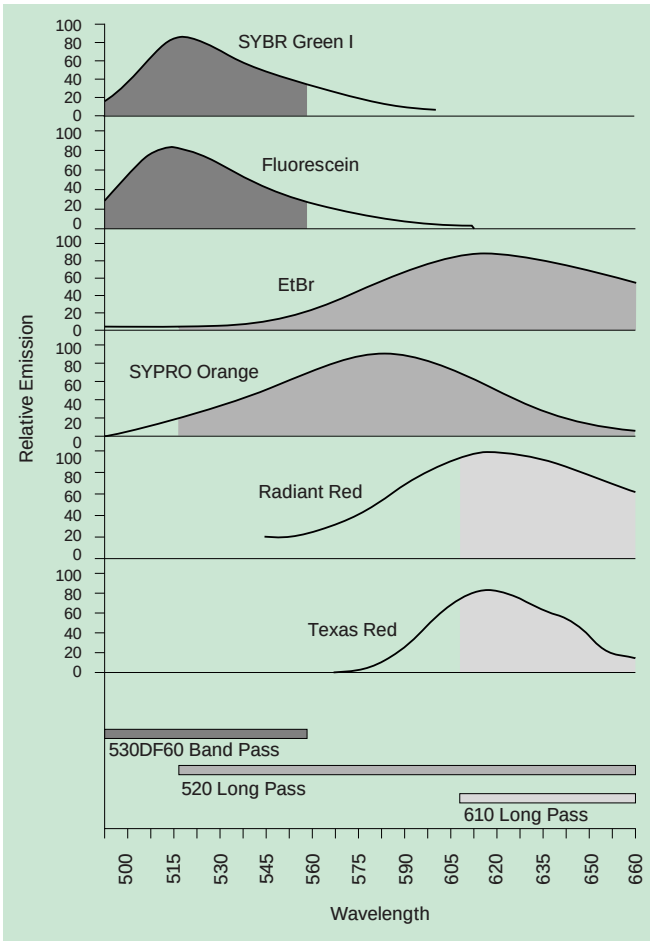


Fig. 3. Dye emission spectra.

The fluorescence of the material bearing the sample also needs to be considered. Most materials scatter light and will fluoresce when illuminated. Light scattering and matrix fluorescence are greater for agarose than for polyacrylamide and greater for nylon than for nitrocellulose. Highest levels of the background sources are in the blue (<500 nm) and decrease toward the red (>600 nm). While dyes which fluoresce in the blue, for example DAPI, Hoechst, and coumarin, can be visualized using the proper filter on the Fluor-S MultiImager system, the dye fluorescence will be masked somewhat by the light emanating from the matrix.

Labeling Strategies

One approach to labeling nucleic acids with a dye is to incorporate fluorescent nucleotides into the molecule. While multiple dye molecules can be incorporated, this generally results in smeared bands on a gel because of variability in the number of dye molecules incorporated into each target molecule. The alternative is to label with a single dye molecule. This can be done enzymatically, *e.g.* end-labeling DNA, or by chemical attachment, *e.g.* synthesis of PCR primers containing fluorescent moieties at their ends.

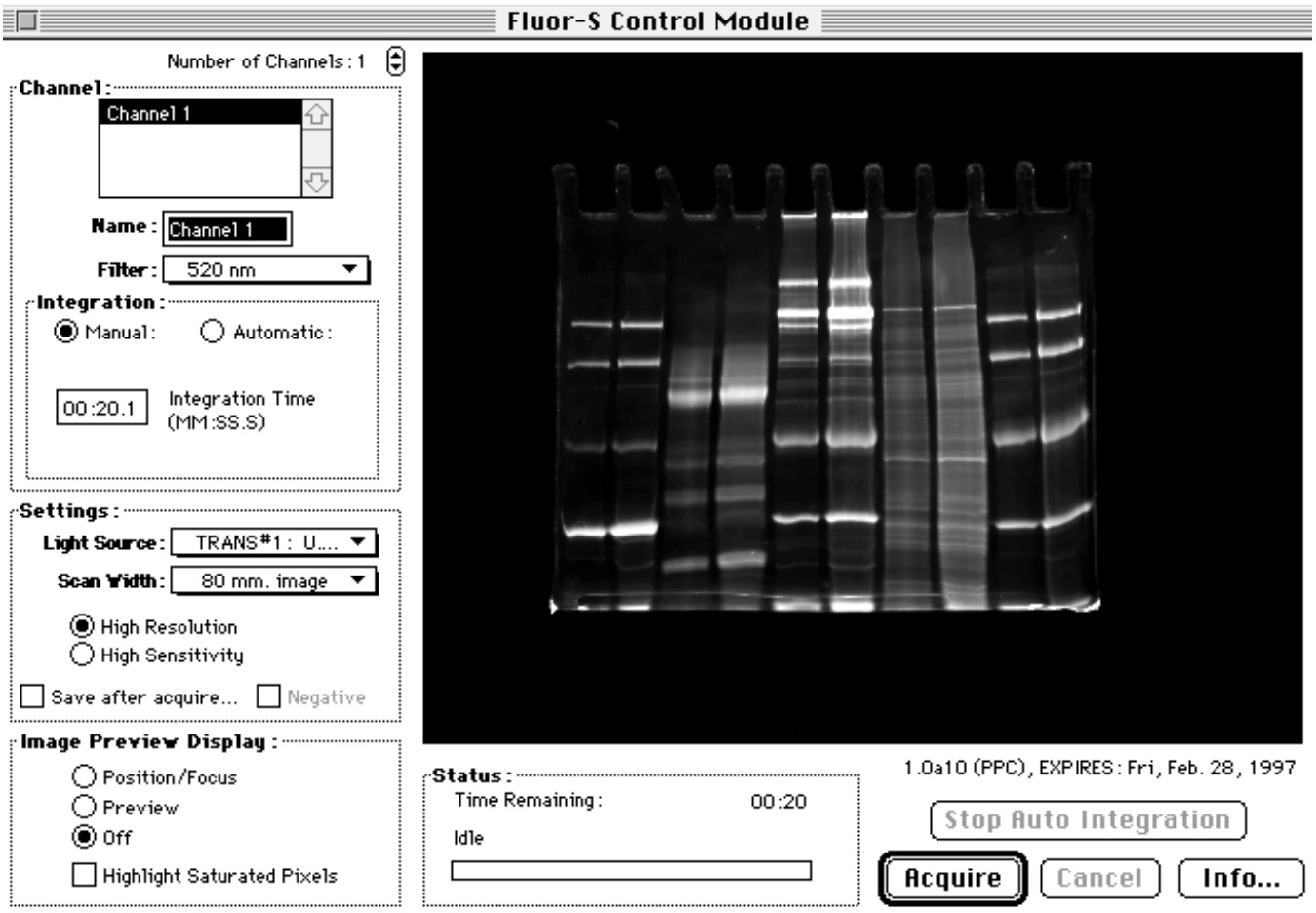


Fig. 4. Fluor-S control module window in Multi-Analyst Macintosh.

The Fluor-S MultiImager system can detect as little as 2–4 fmoles of fluorescein. When DNA is end-labeled, there are one or two fluorophores per DNA molecule. This translates to roughly 2–8 ng of a 1 kb fragment or close to a microgram of a 100 kb fragment. Since the latter amount of DNA is not separated cleanly on a gel, detection of end-labeled DNA is limited to fragments of approximately 10 kb or less. When an intercalating dye is used, many more molecules of dye are bound to each DNA fragment, dramatically improving the limit of detection. For example, ethidium bromide post-staining can detect 0.02 fmoles of a 1 kb DNA fragment, a 100-fold improvement over detection of end-labeled DNA. When pre-staining a sample before electrophoresis, be aware that the mobility of the stained material can be dramatically affected by the presence of the dye. Fluorescence of end-labeled DNA molecules is relatively low compared to DNA stained with an intercalating dye, but the electrophoretic mobility is accurate.

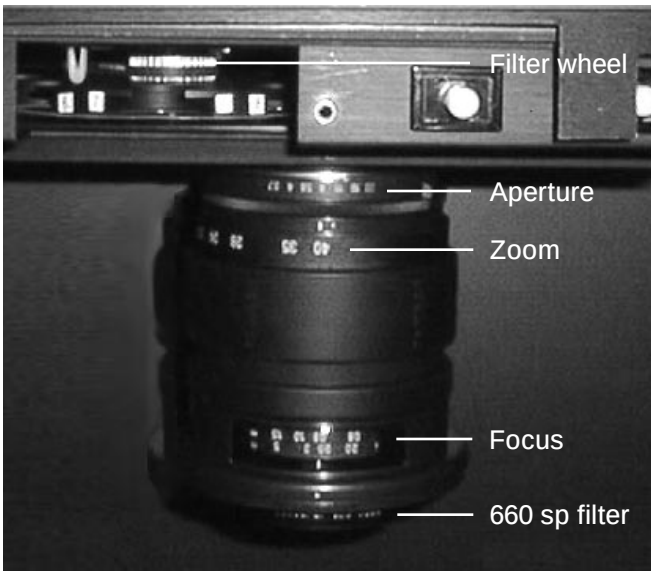


Fig. 5. Fluor-S zoom lens.

A Guide to Fluor-S MultiImager Operation

1. Prepare for image acquisition

- Turn on power to the instrument and the computer.
- Open the Multi-Analyst application.
- Pull down the File menu, and Acquire/Select the Fluor-S MultiImager. The Fluor-S MultiImager Control Module window will be displayed, as shown in Figure 4 (Macintosh version).

2. Select Channel and filter

- For single-color imaging, choose 1 as the number of channels, then select the appropriate filter for your application (see Table 1).
- For multi-color imaging, first determine the number of images to be captured by selecting the Number of Channels, then select Channel 1 and a filter to use for that channel. For additional images, select a different channel and a different filter to use for each channel.

Selection of the emission filter to be used for a particular application is crucial for obtaining the best image. Table 1 lists the standard filter to use for the applications listed. This is not an exhaustive list of available dyes or stains for every application; the rule of thumb is to select a filter which allows collection of the maximum amount of light emitted from a sample.

3. Position the sample and focus

- Select Position/Focus in the Image Preview Display of the Control Module.
- Position the sample in the center of the platen.
- Select the zoom setting and focus the lens with the aperture fully open (see Figure 5).
- Set Image Preview Display to Off when finished.

White light epi-illumination is used to position the sample in the center of the imaging area and to focus the image. A ruler can be placed next to the sample to help focus the image. The Fluor-S MultiImager sample platen can accommodate samples as large as 35 x 43 cm, with movement around the platen in order to image a region of interest. With the standard 20–40 mm zoom lens, samples from 15 x 12.5 cm to 30 x 25 cm can be imaged. For imaging samples smaller than 15 x 12.5 cm, or for imaging small regions of larger samples at high resolution, other F-mount lenses of longer focal length can be used. When using scanning illumination, the maximum size limit for the imaging area is 30 x 25 cm, the size of the transparent region of the platen.

Table 1. Filter Selection Guide

	RECOMMENDED LIGHT SOURCE				
APPLICATION	UV SCAN	UV EPI	WHITE SCAN	WHITE EPI	FILTER*
Ethidium bromide	X				520 LP
SYBR Green I	X				520 LP
Fluorescein	X				530 BP
Texas Red	X				610 LP
Radiant Red	X				610 LP
SYPRO Orange	X				520 LP
Coomassie Brilliant Blue			X		Clear
Silver Stain			X		Clear
Copper Stain			X		Clear
Zinc Stain			X		Clear
Chemiluminescence					Clear
Fluorescent Blotting		X			530 BP
AttoPhos		X			520 LP
Digital documentation—photos+blots				X	Clear
Digital documentation—X-ray film			X		Clear

* BP = Band Pass
LP = Long Pass

4. Select instrument settings

- In the Settings window of the Control Module, pull down to select your light source.
- When using scanning-illumination, select a Scan Width.
- Select High Resolution or High Sensitivity.

The recommended illumination source for many applications is shown in Table 1. When scanning samples less than 30 cm across, the scanning area should be restricted to one of the three smaller subsets of the 30 cm scanning width for more efficient and rapid data collection. In High Resolution mode, images are ~3.0 MB. In High Sensitivity mode (where the data from four pixels are combined) the image files are ~750 KB.

5. Set integration time

- In the Integration window, select either Manual or Automatic integration.
- For Manual integration, set the time of integration.
- For Automatic integration, set the percent or number of pixels and the percent of full range.
- Select Preview in the Image Preview Display.
- Adjust aperture of the lens as necessary.

For most low light applications, such as fluorescence and chemiluminescence, the aperture of the lens should be fully open, *i.e.* with the lowest number f-stop. Integration times are in the several seconds to several minutes range with the lens fully open for these low light applications.

For white light applications, significantly more light is presented to the CCD. Therefore, one limits the exposure by scanning with a short scan time, 1–5 seconds, as well as by decreasing the aperture of the lens (higher numbers). With white light epi-illumination, integration times can be as short as 100 milliseconds. Close down the aperture of the lens for further control of light level. For accurate quantitation, no signal from the sample should saturate the CCD. In order to easily determine if an integration time is resulting in saturation of the CCD, the Highlight Saturated Pixels feature is useful. Turn on the Highlight Saturated Pixels and set the integration time and aperture controls to keep the background just below saturation. In Automatic integration mode, the instrument collects multiple images and sums the images after predetermined time points. Image collection stops when the summed images reach the set level of signal below saturation. For multi-color imaging, each Channel can be previewed independently to determine the optimal integration time for each Channel.

6. Acquire the image

- Capture the image using the Acquire (Macintosh) or Capture (PC) button on the Fluor-S MultiImager control module.

During acquisition, the time remaining in the image capture is presented in the Status window. Acquire will automatically capture all channels in order and subtract the dark current. If all channels are integrated for the same time, a single dark exposure is taken and subtracted from each channel. If integration times are different for each channel, a separate dark exposure will be taken for each channel.

Ethidium Bromide (EtBr) Stain

EtBr, a non-covalent intercalating dye, is by far the most widely used stain for dsDNA in gels. It can also be used to stain both ssDNA and RNA in gels and will stain nucleic acids in solution. The absorption maxima of ethidium bromide is at 280 nm and the emission maxima is ~600 nm.

Methods

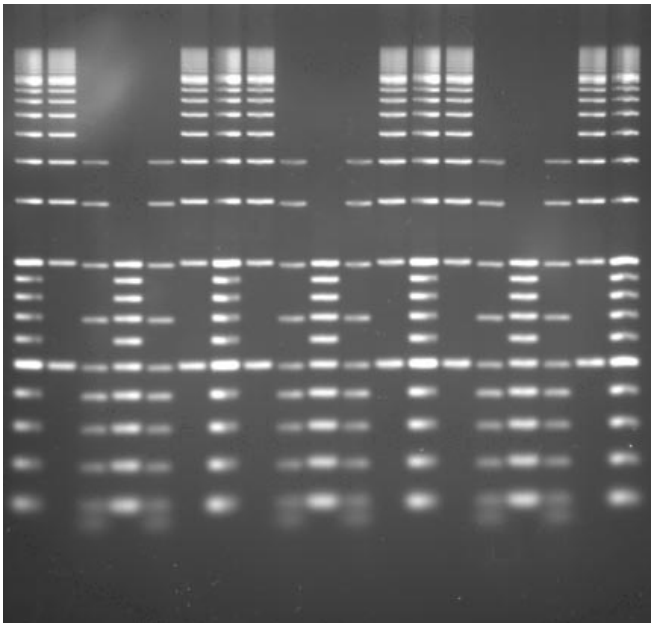
- Stain gel with 0.5 µg/ml EtBr in ddH_2O for 15 minutes with gentle shaking.
- Destain in ddH_2O for 15 minutes.
- Gels can also be stained during the electrophoresis run by adding 0.5 µg/ml EtBr to both the gel solution and the running buffer.
- Use UV scanning illumination with the lens aperture fully open.
- Use the 520 long pass filter.

For Optimal Results on the Fluor-S MultiImager System

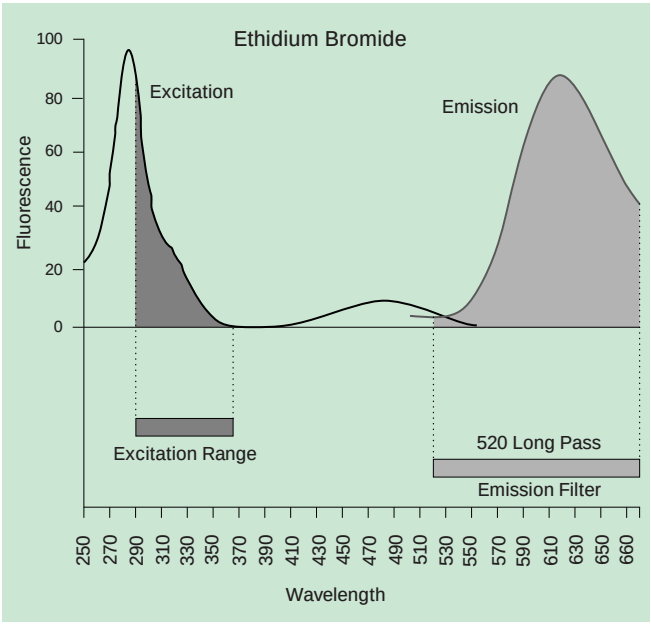
- Typical exposure times are from a few seconds to a couple of minutes, depending on sample concentration. Exposure times for polyacrylamide are shorter than for agarose due to higher background fluorescence in agarose.
- Optimize viewing of the image with the palette and transformation controls in the software.
- Long staining times or use of more than the recommended stain concentration can result in higher stain background in the gel.
- Detection of ssDNA and RNA with EtBr is not as sensitive as dsDNA. Consider using Radiant Red stain for detecting ssDNA and RNA.
- Efficient destaining of the gel can minimize background and improve image quality.

Detection Limits

Detection limits for dsDNA on the Fluor-S MultiImager system are 10–25 pg on polyacrylamide gels and 20–50 pg on agarose gels.



EtBr. A series of 100 bp, 500 bp and AmpliSize™ DNA standards electrophoresed on a 2% agarose gel with TBE buffer and post stained with ethidium bromide. The image was collected for 30 seconds using UV scanning illumination, 300 mm scan distance in the high resolution mode using the 520 long pass filter.



Excitation and detection of EtBr on the Fluor-S MultiImager system.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
161-0433	Ethidium Bromide Solution, 10 ml, 10 mg/ml
161-0430	Ethidium Bromide Tablets
170-8200	AmpliSize Standard
170-8201	20 bp Molecular Ruler
170-8202	100 bp Molecular Ruler
170-8203	500 bp Molecular Ruler
170-8204	1 kp Molecular Ruler
170-8205	2.5 kp Molecular Ruler

SYBR Green I Stain

SYBR Green I stain is a DNA intercalating dye. An advantage of SYBR Green I stain over ethidium bromide is that the fluorescence enhancement upon binding to DNA is greater, thus a lower background from the unbound stain is seen in the gel.

Methods

- Post-stain gel with 1:10,000 dilution of stock SYBR Green I stain in buffer (TE, TBE, or TAE) for 15 minutes with gentle shaking.
- Destaining is not necessary with SYBR Green I stain.
- Use UV scanning illumination with the lens aperture fully open.
- Use the 530 band pass filter for best visualization.

For Optimal Results on the Fluor-S MultiImager System

- Typical exposure times are from a few seconds to a couple of minutes, depending on sample concentration. Exposure times for agarose are longer than for polyacrylamide.
- SYBR Green I stain binds to glass and some plastics. Use dedicated polypropylene containers for staining. Do not use detergents to wash the plastic; just rinse with distilled water.

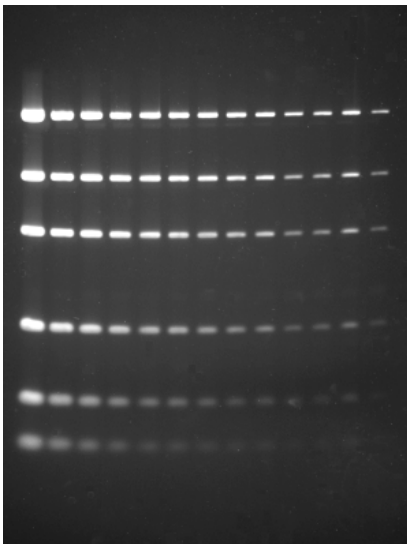
Detection Limits

Detection limits for dsDNA on the Fluor-S MultiImager system are 5–10 pg on polyacrylamide gels and 10–20 pg on agarose gels.

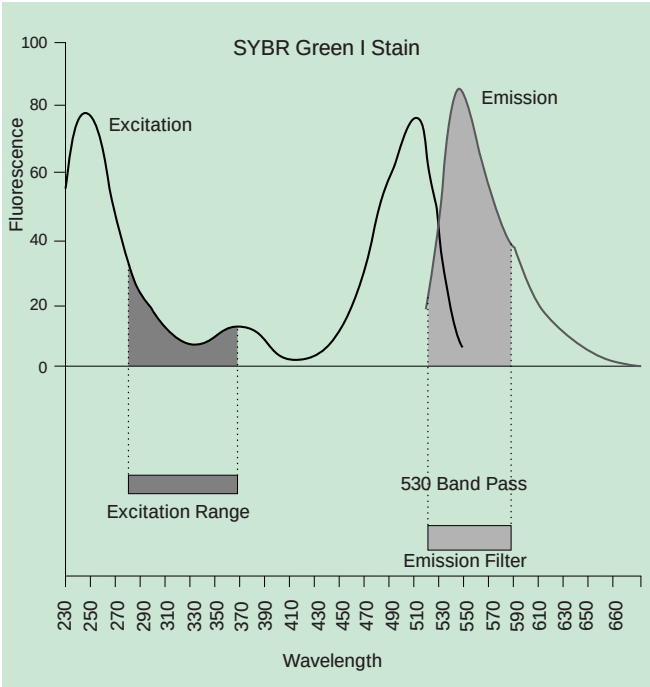
Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION

SYBR Green is available from Molecular Probes (Eugene, OR)



SYBR Green I stain. Dilution series of a DNA Mass Ladder electrophoresed on a 2% agarose gel with TBE buffer and post-stained with SYBR Green I stain. The highest concentration lane is 40 ng, 24 ng, 16 ng, 8 ng, 4 ng, and 2 ng of DNA, starting with the top band. Each successive lane is a two fold dilution of the previous lane. The image was collected for 30 seconds with UV scanning illumination, 80 mm scan distance, in the High Resolution mode using the 530 bp filter.



Excitation and detection of SYBR Green I stain on the Fluor-S MultiImager system.

Multi-Color Detection with Fluorescein and Texas Red Label

Multi-color detection of DNA samples labeled with different fluorophores allows one to multiplex unknown samples together with molecular weight standards in the same lane. This results in the most accurate size determination. Multiplexing also allows increased throughput by running multiple samples in the same lane of a gel. The standard filters on the Fluor-S MultiImager system allow clear distinction of fluorescein-labeled and Texas Red-labeled samples.

Methods

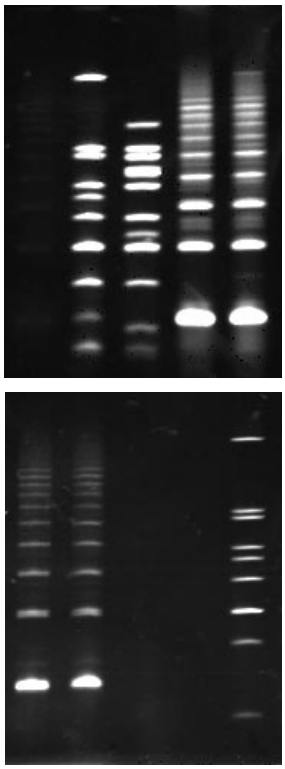
- Mix and load samples which are end-labeled with different fluorophores on the same lane of a gel.
- Use UV scanning illumination with the lens aperture fully open.
- Image the separate fluorophores by collecting multiple channel images first with the 610 long pass filter (for Texas Red label), then with the 530 band pass filter (for fluorescein).
- Note that the integration time may be different for the different filters. The background from the gel will also be higher in the fluorescein channel.

For Optimal Results on the Fluor-S MultiImager System

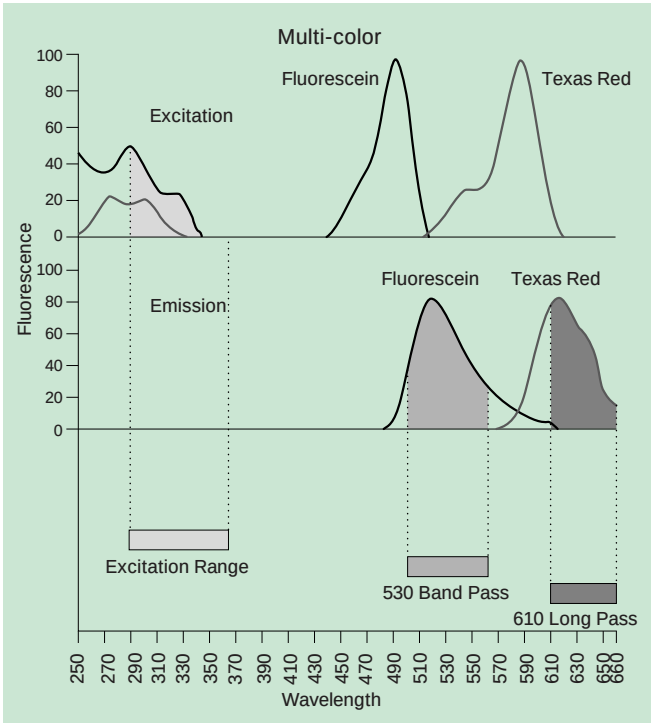
- Typical exposure times are from a few seconds to a couple of minutes, with exposure times for polyacrylamide shorter than for agarose.
- When working with end-labeled DNA, as fragment size increases, load more mass of the DNA in a lane to obtain the same signal intensity.
- Attempt to load equivalent numbers of moles of each labeled sample to equalize signal intensity.

Detection Limits

The detection limit for both fluorescein and Texas Red end-labeled DNA fragments on the Fluor-S MultiImager system is from 2–4 fmoles per fragment.



Multi-color detection. Independent detection of DNA end-labeled with fluorescein or Texas Red electrophoresed on a 5% polyacrylamide gel in TBE buffer. Top: Image of the Texas Red end-labeled DNA. The image was collected for 2 minutes with UV transillumination, 80 mm scan distance, in the High Sensitivity mode using the 610 long pass filter. Bottom: The same gel lanes as in the top image but imaging of the fluorescein end-labeled DNA. The image was collected for 60 seconds with UV transillumination, 80 mm scan distance, in the High Sensitivity mode using the 530 band pass filter.



Excitation and detection of multi-color on the Fluor-S MultiImager system.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
170-3123	FITC Low Molecular Weight Fluorescent DNA Standard
170-3124	Texas Red Low Molecular Weight Fluorescent DNA Standard
170-8216	100 bp Fluorescein Ruler
170-8217	100 bp Texas Red Ruler
170-8218	500 bp Fluorescein Ruler
170-8219	500 bp Texas Red Ruler
170-8221	DNA Labeling Kit, Texas Red-dCTP
170-8222	DNA Labeling Kit, Texas Red-dUTP
170-8223	DNA Labeling Kit, Fluorescein-dCTP
170-8224	DNA Labeling Kit, Fluorescein-dUTP

Radiant Red Stain

Radiant Red stain is a new fluorescent RNA stain available exclusively from Bio-Rad Laboratories. It is a fast, sensitive, and easy to use stain that substantially out-performs ethidium bromide staining of RNA in formaldehyde gels. It is also much less expensive and more sensitive than other single-strand specific stains on formaldehyde gels.

Methods

- Prestain RNA by addition of 1 µl of stock Radiant Red stain to 10 µl of sample.
- Poststain by diluting 100 µl stock Radiant Red stain in 100 ml buffer.
- Stain gel at room temperature 30 minutes with gentle agitation.
- Rinse gel 60 seconds in ddH_2O.
- Use UV scanning illumination with the lens aperture fully open.
- Use the 610 long pass filter to visualize the gel.

For Optimal Results on the Fluor-S MultiImager System

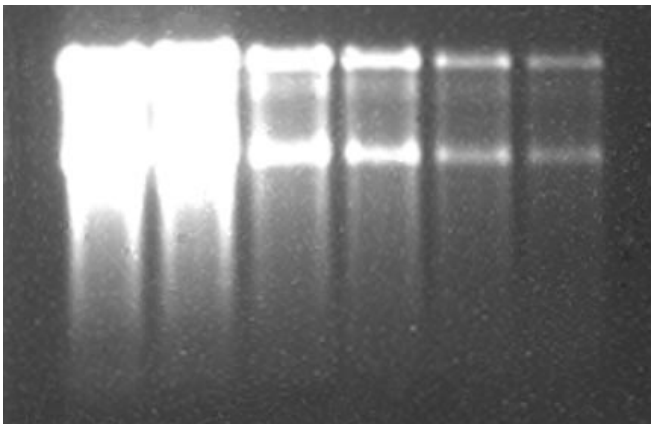
- Typical exposure times are from a few seconds to a couple of minutes, with exposure times for polyacrylamide shorter than for agarose.
- Staining should be done in plastic rather than glass.
- Radiant Red stain can also be used to stain glyoxal RNA gels.

Detection Limits

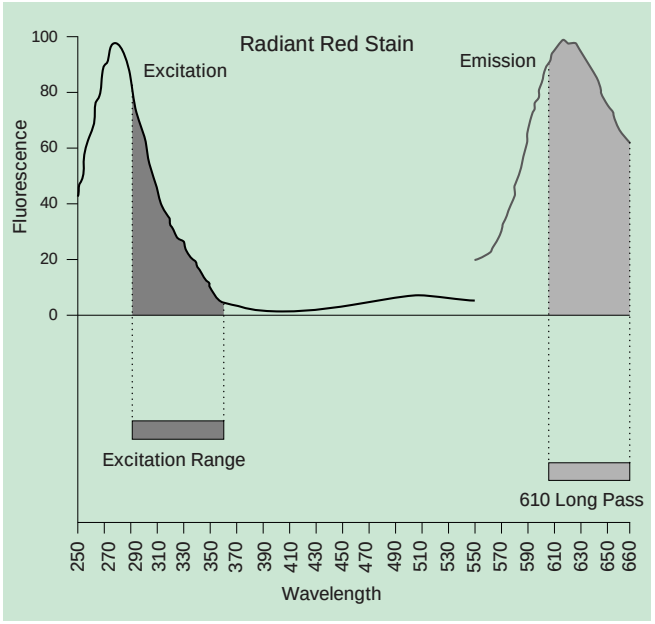
In formaldehyde gels, as little as 10 ng of RNA can be visualized.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
170-3122	Radiant Red RNA Stain, 10 ml



Radiant Red stain. Two-fold serial dilution of E. coli *ribosomal RNA pre-stained with Radiant Red stain and electrophoresed on a 1% agarose gel in buffer containing 1x MOPS and 2.2 M formaldehyde. The highest concentration of RNA is 4 µg. The image was collected for 60 seconds using UV scanning illumination, 160 mm scan distance, in the High Sensitivity mode using the 610 long pass filter.*



Excitation and detection of Radiant Red stain on the Fluor-S MultiImager system.

SYPRO Orange Stain

SYPRO Orange stain is used to detect proteins following native or denaturing polyacrylamide gel electrophoresis. SYPRO Orange-stained proteins may be blotted or electro-eluted without destaining. Furthermore, stained proteins blotted to membranes may be visualized by epi-illumination of the membrane. SYPRO Orange staining does not interfere with later western blotting or protein sequencing. The exact mechanism of the interaction between proteins and SYPRO Orange stain is unknown, but it is mediated by the presence of SDS. For that reason, native gels are first soaked in an SDS solution before incubation in SYPRO Orange staining solution.

Methods

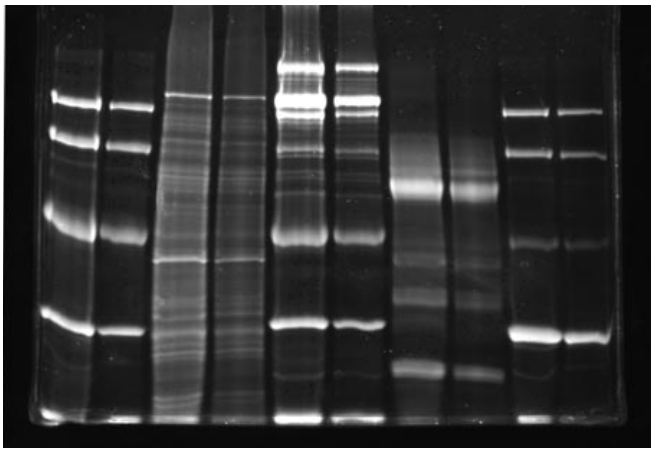
- Post-stain gel in 1:5,000 dilution of SYPRO Orange stain in 7.5% acetic acid for 30 minutes with gentle shaking.
- If the proteins are to be eluted or blotted, substitute a 25 mM Tris, 192 mM glycine solution for the acetic acid. Staining may also be carried out in Towbin buffer (25 mM Tris, 192 mM glycine, 20% methanol).
- If the gel was native, first soak the gel in a 0.05% SDS solution for 30 minutes. After 30 minutes, transfer the gel to a 1:5,000 dilution of SYPRO Orange stain. Make the SDS solution in the same solvent that the gel will eventually be stained in.
- Rinse the gel for 30–60 seconds in fresh 7.5% acetic acid or fresh buffer.
- Use UV scanning illumination with the lens aperture fully open.
- Use the 520 long pass filter.

For Optimal Results on the Fluor-S MultiImager System

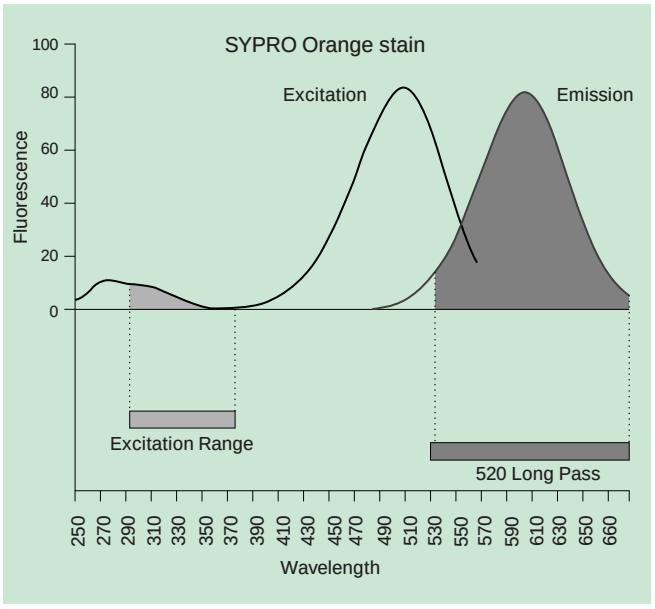
- Typical exposure times are less than 1 minute.
- The fluorescent response is most linear when staining is carried out in acetic acid.

Detection Limits

Detection limits are a function of the protein being stained, but most proteins may be detected in the 1–10 ng range.



SYPRO Orange protein stain. A 12% Tris-Glycine Ready Gel was loaded with 10 µl and 5 µl of a dilution of Bio-Rad's Low Range SDS-PAGE Standards, 10 µg and 5 µg of E. coli *lysate, 10 µl and 5 µl of a dilution of Broad Range SDS-PAGE Standards, 10 µg and 5 µg snake venom (Sigma) and 10 µl and 5 µl of a dilution of the SYPRO Orange Low Range SDS-PAGE Standards. Following electrophoresis the gel was stained for 30 minutes in a 1:5,000 dilution of SYPRO Orange protein stain in 7.5% acetic acid. Detection was carried out using UV scanning illumination and the 520 long pass filter with 20 seconds integration in the High Resolution mode.*



Excitation and detection of SYPRO Orange stain on the Fluor-S MultiImager system.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
170-3120	SYPRO Orange Protein Stain, 500 µl
161-0330	SDS-PAGE Standard for SYPRO Orange Stain, low range
161-0331	SDS-PAGE Standard for SYPRO Orange Stain, high range
161-0332	SDS-PAGE Standard for SYPRO Orange Stain, broad range

Sensitivity of SYPRO Orange, Coomassie Blue, Silver, Copper, and Zinc Stain

	SENSITIVITY	BENEFITS
SYPRO Orange stain	1–10 ng/band	Highly sensitive fluorescent stain; will not stain nucleic acids
Coomassie blue	50–500 ng/band	Simple, fast, consistent
Silver stain	1–10 ng/band	Highly sensitive; can also stain nucleic acids
Copper stain	10–100 ng/band	Simple, fast, reversible, subsequent electro-elution or blotting is possible. SDS-PAGE only
Zinc stain	10–100 ng/band	Simple, fast, reversible; subsequent electro-elution or blotting is possible. SDS-PAGE only

Coomassie Brilliant Blue R-250 Stain

Coomassie Blue stain is a simple, fast, and consistent system for detecting proteins in polyacrylamide gels after electrophoresis. Coomassie Brilliant Blue R-250 stain is the most popular of the protein dyes.

Methods

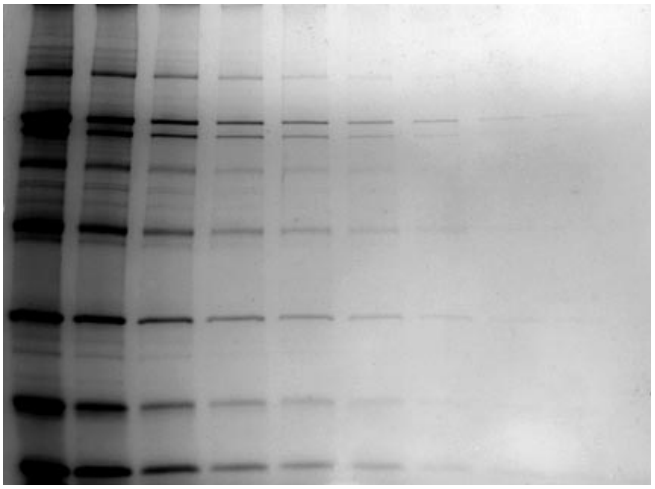
- Stain gel for 30–60 minutes in Coomassie stain (0.25% Coomassie blue R-250, 40% methanol, 10% acetic acid).
- Destain gel in 40% methanol, 10% acetic acid until background is clear (~3–4 hours).
- Use white light scanning illumination with the white light scanning plate.
- Use the clear filter.
- Control the exposure with both integration time and aperture.
- Coomassie stained gels can also be imaged with epi-illumination when placed on the white light scanning plate.

For Optimal Results on the Fluor-S MultiImager System

- When using white light scanning illumination, set the aperture, and adjust the scan time until the background is just below saturation.

Detection Limits

Detection limits for Coomassie Brilliant Blue R-250 stained proteins on the Fluor-S MultiImager system are 50–500 ng/protein band on polyacrylamide gels.



Coomassie stain. Two-fold dilution series of Bio-Rad Broad Range SDS-PAGE Standards run on a 12% Tris-Glycine Ready Gel and stained with Bio-Rad's Coomassie Brilliant Blue R-250 Staining kit. The protein concentration on lane one ranges from 700 to 1,700 ng for the individual proteins. The gel was placed on a white plastic background and the image was collected using white light epi-illumination for a 1.7 second exposure, an f-stop setting of 5.6, in the High Resolution mode.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
161-0435	Coomassie Brilliant Blue R-250 Staining Solutions Kit

Silver Stain

Silver stain is a highly sensitive, general protein stain for detecting proteins or nucleic acids in polyacrylamide and dried agarose gels after electrophoresis. The high sensitivity of silver stain allows the detection of trace proteins in dilute samples.

Methods

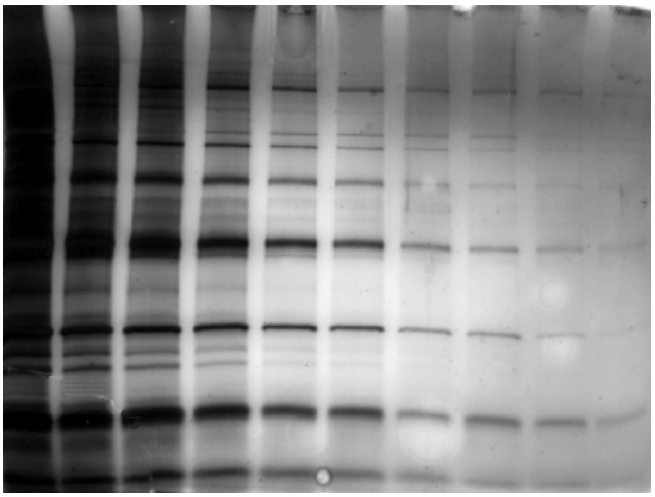
- Fix gel with Fixative Enhancer solution (50% methanol, 10% acetic acid).
- Rinse gel with ddH_2O.
- Develop the gel in silver stain.
- Stop the silver stain development by washing gel in 5% acetic acid.
- Use white light scanning illumination with the white light scanning plate.
- Use the clear filter.
- Control exposure with both integration time and aperture.
- Silver stained gels can also be imaged with white light epi-illumination when placed on the white light scanning plate.

For Optimal Results on the Fluor-S MultiImager System

- When using white light scanning illumination, set the aperture and adjust the integration time until the background is just below saturation.

Detection Limits

Detection limits for silver stained proteins on the Fluor-S MultiImager system are 1–10 ng/protein band on polyacrylamide gels.



Silver stain. Two-fold dilution series of Bio-Rad's Broad Range SDS-PAGE Standards electrophoresed on a 12% Tris-Glycine Ready Gel and stained with Bio-Rad's Silver Stain Plus kit. The protein concentration on lane one ranges from 350 to 850 ng for the individual proteins. The gel was placed on the white light scanning plate and the image was collected using white light epi-illumination for a 1.5 second exposure, an f-stop setting of 5.6, in the High Resolution mode.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
161-0449	Silver Stain Plus Kit
161-0443	Silver Stain Kit

Sensitivity of SYPRO Orange, Coomassie Blue, Silver, Copper, and Zinc Stain

	SENSITIVITY	BENEFITS
SYPRO Orange stain	1–10 ng/band	Highly sensitive fluorescent stain; will not stain nucleic acids
Coomassie blue	50–500 ng/band	Simple, fast, consistent
Silver stain	1–10 ng/band	Highly sensitive; can also stain nucleic acids
Copper stain	10–100 ng/band	Simple, fast, reversible, subsequent electro-elution or blotting is possible. SDS-PAGE only
Zinc stain	10–100 ng/band	Simple, fast, reversible; subsequent electro-elution or blotting is possible. SDS-PAGE only

Copper Chloride Stain

Copper Chloride stain is a simple, rapid, and sensitive system for detecting proteins in polyacrylamide gels after electrophoresis. The proteins are negatively stained showing clear protein bands on opaque background. Proteins can be recovered from the gel after removal of excess copper by chelation destaining.

Methods

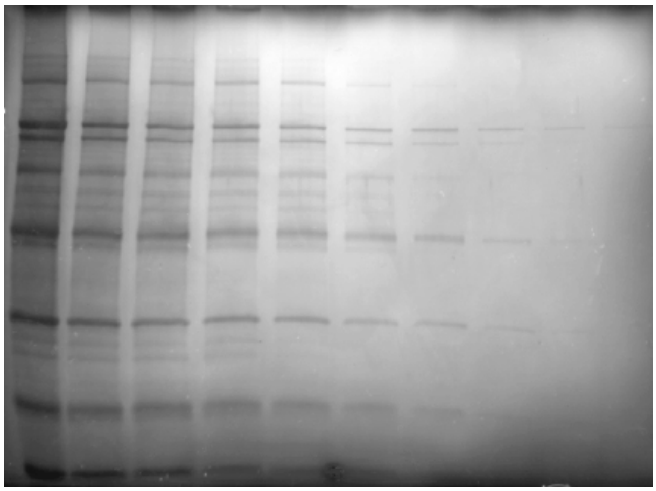
- Wash gels in deionized water for 3–5 minutes.
- Stain the gel in copper chloride solution for 5 minutes.
- Wash the gels in ddH_2O for 3 minutes.
- Discard wash and replace with fresh ddH_2O.
- Copper chloride-stained gels should be imaged with epi-illumination on a black background.
- Use the clear filter.
- Control exposure with both integration time and aperture.

For Optimal Results on the Fluor-S MultiImager System

- When using epi-illumination, set the aperture and adjust the integration time until the background is just below saturation.

Detection Limits

Detection limits for copper stained proteins on the Fluor-S MultiImager system are 10–100 ng/protein band on an polyacrylamide gel.



Copper stain. Two-fold dilution series of Bio-Rad's Broad Range SDS-PAGE Standards run on a 12% Tris-Glycine Ready Gel and stained with Bio-Rad's Copper Stain & Destain kit. The protein concentration on lane one ranges from 700 to 1,700 ng for the individual proteins. The gel image was collected using white light epi-illumination for a 3.5 second exposure, an f-stop setting of 4, in the High Resolution mode.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
161-0470	Copper Stain and Destain Kit

Zinc Stain

Zinc stain is a simple, rapid, and sensitive system for detecting proteins in polyacrylamide gels after electrophoresis. The proteins are negatively stained showing clear protein bands on an opaque white background.

Methods

- Soak gels in Solution A (Imidazole) for 10 minutes.
- Develop the gels in Solution B (Zinc Sulfate) for 30 seconds.
- Wash the gels in ddH_2O for 3 minutes.
- Discard wash and replace with fresh ddH_2O.
- Use the clear filter for white light imaging.
- Zinc-stained gels should be imaged with epi-illumination on a black background.
- Control exposure with both integration time and aperture.
- Alternatively, because the zinc-imidazole complex fluoresces under UV light, the gel can be visualized using UV scanning or epi-illumination with the 520 long pass filter.

For Optimal Results on the Fluor-S MultiImager System

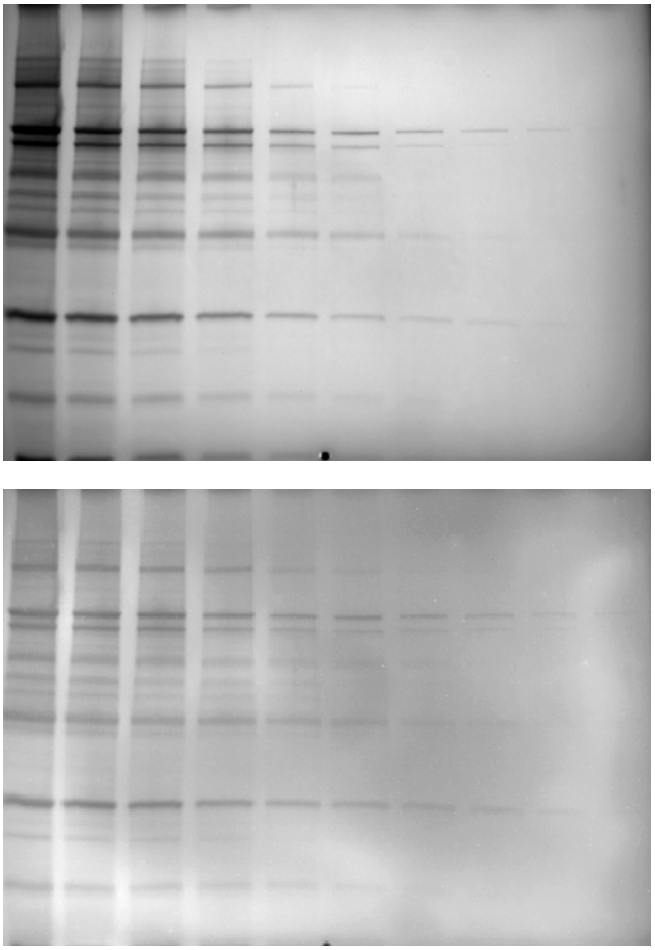
- When using epi-illumination, set the aperture and adjust the integration time until the background is just below saturation.

Detection Limits

Detection limits for zinc stained proteins on the Fluor-S MultiImager system is 10–100 ng/protein band on polyacrylamide gels.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
161-0440	Zinc Stain and Destain Kit



Zinc stain. Two-fold dilution series of Bio-Rad's Broad Range SDS-PAGE Standards run on a 12% Tris-Glycine Ready Gel and stained with Bio-Rad's Zinc Stain & Destain kit. The protein concentration on lane one ranges from 700 to 1,700 ng for the individual proteins. Top: The gel image was collected using white light epi-illumination for a 1.5 second exposure, an f-stop setting of 4, in the High Resolution mode. Bottom: The gel image was collected using UV scanning illumination for a 50 second exposure, an f-stop setting of 2.7, 160 mm scan distance, in the High Sensitivity mode with the 520 nm long pass filter.

Sensitivity of SYPRO Orange, Coomassie Blue, Silver, Copper, and Zinc Stain

	SENSITIVITY	BENEFITS
SYPRO Orange stain	1–10 ng/band	Highly sensitive fluorescent stain; will not stain nucleic acids
Coomassie blue	50–500 ng/band	Simple, fast, consistent
Silver stain	1–10 ng/band	Highly sensitive; can also stain nucleic acids
Copper stain	10–100 ng/band	Simple, fast, reversible, subsequent electro-elution or blotting is possible. SDS-PAGE only
Zinc stain	10–100 ng/band	Simple, fast, reversible; subsequent electro-elution or blotting is possible. SDS-PAGE only

Chemiluminescent Detection of Blots

Southern, northern, western, and dot blots detected with chemiluminescent substrates may be documented and quantitated on the Fluor-S MultiImager system.

Methods

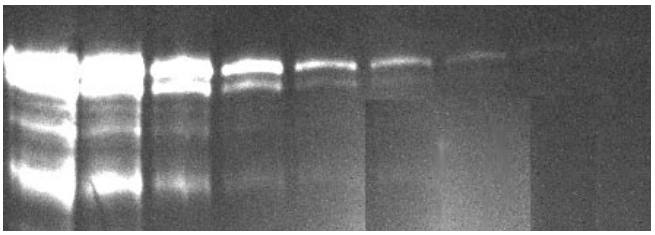
- Carry out detection as usual up to the point that you would normally put the blot on film.
- Select chemi: no light source.
- Place the blot face up in the Fluor-S MultiImager system.
- Open the aperture completely and integrate without a filter; remove the 660 SP filter from the lens.

For Optimal Results on the Fluor-S MultiImager System

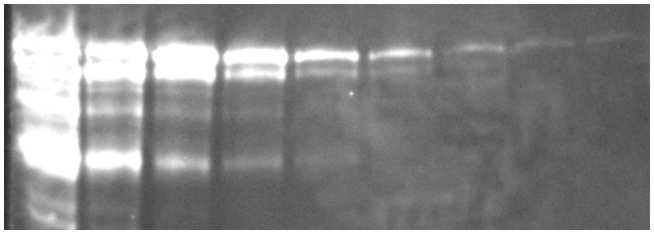
- Typical integration times are 1–10 minutes.
- Use the optional 50 mm f1.8 lens.
- Use the transformation and palette controls to optimize the image.

Detection Limits

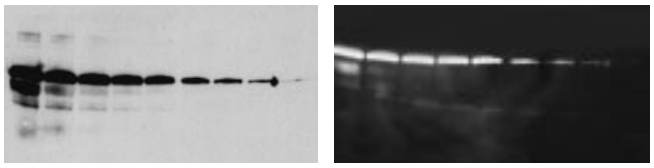
Detection limits are a function of the experimental system, including the protein, the concentration and specificity of both antibodies and the substrate chosen.



*ECL. A serial dilution of the protein transferrin (Sigma) was electrophoresed on a 10% polyacrylamide Tris-Glycine Ready Gel and blotted to a pure nitrocellulose membrane. 256 ng of transferrin were loaded in the first lane; each successive lane contains a further two-fold dilution. Following blocking in 5% non-fat dry milk, the blot was incubated in a 1:3,000 dilution of anti-transferrin (DAKO) and then incubated in a 1:3,000 dilution of a goat-anti-rabbit IgG-horse radish peroxidase-conjugated (GAR-HRP) secondary antibody. After secondary antibody application and washes, the blot was incubated in 6 ml of the ECL substrate (Amersham) for one minute. Detection was carried out for 5 minutes using the 50 mm lens without any filters in High Sensitivity mode (*i.e.*, the 660SP filter removed from the lens, and an empty filter wheel position).*



*Ultra Super Signal. A serial dilution of the protein transferrin (Sigma) was electrophoresed on a 10% polyacrylamide Tris-Glycine Ready Gel and blotted to a pure nitrocellulose membrane. 256 ng of transferrin were loaded in the first lane; each successive lane contains a further two-fold dilution. Following blocking in 5% non-fat dry milk, the blot was incubated in a 1:3000 dilution of anti-transferrin (DAKO) and then incubated in a 1:3000 dilution of GAR-HRP secondary antibody. After secondary antibody application and washes, the blot was incubated in 6 ml of the Ultra Super Signal substrate (Pierce) for 5 minutes. Detection was carried out for 1 minute in the High Sensitivity mode using the 50 mm lens without any filters (*i.e. *the 660SP filter removed from the lens, and an empty filter wheel position).*



*CDP-*Star. *Two-fold serial dilutions of human serum, beginning at 1:25 in lane 1, were loaded onto a 10% Polyacrylamide Tris-Glycine Ready Gel and electrophoresed at 200 V for 45 minutes. The proteins were electroblotted to pure nitrocellulose membranes which were then blocked in 5% blotto. The anti-transferrin (DAKO) primary antibody was diluted 1:5,000 as was the GAP-AP secondary antibody. After the final washes, detection was carried out with the Immun-Star kit using CDP-*Star *and the enhancer. The blot was incubated in the substrate plus enhancer for five minutes, then put on film for 30 minutes (left). Immediately after the film exposure, the same blot was placed into Fluor-S MultiImager system for a 45 minute integration (right).*

AmpLight™ Western Blotting Kits

Very sensitive immunodetection of proteins blotted to nitrocellulose may be carried out using Bio-Rad's new AmpLight fluorescent western blotting kit or the AmpLight chemiluminescent western blotting kit. Both kits use a proprietary amplification reagent which is compatible with either fluorescent or chemiluminescent detection. Use of the amplification reagent before chemiluminescent detection can result in a four-fold increase in overall sensitivity and in improved image quality.

Methods

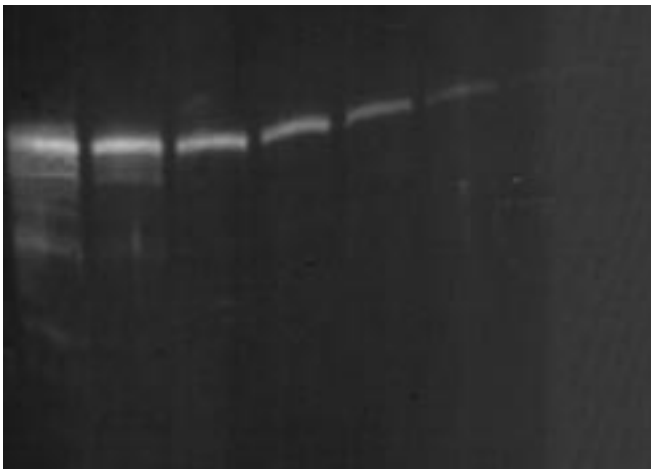
- Carry out electrophoresis as usual.
- Blot as usual.
- Soak membrane in the provided blocking solution for an hour
- Incubate in a dilution of primary antibody for an hour, then wash twice for 5 minutes with PBST (phosphate buffered saline with 0.05% Tween-20). Incubate for 1 hour in a dilution of secondary antibody and then repeat the wash steps.
- Incubate the blot for 10 minutes in the Bio-Rad Amplification Reagent (BAR) and then wash 2–4 times in DMSO/PBST followed by 1–2 washes in PBST alone.
- Incubate the blot for 30 minutes in Streptavidin-HRP followed by 2 washes in PBST.
- For chemiluminescent detection, incubate the blot in the ChemiDetect substrate for 1 minute, and for fluorescent detection incubate the blot for 10 minutes in the FluorDetect reagent followed by 2–4 washes in DMSO/PBST and 1–2 washes in PBST.

Getting the Best Results on the Fluor-S MultiImager System

- For fluorescent detection, use UV epi-illumination with the 530DF60 filter and High Resolution mode.
- For chemiluminescent detection, use the 50 mm lens without the 660 LP filter, the clear filter and High Sensitivity mode.
- Use pure nitrocellulose rather than supported nitrocellulose.
- For most sensitive detection, extend integration time until background approaches half way to saturation (2,000 counts).
- For most linearity, integrate until the most concentrated band approaches saturation.

Detection Limits

Detection limits are a function of the experimental system, including the protein, the antibody concentration, and specificity and the detection reagent chosen.



AmpLight Fluorescent Western Blotting Kit. A serial dilution of transferrin (Sigma) was electrophoresed on a 10% acrylamide Ready Gel and blotted to a pure nitrocellulose membrane. 16 ng of transferrin were loaded in the first lane; each successive lane contains a further two-fold dilution. Following blocking in a 3% blocking solution, the blot was incubated in a 1:3,000 dilution of anti-transferrin (DAKO) and then incubated in a 1:3,000 dilution of a goat-anti-rabbit IgG-horseradish peroxidase-conjugated (GAR-HRP) secondary antibody. The blot was next incubated in the Bio-Rad Amplification Reagent (BAR) for 10 minutes, rinsed, then incubated in streptavidin-HRP for 30 minutes and rinsed again. Following a 10 minute incubation in the FluorDetect reagent and a rinse, the membrane was placed on the Fluor-S MultiImager system. Detection was carried out using UV epi-illumination, the 530 band pass filter and 60 seconds integration in High Resolution mode.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
170-8234	AmpLight Chemiluminescent Western Blotting Kit
170-8232	AmpLight Fluorescent Western Blotting Kit

Chemifluorescent (AttoPhos) Detection of Blots

Western and dot blots detected with the chemifluorescent substrate, AttoPhos, may be documented and quantitated on the Fluor-S MultiImager system.

Methods

- After primary antibody application, choose an appropriate secondary antibody conjugated with alkaline phosphatase.
- Wash the blot with PBST two or three times following secondary antibody application.
- Dissolve 36 mg of the AttoPhos detection reagent in 60 ml of AttoPhos dilution buffer.
- Incubate the membrane for 20 minutes with 0.25 ml diluted AttoPhos reagent per cm^2 of membrane.
- Drain off the excess AttoPhos reagent.
- Illuminate the blot using UV epi-illumination.
- Use the 520 long pass filter for emission.

For Optimal Results on the Fluor-S MultiImager System

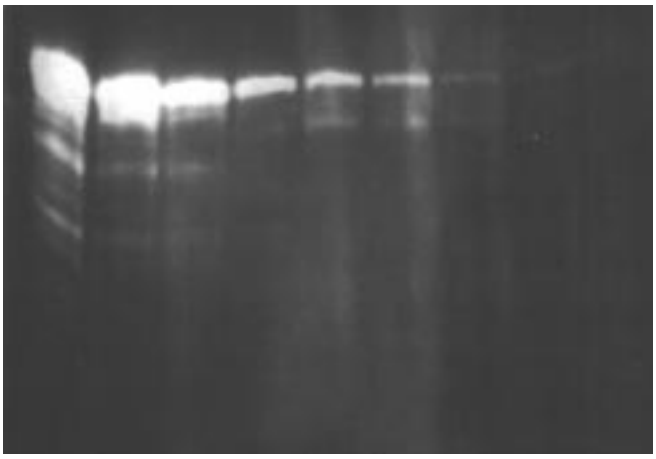
- Use pure nitrocellulose membranes rather than supported nitrocellulose.

Detection Limits

Detection limits are a function of the experimental system, including the protein, the concentration and specificity of both antibodies, and the substrate chosen.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
Purchase AttoPhos from JBL Scientific (805) 544-8524	



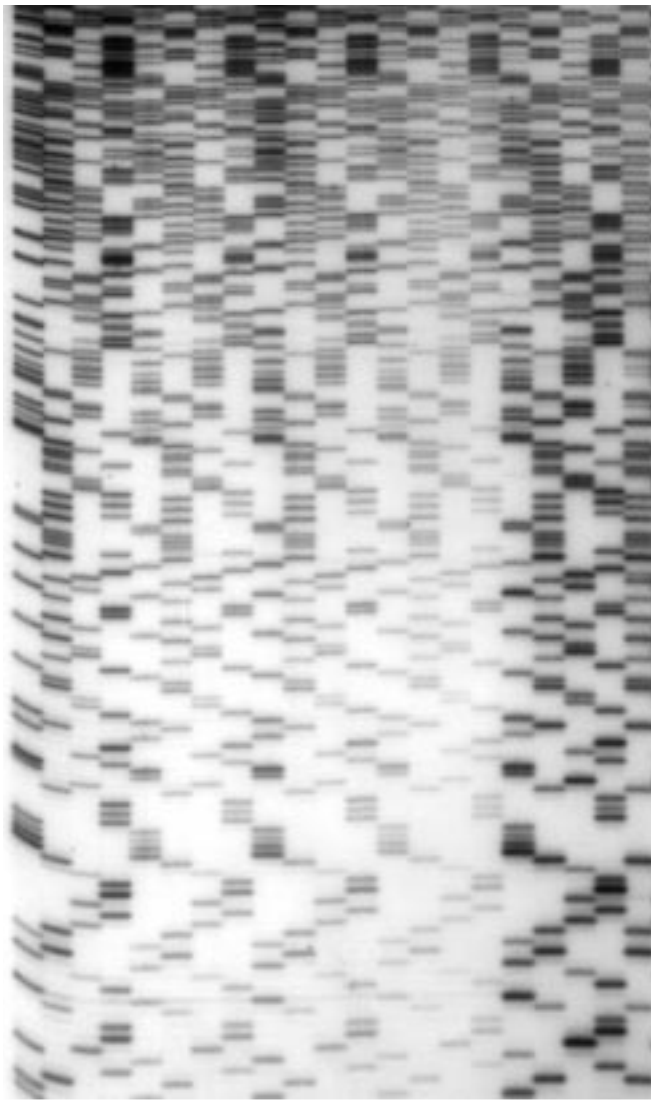
AttoPhos. A serial dilution of the protein transferrin (Sigma) was electrophoresed on a 10% polyacrylamide Tris-Glycine Ready Gel and blotted to a supported nitrocellulose membrane. 64 ng of transferrin were loaded in the first lane; each successive lane contains a further two-fold dilution. Following blocking in 5% non-fat dry milk, the blot was incubated in a 1:3,000 dilution of anti-transferrin (DAKO) and then incubated in a 1:3,000 dilution of a goat-anti-rabbit IgG alkaline phosphatase-conjugated (GAR-AP) secondary antibody. After secondary antibody application and washes, the blot was incubated in 3 ml of a 0.6 mg/ml solution of the AttoPhos substrate (JBL Scientific) for 20 minutes. Detection required 5 seconds of integration using UV epi-illumination and the 520 LP filter in High Resolution mode.

Digital Documentation

The Fluor-S MultiImager system has both white light scanning illumination and white light epi-illumination for the digital documentation of both photographs and X-ray film.

Methods

- For X-ray film or other transparent samples use white light scanning illumination with the white light scanning plate.
- For X-ray film or other transparent background samples, collect images until the background approaches saturation.
- For photographs or opaque samples use white light epi-illumination.
- Control exposure with both integration time and aperture.
- Use the clear filter.



X-Ray film. A ^{35}S labeled DNA sequencing reaction was run on a sequencing gel and then exposed to X-ray film. The DNA sequencing x-ray film image was collected using white light scanning illumination for a 8 second exposure, an f-stop setting of 16, 300 mm scan distance, in the High Resolution mode.

Ordering Information

CATALOG NO.	PRODUCT DESCRIPTION
Fluor-S Instrument	
170-7700	Fluor-S MultiImager-PC, 100-240 V, includes Multi-Analyst PC software, SCSI interface cable, and instructions
170-7701	Fluor-S MultiImager-Macintosh, 100-240 V, includes Multi-Analyst Macintosh software, SCSI interface cable, and instructions
170-7705	50 mM Lens
170-7706	105 mM Lens
Nucleic Acid Stains	
161-0430	Ethidium Bromide Tablets
161-0433	Ethidium Bromide Solution, 10 ml, 10 mg/ml
170-3122	Radiant Red RNA Stain, 10 ml
DNA Standards	
170-3123	FITC Low MW Fluorescent DNA Standard
170-3124	Texas Red Low MW Fluorescent DNA Standard
170-8216	100 bp Fluorescein Ruler
170-8217	100 bp Texas Red Ruler
170-8218	500 bp Fluorescein Ruler
170-8219	500 bp Texas Red Ruler
170-8200	AmpliSize DNA Size Standard
170-8201	20 bp Molecular Ruler
170-8202	100 bp Molecular Ruler
170-8206	100 bp PCR Ruler
170-8203	500 bp Molecular Ruler
170-8204	1 K bp Molecular Ruler
170-8205	2.5 K bp Molecular Ruler
170-8207	Mass Ruler
170-3465	Low Range DNA Size Standard
170-8210	Mid Range DNA Size Standard, 1–4.2 kb ladder
170-8220	Mid Range DNA Size Standard, 1–14.1 kb ladder
170-3470	High Range DNA Size Standard
Fluorescent Labeling Kits	
170-8221	DNA Labeling Kit, Texas Red-dCTP
170-8222	DNA Labeling Kit, Texas Red-dUTP
170-8223	DNA Fluorescein, Fluorescein-dCTP
170-8224	DNA Labeling Kit, Fluorescein-dUTP

CATALOG NO.	PRODUCT DESCRIPTION
AmpLight Western Blotting Kits	
170-8234	AmpLight Chemiluminescent Western Blotting Kit
170-8232	AmpLight Fluorescent Western Blotting Kit
Protein Stains	
170-3120	SYPRO Orange Protein Stain, 500 µl
161-0435	Coomassie Brilliant Blue R-250 Staining Solutions Kit
161-0449	Silver Stain Plus Kit
161-0443	Silver Stain Kit
161-0470	Copper Stain and Destain Kit
161-0440	Zinc Stain and Destain Kit
Protein Standards	
161-0330	SDS-PAGE Standard for SYPRO Orange Stain, low range
161-0331	SDS-PAGE Standard for SYPRO Orange Stain, high range
161-0332	SDS-PAGE Standard for SYPRO Orange Stain, broad range
161-0314	Silver Stain SDS-PAGE Standards, low range
161-0315	Silver Stain SDS-PAGE Standards, high range
161-0305	Prestained SDS-PAGE Standards, low range
161-0309	Prestained SDS-PAGE Standards, high range
161-0318	Prestained SDS-PAGE Standards, broad range
161-0326	Polypeptide SDS-PAGE Standards
161-0304	SDS-PAGE Standards, low range
161-0303	SDS-PAGE Standards, high range
161-0317	SDS-PAGE Standards, broad range
161-0320	2-D SDS-PAGE Standards
161-0310	IEF Standards, pI 4.6–9.6

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AmpliSize®
Fluor-S™
Multi Analyst™
Radiant™

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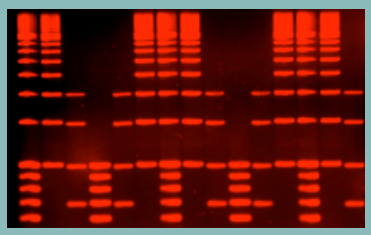
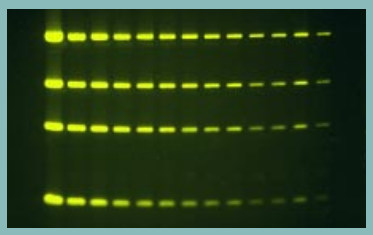
SYPRO, SYBR and Texas Red are trademarks of Molecular Probes, Inc.

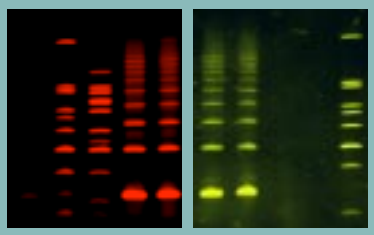
The Versatility of Fluor-S™ Imaging



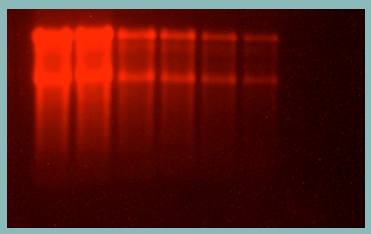
Ethidium Bromide Stain



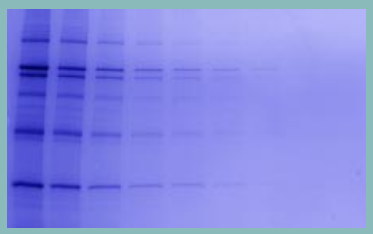
SYBR Green Stain



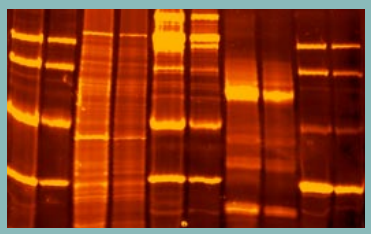
Multiplexing with
Texas Red and Fluorescein



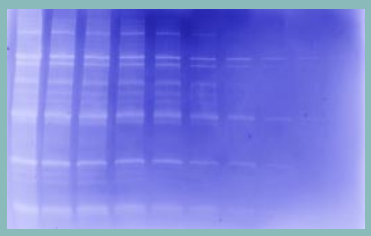
Radiant Red Stain



Coomassie Blue Stain



SYPRO Orange Stain



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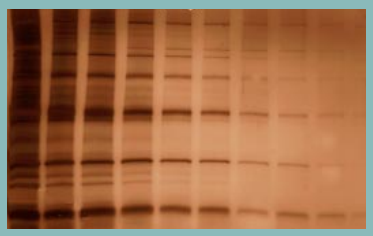
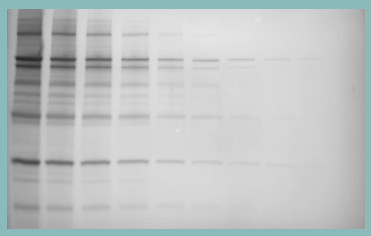
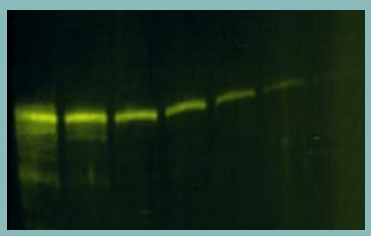


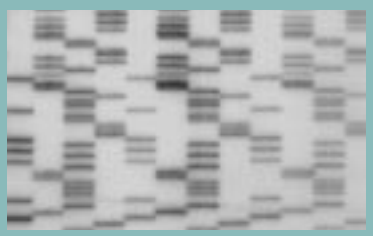
Silver Stain



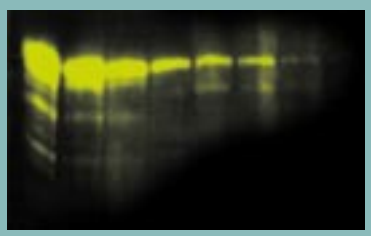
Zinc Stain



AmpLight Fluorescent
Western Blotting Kit



Digital Documentation



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