

Clare Chemical Research

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Dark Reader™ Transilluminators

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Dark Reader technology is the subject of issued US patents 6198107 and 6512236 as well as US and international patents pending.

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Safety Instructions

Keep these instructions available for easy reference by any user of this Dark Reader unit. For further assistance contact:

Clare Chemical Research
18390 Hwy 145, PO Box 180, Dolores, CO 81323
Tel: 970 882 7499
Fax: 970 882 7068
email: support@clarechemical.com
web: www.clarechemical.com

*** This transilluminator is only to be operated at the voltage specified on the accompanying Parts Checklist. The operating voltage is also listed on the side on the unit.

*** This unit is only intended for research and development purposes only.

*** The Dark Reader transilluminator is designed to be used by individuals who are experienced in using transilluminators to view fluorescent samples. Do not let untrained personnel operate this device!

*** Measurements show that the Dark Reader emits less UV light than the standard fluorescent tubes used in most offices and laboratories. However, the blue light emitted is fairly intense and it is unwise to look at any bright light source for extended periods of time. Always wear the viewing glasses or use the amber screen.

*** Unplug from the mains socket before attempting to open the Dark Reader box. The only replaceable component in the shell is the lamp. Seek advice from Clare Chemical technical support before attempting to open the box.

*** The Dark Reader transilluminator is not designed to be used in the bath! The Dark Reader is not waterproof. As with any electrical device, great caution must be taken when using near liquids. Mop up liquid spills immediately. (Disconnect the unit from the power supply first.)

*** Turn off after use to prevent over-heating. The Dark Reader should not be left switched on for a continuous period exceeding 1 hour. Do not locate the unit in an enclosed space that will prevent air circulation.

*** The Dark Reader has not been designed to withstand substantial impact. Do

not drop it on the floor!

*** Though data published by researchers at Molecular Probes show that SYBR Green and SYBR Safe stains are significantly safer than ethidium bromide, it should be remembered that any dye that stains DNA is potentially hazardous. Gloves should be worn when handling solutions or gels containing such dyes. Always follow the manufacturer's instructions regarding dye handling.

*** Contact of the Dark Reader and its parts with organic solvents or concentrated acids can damage the unit. Do not let organic solvents or acids come into contact with the Dark Reader.

*** The surface of the transilluminator should be cleaned only with soap and water or ethanol soaked onto a soft cloth or tissue paper. Disconnect the unit before cleaning.

*** The glasses and amber screen are ONLY for viewing in conjunction with Dark Reader products. The glasses and screen are NOT safety devices and do NOT provide eye protection. Do not use with UV sources.

Basic Instructions for Use

Questions? - Do not hesitate to contact us:

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Overview

Familiarize yourself with the parts of the Dark Reader as describes on the accompanying Parts Checklist sheet and read the Safety Instructions (pages 1-2 of this manual) before using your Dark Reader transilluminator!

To achieve maximum sensitivity, viewing fluorescence should be done in a darkened room.

Set up the transilluminator on a level surface and plug the power cord into a grounded wall socket.

Place the gel or other fluorescent samples on the Dark Reader transilluminator surface.

Place the amber screen on top of the gel. Alternatively, the glasses can be worn.

Switch on the Dark Reader transilluminator and view the gel.

After viewing / photographing the gel, turn off the Dark Reader.

Recommended DNA Stains

The new generation of DNA stains, such as SYBR® Green, SYBR Gold and GelStar®, are intrinsically much more sensitive than ethidium bromide. When used in conjunction with a Dark Reader transilluminator, the new stains are especially effective. Indeed, we recommend that you load about 5 times less DNA on your gel to avoid the 'sudden' appearance of minor DNA bands you never knew you had in your samples!

A Brief Method

The new stains are all used in a similar manner. (Always follow the manufacturer's detailed instructions carefully.) Make a 1:10,000 dilution of stain in 1 x TAE or TBE buffer. (Unused stain can be stored in a dark bottle at 4 °C for about 1 week.) Gently rock the agarose gel in the dye solution for 20 - 30 min. and then view on the Dark Reader transilluminator. No destaining is required. For maximum sensitivity, the gel should be removed from the tray, but because the Dark Reader visible excitation light passes through many kinds of plastic and glass, there is often no need to remove the gel from the container in order to view the major DNA fragments. If the gel or Dark Reader surface warms up after an extended period, it may be necessary to occasionally remove condensation off the underside of the amber screen to achieve the best sensitivity.

SYBR Green

SYBR Green stain was the first of the new generation of DNA stains introduced by Molecular Probes. Using a Dark Reader transilluminator it is possible to detect less than 100 pg of SYBR Green-stained DNA by eye and 10 - 20 pg using a CCD or Polaroid camera system. More information is available at: www.clarechemical.com/green.htm

SYBR Green stain can also be used by just adding it to the DNA samples before electrophoresis. This technique has 2 advantages: (i) It drastically reduces the amount of potentially hazardous waste, (ii) It provides results quickly because the staining time is eliminated. Proceed as follows: make a 1:100 dilution of SYBR Green in 1 x TAE. Add 1 microL of this solution to each 9 microL of DNA sample. Load the gel and run in 2 hours or less. The dye is not added to the gel or running buffer. The disadvantages of this technique are: (i) the detection sensitivity is significantly reduced to about 300 pg of dsDNA, (ii) the migration rates of DNA fragments become unpredictable and this technique cannot be used to determine the sizes of DNA bands accurately.

SYBR Gold

SYBR Gold stain was also developed by Molecular Probes. Our tests on the sensitivity of DNA detection show that SYBR Gold is the most sensitive of the new DNA stains and it is possible to see (by eye) less than 75 pg of DNA. Furthermore, SYBR Gold enters agarose gels very rapidly and major DNA bands become visible in less than 2

minutes after adding staining solution. More information is available at: www.clarechemical.com/gold.htm

GelStar

GelStar stain (Cambrex) can be used as a post-stain, just like both of the SYBR dyes, but also works very well as an 'in-gel' stain if it is added to the agarose before electrophoresis. This is done as follows: melt the required amount of agarose; allow to cool to ~ 60 °C, add GelStar to give a 10,000-fold dilution; pour the gel. The presence of GelStar in the gel does not significantly affect the relative migration behavior of DNA bands. In-gel staining has the advantage that the DNA bands can be viewed immediately after the gel has been run. More information is available at: www.clarechemical.com/gelstar.htm

Ethidium bromide

Ethidium bromide detection of DNA using a Dark Reader transilluminator is less sensitive than with a 312 nm UV device. It is possible to detect ~5 ng of ethidium bromide-stained DNA using the Dark Reader by eye and about 600 pg using a CCD camera. It is important not to use an excessive amount of ethidium bromide. (0.1 - 0.2 microgram / mL is optimal.) Also, the room needs to be well darkened. More information is available at: www.clarechemical.com/ethidium.htm

Recommended Protein Stains

Several new fluorescent protein stains have been recently developed by Molecular Probes, Inc. These SYPRO® stains display excellent sensitivity similar to that of silver staining, less protein-to-protein variability than silver, a greater quantitation range, a simple one step staining procedure, and do not interfere with subsequent downstream characterization techniques. Most of the SYPRO stain family can be very effectively detected using a Dark Reader transilluminator.

SYPRO Ruby

The family of SYPRO Ruby stains can be used to detect proteins in SDS-polyacrylamide gels, isoelectric focusing gels and on membranes. About 2 ng of SYPRO Ruby-stained protein can be detected directly by eye in an SDS-polyacrylamide gel using a Dark Reader transilluminator and about 8 ng after transfer to a PVDF membrane. More information is available at: www.clarechemical.com/ruby.htm

SYPRO Orange

SYPRO Orange is a more economical alternative to SYPRO Ruby for SDS gels. To ensure maximum sensitivity it is important to run the gel using 0.05% SDS rather than the more typical 0.1%. The detection limit for Orange-stained proteins using a DR transilluminator is around 2 - 4 ng both by eye and using either a CCD or Polaroid

camera. More information is available at: www.clarechemical.com/sypro.htm

Excising DNA and Protein Bands

Obviously this should be done using the Dark Reader viewing glasses. Before cutting out a band, placing a sheet of glass under the gel will protect the blue screen of the transilluminator from scratching.

You can relax a little while band cutting! Various experiments show that:

1. Transformation efficiencies are increased over 100-fold when SYBR Gold-stained DNA samples are exposed on a Dark Reader transilluminator rather than a UV device.

2. Photobleaching of SYPRO Orange-stained proteins is significantly reduced when using a Dark Reader transilluminator compared to UV.

Photography and Imaging

Because the amber screen acts as an optical filter, any additional filter attached to the camera must be removed. An exposure time of 1 - 4 seconds at an f-stop of 5.6 is optimal using a Polaroid camera and 667 film. The exposure time using a CCD camera will vary, depending on the particular model. For example, using a basic Olympus 3000 digital camera with an f-stop of 2.8, typical exposure times are 2 - 5 sec. Additional imaging tips are available at: www.clarechemical.com/imaging.htm and on page 8 of this manual.

Camera Filters

Clare Chemical has available a variety of different sizes of camera filters. These filters have exactly the same optical properties as the amber screen. They have been introduced for those researchers who prefer to photograph fluorescent samples on their Dark Reader transilluminator without the amber screen. Substituting the screen with a camera filter can be helpful, for example, in humid climates where condensation tends to form more rapidly under the screen. More information is available at: www.clarechemical.com/filters.htm

Note that there is only one 'best' filter for use with the Dark Reader transilluminator and that is provided by the Dark Reader amber. This long-pass filter is designed to maximize the fluorescence signal and minimize the background. The actual excitation and emission maxima of a particular fluorophor are not especially relevant. Of course, if a 'black-and-white' camera is being used and the goal is to 'isolate' several different colored fluorophors by recording a series of images, then it will be necessary to experiment with a selection of bandpass filters.

More Information

Other Fluorophors

It is a commonly held misconception that a fluorophor, to work with the Dark Reader, must have an excitation maximum between 420 - 500 nm and an emission maximum above ~520 nm. While these are useful guidelines, it should be emphasized that the DR can also be effectively used to detect fluorophors that have maxima outside the above ranges. The more general criteria for visualizing a fluorophor with a Dark Reader transilluminator are (i) a portion of the excitation spectrum is between about 420 - 500 nm and (ii) a portion of the emission spectrum is above ~520 nm. This encompasses a large number of commonly used fluorophors besides those mentioned above such as Pro-Q® Diamond phosphoprotein stain, Pro-Q Emerald 488 glycoprotein stain, various fluorescein and rhodamine derivatives, Cy3, GFP variants such as EGFP, EYFP and dsRed, alkaline phosphatase substrates such as AttoPhos® and ECF®, dimeric cyanine stains such as YOYO® and TOTO® and some of the Alexa® dye series. There are many other dyes that can be used effectively with the Dark Reader transilluminator and this list is by no means exhaustive.

Viewing Lab Samples

Most lab samples are contained, one way or another, whether it be a gel, tube, plate, etc. More often than not, UV will fail to excite such samples because the container material absorbs UV. However, because the excitation light generated by the Dark Reader transilluminator is visible light, it easily passes through transparent glass and plastic (and even some semi-opaque materials). Consequently, fluorophors can be conveniently viewed in electrophoresis apparatus, 96-well plates, tubes, Petri dishes, cell culture bottles and even on blotting membranes.

Another problem often encountered when attempting to use a UV light source to view fluorescent samples is that the support or the container itself may fluoresce strongly enough to mask the fluorescence from the sample. For example, GelBond® film which is used to reinforce delicate gels, fluoresces under 300 nm light. With the Dark Reader transilluminator, there is minimum membrane fluorescence and the fluorophors in the gel can be viewed without any significant background interference

Where to Purchase Stains

SYBR Green stain (catalog #S-7563), SYBR Gold stain (# S-11494) SYPRO Ruby (# S-12000) and SYPRO Orange

(# S-6650) can be obtained from Molecular Probes, Eugene, Oregon, USA. Phone 1 800 438 2209. www.probes.com. Gelstar stain (# 50535) can be obtained from Cambrex, Inc. Rockland, Maine, USA. Phone 1 800 341 1574. www.cambrex.com

More About Imaging

A Simple Digital Imaging System

By way of example, here is some information about the imaging system currently used in the Clare Chemical lab: The CCD camera is an Olympus 3000. This is an ordinary (3 megapixel, color) 'consumer-level camera that costs about \$500. It has a USB connection that allows images to be downloaded to a computer.

Using the Olympus 3000, it is possible to detect about 10 pg of dsDNA (in color!) with exposure times of about 5 seconds. The maximum exposure time is 16 seconds. The 3 megapixel images provides a spatial resolution of better than 100 micrometers over the area of a standard mini-gel. The color capabilities can be used to distinguish multiple fluorophors in the same image.

On the downside, the CCD chip is 8-bit - 256 shades each of red, green and blue - whereas most 'high-end' cameras are 16-bit grayscale. This does reduce the quantification range of the Olympus camera. (One work-round is to record multiple images at different exposure times and then combine the data sets.) Also, the USB connection is relatively slow and there are no computer control or 'real-time' image acquisition capabilities. In spite of these limitations the Olympus is a very effective little camera.

Camera Filter Attachment

Attaching a filter to a digital camera requires, more often than not, some accessory parts that are not included in the camera package. For example, the Olympus 3000 requires a lens tube (Olympus #CLA-1) that attaches over the retractable lens, and a 43-46 filter step-up ring that attaches to the lens tube and converts the existing threads to 46 mm. With these attachments in place, a standard 46 mm DR filter (Clare Chemical #AF460) can now be attached to the camera.

Other Components

Freeware (Windows only) has recently been released by PineTree Computing that allows a variety of Olympus cameras to be controlled and images monitored 'live' using a PC.
www.pinetreecomputing.com/camctl.asp

If you want to photograph gels or other samples in a well-lit area of the lab, a hood is necessary. A variety of hoods

are available from Peca Products.

Infrared

All lamps emit IR radiation. Unfortunately, this is the region of the spectrum to which CCD chips are most sensitive. All basic digital cameras from companies such as Olympus, Kodak, Fuji, Nikon, etc., contain a built-in IR filter but the more expensive the CCD camera, the less likely, it seems, it will have this filter. The absence of IR filtering will result in an excessive background 'flare' in the recorded images that effectively obscures any fluorescence signal.

IR filters (or 'hot mirrors' which reflect IR rather than absorb it) are readily available from Tiffen in a variety of sizes and can be obtained from your local photographic store for around \$50. Alternatively, IR filters are available from Edmund Industrial Optics.

Tips for Good Photography

The following tips are the results of our experience with a variety of digital cameras:

1. Turn all the auto functions off. Always use manual settings. The auto functions are designed for 'average' conditions. Photographing fluorescent samples is not average and the auto software becomes hopelessly confused.

2. The LCD screen on the back of the camera warms up over time. This warming significantly increases the noise level in the images. Always try to turn on the camera just before use and take a picture immediately.

3. You should not have any filters on the camera except a DR filter (either in the form of the amber viewing screen or a separate camera filter).

4. Wipe the surface of the transilluminator with a little ethanol soaked onto a tissue before use to remove absorbed dye left by previous users.

5. The new generation of DNA dyes are sensitive to dust particles in the agarose. Try to avoid dust in the agarose and running buffer.

6. Because the new dyes are so much more sensitive than EtBr, it is easy to overload gels and get some ugly looking smearing. This is easily avoided by cutting down the DNA loaded by a factor of about 5.

7. Specific manual settings for the Olympus 3000 camera are given below:

- flash off

- zoom in as necessary. (Not digital zoom)

- Macro mode on

- ISO 100

- TIFF file 1600x1200. (This generates a 5.5 Mb file)

- f2.8

- focus manually (we place a piece of white card with fairly large type on the surface of the transilluminator to set the focus if the camera position has been moved since the last session.)

- exposure time set somewhere between 1 and 5 seconds to get the appropriate exposure.

Fixed-Focus Cameras

The most popular fixed-focus camera is the Polaroid DS34. Typically, this is mounted on a hood that contains a built-in correction lens that corrects for the short sample-to-camera distance. If the hood is placed on the Dark Reader amber screen, the distance is increased by about 0.25". At the lowest f-stop value of 4.5, the depth of field will be too shallow to properly focus the image. Increasing the f-stop to 5.6 increases the depth of field sufficiently to bring the image back into focus. A slightly longer exposure time will be required to account for the smaller aperture.

Troubleshooting

Problem	Cause	Solution
Fluorescence is difficult to see.	The room needs to be darker.	Switch off overhead lighting. Move the transilluminator away from windows.
Fluorescent 'smudges' on the transilluminator surface.	Fluorescent dye is present on the surface.	Wipe the surface with a little ethanol. Rinse the gel briefly in water to remove excess dye.
DNA bands are smeared.	The gel is overloaded. The new DNA dyes are much more sensitive than EtBr.	Try loading ~5 times less sample onto the gel.
Fluorescent bands in the gel seem to gradually disappear when viewing the gel.	Condensation is forming on the underside of the amber screen.	Wipe off the condensation. If it continues to form, use the DR glasses or a separate DR camera filter.
No light from the transilluminator.	A lamp may be broken.	Replace the lamp (see page 15). If this fails to correct the problem, contact CCR.
Photograph of gel does not look as good as when just viewing by eye.	There are several possible causes but, in general, if a photograph does NOT look as good as when viewed by eye, photographic conditions need optimizing.	See the Photography & Imaging Section for more information. (Pages 9 and 11) as well as items in this list.
The photo is very dark.	Not enough light is reaching the camera.	Make sure you are using only a DR filter. Increase the exposure time. Decrease the f-stop.
The photo is very light.	Too light is reaching the camera.	Make sure you are using only a DR filter. Decrease the exposure time. Increase the f-stop.
A background 'flare' in images recorded using a CCD camera.	The camera is not equipped with an IR blocking filter.	Obtain a separate IR filter. (See page 12 for details.)
Image is not in focus.	The usual causes are using auto-focus or using too low an f-stop.	With a digital camera, set the focus manually. With a fixed focus camera, increase the f-stop.

Service & Parts

Remember to disconnect the unit from the electric supply before replacing any parts!

If you have any questions, contact Clare Chemical Research.

The fuse and the lamp are the only user-replaceable parts. All other problems require that the unit be returned to Clare Chemical Research for servicing.

Fuse Replacement

The fuse-holder is located on the back of the unit. Fuses are available from Clare Chemical Research. Use the table below to identify the correct fuse. (The transilluminator model is printed on the side of the unit next to the switch.)

Do not use a fuse with other specifications.

Disconnect the unit from the power supply!

Unscrew the fuse-holder cap.

Remove the old fuse from the cap.

Insert the new fuse into the cap.

Screw the cap/fuse back onto the fuse-holder securely.

If the fuse continues to blow, contact Clare Chemical Research

* The VDC transilluminators contain a built-in fuse that is not user-replaceable.

Lamp Replacement

Replacement lamps are available from Clare Chemical Research. Do not use a lamp with other specifications.

NOTE: if a lamp is broken, some of the glass may be a very fine dust. DO NOT WIPE THE INSIDE OF THE BOX! Wear a pair of gloves and protective glasses when cleaning up broken glass. DO NOT TRY TO BLOW THE DUST OUT OF THE BOX! A vacuum cleaner is the best way to remove glass fragments.

General Procedure

Disconnect the unit from the power supply! Wear gloves and protective glasses!

Turn the unit upside-down onto a piece of plastic wrap (to protect the blue screen) and take out the screws that secure the blue screen.

Turn the unit right-side up (hold the blue screen in place)

Remove the blue screen and white diffuser screen from the unit.

After installing the new lamp according to the specific instructions below, reassemble the unit, and replace the screws. Only now can you switch the unit on.

If the new lamp fails to light, contact Clare Chemical Research.

DR45 and DR88 Transilluminators

DR45 and DR88 transilluminators use the blue 9W compact fluorescent lamp.

Remove the lamp from its socket by gently pulling on the lamp.

Put in a new lamp by gently pressing the lamp into the socket. The lamp 'clicks' into place.

DR195 Transilluminators

DR195 transilluminators use two blue 32W compact fluorescent lamps.

Remove the lamp from its socket by gently pulling apart the lamp and the socket.

Twist the lamp and carefully pull to remove the lamp from the clips.

(Note: because of the stresses of shipping, some DR195 units may contain lamps with extra securing features including wire wraps across the clips and a silicone sealant between the lamp and the socket. If present, the wire and sealant should be removed first. (Note that these features are only needed if the unit is being shipped commercially and there is no need to replace.)

Warranty Information

If you are not satisfied with the Dark Reader transilluminator for any reason, return it within 30 days for a full refund (less shipping and handling).

The DR transilluminator parts and workmanship are guaranteed for 1 year from the date of purchase. (See details below.) Please fill out the warranty card and send it back to Clare Chemical.

To obtain warranty service contact Clare Chemical (see p.16) and obtain a Return Form. Ship the unit to Clare Chemical postage prepaid, together with a completed Form. All products returned for warranty service must be carefully repackaged in the original packing materials:

Clare Chemical Research makes the following limited warranties.

Clare Chemical Research products are guaranteed to be free of defects in materials and workmanship under normal use for period of 1 year after the date of original purchase. During this period Clare Chemical will repair or replace a defective product or part without charge to you.

The warrant conditions and limitations are set out below:

The warranty applies only to defect in material or workmanship and does not include normal wear. The warranty applies only to defects which occur during normal use and does not extend to damage to products or parts which results from alternation, repair, modification, faulty installation or service by anyone other than Clare Chemical or an authorized representative; damage to products or parts caused by accident, abuse, or misuse, or maintenance, mishandling, misapplication, or use in violation of instruction furnished by us.

The warranty and remedies set forth above are exclusive and in lieu of all others, whether oral or written, express or implied, Clare Chemical specifically disclaims any and all implied warranties, including, without limitation, warranties of merchantability and fitness for a particular purpose.

In no event shall Clare Chemical be liable for special, incidental, consequential or punitive damages, including, without limitation, damage to other property caused by any defect in this product, inconvenience, loss of goodwill, lost profits or revenue, loss of use of this product or any associated equipment, cost of substitutive equipment, downtime costs or claims of any part dealing with purchaser for such damages, resulting from the use, installation or servicing of this product. Nor is Clare Chemical Research liable or responsible for any personal injuries occurring as a result of the use, installation or servicing of this product This warranty does not supersede any statutory rights that may be available in certain States or Countries.

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