Clare Chemical Research

**www.clarechemical.com**

# Dark Reader™

## Hand Lamp & Spot Lamp

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# Safety Instructions

Keep these instructions available for easy reference by any user of this Dark Reader Lamp. 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 Lamp 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 Lamp. Do not substitute the provided wall-unit. For example, do not use the plug-in wall unit provided with the Dark Reader DR88 transilluminator which has a 12V output.

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

*** Dark Reader Lamps are designed to be used by individuals who are experienced in using such equipment to view fluorescent samples. Do not let untrained personnel operate this Lamp!

*** 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 Dark Reader viewing glasses when the Lamp is switched on. Do not point the Lamp at another individual who is not wearing Dark Reader glasses.

*** Do not attempt to open the Lamp. There are no user-replaceable parts inside.

*** Dark Reader Lamps are 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.

*** Dark Reader Lamps are remarkably robust but they have not been designed to withstand substantial impact. Do not drop 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 likely to be hazardous. Gloves should be worn when handling solutions or gels containing such dyes. Always follow the manufacturer's instructions regarding dye handling.

*** Contact of Dark Reader Lamps 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 blue filter of the Lamp should be cleaned only with soap and water or ethanol soaked onto a soft cloth or tissue paper. Disconnect the unit from the power supply before cleaning.

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

*** 9V batteries: Do not open battery, dispose of in fire or short circuit - may ignite, explode, leak or get hot causing personal injury. Note - keeping batteries in your pocket can lead to a short-circuit if the contacts are exposed! Keep away from small children. Disposal: Dispose in accordance with all applicable federal, state, and local regulations.

# 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 Lamp!

Viewing of fluorescent samples is best done in a darkened area.

Attach the Hand Lamp to the DC input jack of the AC/DC wall transformer.

Put on the glasses.

Switch on the Lamp.

Hold the Lamp over the samples to be viewed.

After viewing, switch off the Lamp.

# Green & Red Fluorescent Proteins

The optical performance of Dark Reader technology is perhaps most spectacular when viewing Fluorescent Proteins. The new generation of red-shifted GFPs have excitation and emission properties that are very well suited for viewing with the Dark Reader Lamps. For example, EGFP (ex/em = 488/507 nm) can be detected, by eye, down to concentrations of less than 100 pM. ECFP, EYFP and DsRed are also highly fluorescent under Dark Reader light. Be aware that the excitation maximum of wild-type GFP is in the UV region at around 390 nm resulting in minimal fluorescence using a Dark Reader.

# DNA & Protein 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 Lamp, 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!

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 Lamp.

# More 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 Lamp 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 Lamp and this list is by no means exhaustive

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# 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 Lamp 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 Lamp, there is minimum membrane fluorescence and the fluorophors in the gel can be viewed without any significant background interference

# Photography and Imaging

Always use a Dark Reader camera filter. The amber filters have the same optical properties as the amber viewing glasses and have been optimized for use with Dark Reader equipment. More information is available at: www.clarechemical.com/filters.htm

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.

## 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

Almost all light sources emit some 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 can 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.

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 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.

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 Lamp 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.

# Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| Fluorescence is faint and difficult to see. | The room needs to be darker. | Switch off overhead lighting. Move away from windows. |
| 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. |
| Photograph of sample 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. |
| 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 Lamps. Your unit should be returned to Clare Chemical Research for any necessary repairs.

Available accessories for use with the Lamps include Dark Reader camera filters and viewing glasses. More details are available on the following web pages:
www.clarechemical.com/filters.htm
www.clarechemical.com/glasses.htm

# Warranty Information

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

The DR Lamp 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.

# Contact Information

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