Clare Chemical Research
www.clarechemical.com

Dark Reader® Transilluminators

DR22A, DR46B, DR89X, DR196

Safety Instructions

Keep these instructions available for easy reference by any user of this Dark Reader unit. For further assistance contact:
Clare Chemical Research
Tel: 970 882 7499
info@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 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!

*** The Dark Reader emits no UV light. However, the blue light emitted by the Dark Reader is relatively intense. Do not look directly at the transilluminator for extended periods of time without the amber screen in place or wearing the viewing glasses. Always wear the viewing glasses or use the amber screen.

*** There are no user-replaceable parts in the DR22A, DR46B, DR89X or DR196 transilluminators. If the transilluminator is not functioning correctly, contact Clare Chemical as outlined above.

*** 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. (Unplug from the wall socket 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 for several of the new DNA stains show they are significantly safer than ethidium bromide, it should be remembered that any dye that binds to 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 water or ethanol soaked onto a soft cloth or tissue paper. Disconnect the unit from the wall socket 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 UV protection. Do NOT use the glasses or screen with UV sources.

Specifications

Dark Reader DR22A Transilluminator
Viewing surface dimensions: 13 x 12 cm
Overall dimensions: 16 x 14.5 x 7 cm
Weight: 0.45 kg approx
Optical: Multiple blue LEDs
Power consumption: 12V DC, 0.25A, 3W
Included accessories: Amber screen and DR viewing glasses.

Dark Reader DR46B Transilluminator
Viewing surface dimensions: 19 x 15 cm
Overall dimensions: 22.5 x 18.5 x 8 cm
Weight: 0.7 kg approx
Optical: Multiple blue LEDs
Power consumption: 12V DC, 0.42A, 5W
Included accessories: Amber screen and DR viewing glasses.

Dark Reader DR89X Transilluminator
Viewing surface dimensions: 25 x 22 cm
Overall dimensions: 29.5 x 26 x 12 cm
Weight: 1.4 kg approx
Optical: Multiple blue LEDs
Power consumption: 12V DC, 1.0A, 12W
Included accessories: Amber screen and DR viewing glasses.

Dark Reader DR196 Transilluminator
Viewing surface dimensions: 46 x 30.5 cm
Overall dimensions: 50.8 x 34.8 x 12.5 cm
Weight: 6.4 kg approx
Optical: Multiple blue LEDs
Power consumption: 12V DC, 2.0, 24W
Included accessories: Amber screen and DR viewing glasses.

Basic Instructions for Use

Questions? - Do not hesitate to contact us:
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email: support@clarechemical.com
web: www.clarechemical.com

Familiarize yourself with the parts of the Dark Reader as described on the accompanying Parts Checklist sheet and read the Safety Instructions (page 1 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 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.

DNA Stains

Some of the new generation of DNA stains, such as SYBR® Green and GelStar®, are much more sensitive than ethidium bromide. When used in conjunction with a Dark Reader transilluminator, the new stains are especially effective. Load about 5 times less DNA on your gel to avoid the appearance of minor DNA bands that can result in a smear.
More information is available at: clarechemical.com/applications/dna-gels

A Typical Method

Many of the new DNA stains are used in a similar manner. (Always follow the manufacturer's detailed instructions carefully.) Make a 1:10,000 dilution of stain in 1x TAE or TBE buffer. (Do not make more stain solution than needed as many of the stains are not stable in aqueous solution.) 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 uses visible excitation light which 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. Alternatively, place a piece of plastic wrap over the gel to prevent condensation on the amber screen.

Viridi DNA Stains

Viridi Vivid and Viridi Easy are the new family of DNA stains available exclusively from Clare Chemical Research. The Viridi stains are specifically formulated for exceptional performance with Dark Reader transilluminators. For more information visit: clarechemical.com/products/viridi

Viridi stains are less toxic than ethidium bromide and are non-carcinogenic by the Ames-test. In addtition, the results are negative in both the mouse marrow chromophilous erythrocyte micronucleus and mouse spermary spermat-ocyte chromosomal aberration tests.

Both Viridis are stable in aqueous solution making them easy to handle and conveniently stored in the refrigerator. (The majority of SYBR Stains, GelStar, etc. are unstable and must be stored in DMSO at -20 ^{o}C)

The **Viridi Vivid** formulation is one of the most sensitive DNA stains and it is possible to see as little as 100 - 200 pg DNA. It can be used either as a staining bath after electrophoresis, or it can be added to the agarose before running the gel. In-agarose staining has the advantage that the DNA bands can be viewed immediately after the gel run has been completed.

Viridi Easy is the sample loading buffer formulation. It doesn't have the extreme sensitivity of Vivid, but is is very easy to use - you just add it to the DNA samples, run the gel and view. It's very quick and simple with minimal clean-up too.

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 100-200 pg of SYBR Green-stained DNA by eye and less using a digital camera system. It is not recommended to add SYBR Green to the agarose before pouring the gel as it can interfere unpredictably with DNA migration rates.

GelStar

Like Viridi Vivid, GelStar can be used in-agarose with only minimal distortions to DNA migration rates. It's sensitivity as is almost as good as Vivid's. There is no published safety data available for GelStar.

GelGreen

GelGreen has a number of attractive features:
- it can be used as an in-agarose stain.
- is stable in aqueous solution
- shows little mutagenic effect in Ames tests.

GelGreen does not have the sensitivity of Viridi Vivid but is better than SYBR Safe.

SYBR Gold

Our tests on the sensitivity of DNA detection show that SYBR Gold is one of the most sensitive of the new DNA stains and it is possible to see about 100 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. It cannot be used in-agarose because of serious effects on DNA migration rates.

SYBR Safe

SYBR Safe is the least sensitive of the new generation of DNA stains (1-2 ng of dsDNA). But it does have extensive safety data and has been extensively tested for environmental safety and is not classified as hazardous waste under U.S. Federal regulations.

Ethidium Bromide and GelRed

EtBr, GelRed, etc., are UV stains and will look better on a UV box. It is ,of course, possible to see DNA bands using a Dark Reader, but the bands will not be as distinct as with UV excitation. To maximize viewing with EtBr specifically, it is important to:
- view in a completely dark room and give your eyes a few moments to adjust.
- use a lower amount of EtBr to stain the gel (0.2 - 0.5 μg / mL)
- load plenty of DNA (10+ ng / band)

Protein Stains

Several novel fluorescent protein stains are now available including the SYPRO® stains developed by Molecular Probes and ProteOrange from Lumiprobe. The new stains are much more sensitive than methylene blue and are similar to silver staining. Advantages over silver include less protein-to-protein variability, a greater quantitation range, a simple one-step staining procedure, and no interfere with subsequent downstream characterization techniques. Most of these fluorescent protein stains family can be very effectively detected using a Dark Reader transilluminator.
More information is available at: clarechemical.com/applications/protein-gels

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.

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 Dark Reader transilluminator is around 2-4 ng both by eye and using a camera.

GFPs

Many of the new generation of GFP variants have excitation and emission properties that are well-suited for viewing with Dark Reader devices. For example, eGFP (ex/em = 488/507 nm) can be detected, by eye, down to concentrations of less than 100 pM. Other colors such as eYFP and DsRed are also highly fluorescent under Dark Reader lights

Typically, the Hand Lamp or Spot Lamp would be used to view fluorescent proteins in whole organisms such as mice or seedlings. The transilluminators though are ideal for viewing GFPs in Petri dishes, 96-well plates, on membranes, in gels, etc.

Here are some of the factors that influence the viewing of fluorescent proteins:

- Green fluorescent variants are usually a little easier to detect than the red variants because the maximum excitation of the red proteins is often at a longer wavelength than the Dark Reader excitation maximum.

- Perhaps the most important factor in viewing fluorescent proteins is the expression level of the GFP in the organism of interest. GFPs expressed at low levels will, of course, be more difficult to detect.

- There may be some background interference from the organism itself. For example, chlorophyll in leaves will fluoresce red and this may make it difficult to identify red fluorescent proteins.

- The size of the samples - e.g., embryos and seedlings - may be physically too small to clearly see any fluorescence by eye.

Photography and Imaging

Basics

Just about any digital camera can be used. For quick documentation, it is possible to use the camera in cell phones to photograph gels. For example, if you have a dark room, put the amber screen over the gel and hold the cell phone as steady as possible about 30 cm directly above the gel.

For better reproducibility, sensitivity and resolution, a digital camera with manual control of exposure time, focus and f-stop is required. The Canon G-series cameras have long been popular with researchers. You will also need either:
(a) a dark room and a copy-stand to hold the camera over the gel at a fixed distance, or

(b) a hood to place over the transilluminator and then set the camera on top of the hood. Hoods are available from PecaScientific.com and ViewPointLabs.com

You should be fine using the amber screen instead of a camera filter. The amber screen and the camera filters have exactly the same optical properties. If the amber screen is over a gel for an extended period of time, condensation can form on the underside of the screen that can obscure any fluorescent bads. To prevent this place a piece of plastic wrap over the gel.

Because the amber screen acts as an optical filter, any additional filter attached to the camera should be removed. The exposure time using a digital camera will vary, depending on the particular model.

A Simple Digital Imaging System

By way of example, here is some information about one of the simplest imaging system currently used in the Clare Chemical lab:
The camera is a Canon Rebel T3. This is a basic digital SLR camera that costs about $400. Typical settings for photographing a DNA gel would be an exposure time of 1 sec, ISO of 200 and an f-stop of 5.6 or lower. Using the Rebel T3, it is possible to detect about 50 pg of dsDNA stained with Viridi Vivid. The CMOS chip is linear response over about 2 orders of magnitude. There is computer control of the camera and live preview on the monitor.

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. 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.

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, several of the Canon G-series cameras require a ‘lens adapter’ that attaches over the retractable lens.

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. 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).
3. Wipe the surface of the transilluminator with a little ethanol soaked onto a tissue before use to remove absorbed dye left by previous users.
4. The new generation of DNA dyes are sensitive to dust particles in the agarose. Try to avoid dust in the agarose and running buffer.
5. Because the new dyes are so much more sensitive then 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.

Specific manual settings for the Canon T3 camera are given below:
- flash off
- zoom in as necessary
- ISO 200
- f5.6
- 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 0.2 and 2 seconds to get the appropriate exposure.

More Information

Other Fluorophors

It is incorrect to assume that for a fluorophor, to work with the Dark Reader, it must have an excitation max. in the blue wavelengths, and an emission max. above ~520 nm. While these are useful guidelines, it should be emphasized that the Dark Reader can be effectively used to detect fluorophors that have maxima well 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 such as polystyrene). 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

Troubleshooting

Problem	Probable 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 blue 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.	Put a piece of plastic wrap over the gel the gel before placing the amber screen.
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.
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 much light is reaching the camera.	Make sure you are using only a DR filter. Decrease the exposure time. Increase the f-stop.
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

There are no user-replaceable parts in the DR22A, DR46B, DR89X or DR196 transilluminators. Contact Clare Chemical if your transilluminator is not functioning.

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 Dark Reader transilluminator parts and workmanship are guaranteed for 1 year from the date of purchase.

To obtain warranty service contact Clare Chemical 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 alteration, 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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