LUM

PARADIGM™ Dual Color Luminescence Detection Cartridge

User's Manual

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- The Company makes no warranty with respect to components or accessories not manufactured by it. In the event of defect in any such component or accessory, the Company will give reasonable assistance to Purchaser in obtaining from the manufacturer's own warranty.
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- The Company shall be released from all obligations under all warranties, either expressed or implied, if any product covered hereby is repaired or modified by persons other than its own authorized service personnel, unless such repair by others is made with the written consent of the Company.
- If the product is a reagent or the like, it is warranted only to conform to the quantity and content and for the period (but not in excess of one year) stated on the label at the time of delivery.

It is expressly agreed that the above warranty shall be in lieu of all warranties of fitness and of the warranty of merchantability, and that the company shall have no liability for special or consequential damages of any kind or from any cause whatsoever arising out of the manufacture, use, sale, handling, repair, maintenance, or replacement of any of the products sold under the sales agreement.

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Parts replaced during the warranty period are warranted to the end of the instrument warranty.

NOTE

Performance characteristics and specifications are only warranted when Beckman Coulter, Inc. replacement parts are used.

Safety Information

WARNING

If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the equipment may be impaired.

Warning and Caution Definitions

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle.

The exclamation point symbol is an international symbol which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing is attempted.

WARNING

A WARNING calls attention to a condition or possible situation that could cause injury to the operator.

CAUTION

A CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator's work.

When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a service engineer for any servicing of equipment requiring the removal of covers or panels.

High Voltage

This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) wall outlets. Use only multiplug power strips provided by the manufacturer.

Disposal of Electronic Equipment

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- the device was put on the European Market after August 13, 2005.
- the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Laser Light

This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Laser Specifications

The Paradigm Multimode Detection Platform is rated a Class 1 Laser Product, because it houses a laser module. During normal operation, the laser light is not accessible to the user.

Figure 1.1 Label on rear of instrument

The embedded laser module has the following specifications:

Laser type:	diode laser
Wavelength:	650nm
Max. output power:	2 mW, cw
Laser Class:	Class 2M (IEC60825-1, ed. 1.2: 2001)
Fan angle:	55°±5°

The embedded laser module is ON, only if the microplate chamber flap is closed (hardware interlock). The user or the service engineer is not exposed to any radiation from the embedded laser module during operation, maintenance, or service. The closed microplate chamber acts as the protective housing.

Chemical and Biological Safety

Normal operation of the instrument may involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility's waste disposal procedures.
- Operate the instrument in accordance with the instructions outlined in this manual, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

NOTE

Observe all warnings and cautions listed for any external devices attached or used during operation of the instrument. Refer to applicable external device user's manuals for operating procedures of that device.

Moving Parts

To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the instrument.
- Keep the instrument work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user's manual for the instrument. Prior to cleaning equipment that has been exposed to hazardous material:

- Appropriate Chemical and Biological Safety personnel should be contacted.
- The Chemical and Biological Safety information contained in this user's manual should be reviewed.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

IMPORTANT

It is your responsibility to decontaminate components of the instrument before requesting service by a service engineer or returning parts to Beckman Coulter for repair. Beckman Coulter will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

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Dual Color Luminescence Detection Cartridge

Overview

The Dual Color Luminescence Detection Cartridge is a detection cartridge for use with the PARADIGM™ Detection Platform. Installing the Dual Color Luminescence Detection Cartridge enables detection methods for glow luminescence and Promega's Chroma-Glo[1] reagents.

NOTE

For glow luminescence measurements which do not require emission filters, the Glow Luminescence Detection Cartridge (PN A41584) should be used.

A luminescence detection cartridge contains the optics and electrical components needed to perform specific measurement modes or for specific applications. The PARADIGM Detection Platform requires these detection cartridges to enable different measurement modes.

The PARADIGM Detection Platform is a modular multimode microplate detector. User-installable and removeable detection cartridges allow the detector to be configured for specific applications and easily expand the capabilities of the detector at any time.

1. Chroma-Glo is a trademark of Promega Corporation, Madison, WI, USA.

The Dual Color Luminescence Detection Cartridge occupies one (1) slot. It may be installed in either the top read detection cartridge transport for top reading, or in the bottom read detection cartridge transport for bottom reading; however, it may NOT be installed in the rearmost slot on either the top or bottom detection cartridge transport. Refer to *Installing Detection Cartridges* on page 1-18 of the *PARADIGM™ Detection Platform User's Manual*, for installation instructions.

NOTE

It is recommended to install the Dual Color Luminescence Detection Cartridge in the top read detection cartridge transport when running glow luminescence detection methods.

About Dual Color Luminescence

In a glow luminescence method, the intensity at which light is emitted from a chemiluminescent or bioluminescent reaction is measured. The light output is measured as the rate of photons per time and is expressed as counts per second. In glow luminescence reactions, the light output decays slowly with time.

In a dual color luminescence method, dual labels (such as gene reporters) causing the emission of different wavelength bands at the same time are measured.

The Dual Color Luminescence Detection Cartridge contains the components for measuring the light intensity from luminescence. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence. The PARADIGM Detection Platform incorporates a dual emission design to measure both emission channels simultaneously, improving precision and reducing read times.

CAUTION

Luminescence light levels visible to the human eye may cause damage to the detection system.

NOTE

The Dual Color Luminescence Detection Cartridge is not suitable for measurement of flash luminescence reactions, which require injectors.

Applications of Chroma-Glo Dual Color Luminescence

The Chroma-Glo assay system is a further development of the Dual-Glo[1] luciferase gene reporter assays. Both types of assays are designed to normalize the activity of the one, more specific reporter of gene expression with the activity of another reporter, in order to cancel out variations in the experimental (biological) assay parameters. In the Dual-Glo Luciferase assay system, the one reporter is activated, then measured, and during the activation of the other reporter the emission induced by the first reporter is extinguished. Therefore, the Dual-Glo Luciferase assay system requires no emission filters, and may be measured using the Glow Luminescence Detection Cartridge (A41584). The Dual Color Luminescence Detection Cartridge (A41585) described herein contains emission filters designed for the Chroma-Glo reagents.

One major benefit of the Chroma-Glo assay system over the Dual-Glo luciferase system is it requires only a single reagent addition step. However, this also requires a compensation for overlap in the emission spectra induced by the concurrent activity of the Chroma-Glo dual luciferases (see *Color Separation and Red to Green Ratios* on page 7) .

For typical applications and a discussion on the advantages of the one over the other assay system, refer to the manufacturer's website at http://www.promega.com.

1. Dual-Glo is a trademark of Promega Corporation, Madison, WI, USA.

Analyzing Dual Color Luminescence Data

The conversion rate of photons to counts is individual for each detector. Therefore, raw data from the same plate may appear significantly different from one instrument to the next. In addition, the data format used by other manufacturers may not be counts/second and may be different by several orders of magnitude. It is important to know that the number of counts and the size of figures is in no way an indication of sensitivity (see *Detection Limit* on page 8) .

On the PARADIGM Detection Platform, the raw data is normalized to counts per second by dividing the number of counts by the read time per well.

Analyzing and validating dual color luminescence intensity data from Chroma-Glo reagents typically consists of the following:

- *Background Correction* (page 1-5)
- *Color Separation and Red to Green Ratios* (page 1-7)
- *Normalized Ratios and Relative Response Ratios* (page 1-8)
- *Detection Limit* (page 1-8)
- *Sample Volumes and Concentration of Reactants* (page 1-10)
- *Data Optimization* (page 1-10)

Background Correction

The light detected in a luminescent measurement typically has two components: specific light from the luminescent reaction; and an approximately constant level of background light caused by various factors, including the plate material and impurities in the reagents. The background can be effectively measured using blank replicates. Blanks should include the luminescent substrate (chemical energy source) but not the luminescence agent (typically an enzymatic group which makes the substrate glow).

For improved results, it is recommended to run replicates for all blanks, controls, and samples. In this case, the blank value subtracted is the average value of all blanks. Multimode Software performs automatic blank averaging and reduction if blanks are defined on the plate layout in the protocol (refer to *Creating Protocols* on page 7-137 of the *Multimode Analysis Software User's Manual).*

Color Separation and Red to Green Ratios

As suggested by Promega, before ratios can be taken from the Chroma-Glo red and green emission channels, some mutual overlap of the emission spectra needs to be considered. This spectral overlap can be exactly quantified by the help of controls which contain only the one or the other reporter, respectively (red control, green control). The Multimode SW allows to define these controls in the plate layout, and if defined, the color separation will be performed automatically.

The spectral overlap is defined by two coefficients (CT_{GR} and CT_{RG}, see *Calculations Appendix* on page A), which depend on the individual emission filters (the cartridge), and the relative sensitivity of the PMTs (the detection platform) in the red and the green wavelength range. Therefore, the values of these two coefficients need to be established for each detection system (combination of cartridge and PARADIGM Detection Platform). If determined once for a system, the two values may be carried over into further protocols, although it is good practice to provide such controls in replicates for each assay.

Before calculating the color corrected red to green ratios (R/G), the Multimode Software applies a further normalization of the data from the red and green emission channels using filter efficiency values. The filter efficiency values are determined at the factory, and stored in the cartridge's onboard memory (EEPROM). In effect, although changing in time after reagent addition due to the different decay rates, the peak R/G values may become somewhat better equalized when comparing several dual color luminescence cartridges. These filter efficiency values, however, cancel out when the time dependence of the R/G values is compensated by taking ratios between R/G values of samples and controls (see *Normalized Ratios and Relative Response Ratios* on page 8) .

Normalized Ratios and Relative Response Ratios

The decay of the red signal is considerable, with a halflife of about 30 minutes, when compared with the decay of the green signal. Thus, the color corrected red to green ratios (R/G, see *Color Separation and Red to Green Ratios* on page 7) are normalized with the value of, for example, a negative control, which is then named the Normalized Ratio (NR).

$$NR = (R/G)_{sample} / (R/G)_{neg} \qquad \text{(equation 1)}$$

In the case that both a negative and a positive control have been defined in the plate layout, the Multimode Software also calculates a Relative Response Ratio (RRR), quantifying a sample in the range between the negative and the positive control (RRR may become negative in some cases).

$$RRR = [(R/G)_{sample}-(R/G)_{neg}] / [(R/G)_{pos}-(R/G)_{neg}] \times 100\% \qquad \text{(equation 2)}$$

which can be read as

$$RRR = [\ NR_{sample} - 1\ ] / [\ NR_{pos} - 1\ ] \times 100\% \qquad \text{(equation 3)}$$

Detection Limit

The detection limit is the smallest measurable signal above the background. The background can be determined as described above, taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection limit can then be determined as the average blank plus three standard deviations. If the average sample value measures above the detection limit, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{det limit} = 3 \times \text{stdev(blank)} / (\text{standard-blank}) \times \text{conc}_{std} \qquad \text{(equation 4)}$$

where standard is the signal of a standard, blank is the mean of blanks, $conc_{std}$ is the concentration of the standard, and stdev(blank) is the standard deviation of the blanks.

Sample Volumes and Concentration of Reactants

The concentration of the luminescent agent impacts the amount of light output in a luminescent reaction. Light is emitted as a result of a reaction between two or more compounds; therefore, the amount of light output is proportional to the amount of the limiting reagent in the sample.

For example, in a luciferase assay system, when total volume is held constant and luciferase is the limiting reagent, the blanked light output is proportional to the concentration of luciferase in the sample. At very high concentrations of luciferase, the excess requirement for the availability of substrate may become violated because considerable amounts of substrate are used up, resulting in earlier decay of the strongest samples. In effect, it may appear to be a non-linearity of the detector, while it actually is an effect of the assay.

⚠ CAUTION

Very bright samples may exceed the linear dynamic range of the detection system. If such is the case, reading may be performed using an attenuation filter.

Data Optimization

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. Therefore, it is important to specify the read time when comparing measurements.

All low light level detection devices have some measurement noise in common. In order to average out the measurement noise, optimization of the time per plate (or time per well) shall target for accumulating as many counts as possible. Within some range, the reduction of noise (CVs, detection limit) can be accomplished by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations. For maximum sensitivity, the Chroma Glo reagents in particular shall be read within the first hour after preparation.

Performing Measurements

When the Dual Color Luminescence Detection Cartridge is installed in the PARADIGM Detection Platform, detection methods and protocols to perform glow luminescence measurements are enabled in Multimode Software.

The Dual Color Luminescence Detection Cartridge enables several protocol templates in Multimode Software that may be used to create protocols. A few different templates are available depending on whether or not red and green controls are in the plate layout. For details see the template info.

Additional detection methods and protocols using the Dual Color Luminescence Detection Cartridge may be created using Multimode Software.

- For information on creating detection methods using the Dual Color Luminescence Detection Cartridge, refer to *Creating and Editing Detection Methods* on page 5-77 of the *Multimode Analysis Software User's Manual.*
- For information on creating and running protocols using the Dual Color Luminescence Detection Cartridge, refer to *Creating and Running Protocols* on page 7-135 of the *Multimode Analysis Software User's Manual.*

NOTE

Measurement protocols that use multiple cartridges may also be created and run on the PARADIGM Detection Platform.

Microplate Recommendations

The type of microplate and the way it is handled can affect the measurement performance of the instrument. Plate height may not exceed 25 mm. Select a plate type with properties suited for the application and for use with multimode detectors. Table 1.1 lists microplate selection guidelines.

NOTE

For each type of assay (defined by plate type and sample volume), it is recommended to optimize the labware. Because the luminescence cartridge features a read head that moves towards the microplate into close proximity, the actual read height of the labware must be defined accurately (refer to *Creating and Editing Labware* on page 6-115 of the *Multimode Analysis Software User's Manual)*.

Plate handling guidelines include:

- Keep unused plates clean and dry.
- Make sure the strips on strip plates are inserted correctly and level with the frame.

Table 1.1 Microplate selection guidelines

Measurement Technique	Microplate Type	Additional Considerations
Dual Color Luminescence	solid white	When an application specifies a surface treatment, only use plates with the correct treatment. NOTE: White plates provide significantly higher signal than black plates, and are recommended if high sensitivity is required. White plates may, however, exhibit some detectable phosphorescence which increases background after being exposed to light (in particular under neon lights). For maximum sensitivity, it is recommended to prepare plates under reduced ambient light conditions, and/or adapt the plates to dark for 10-30 minutes prior to measurement.

Measurement Specifications

The specifications for measurements using the Dual Color Luminescence Detection Cartridge are shown in Table 1.2.

Table 1.2 Measurement specifications

Parameter	Specification
Detection Cartridge Name	PARADIGM™ Dual Color Luminescence Detection Cartridge
Short Name	LUM
Part Number	A41585
Weight	1.5 lbs (0.7 kg)
Detection Techniques	Dual Color Luminescence
Type	Dual emission
Number of Slots	1
Top/Bottom	Top or Bottom, except for rearmost slot
Light Source	NA
Wavelength Range	EM1: 510-60 EM2: LP610
Substrates	Any compatible with wavelength range
Applications	Designed for use with Promega's Chroma-Glo™ reagents
Check Plate Tests	test names ending with -LUM

Preventive Maintenance and Troubleshooting

To ensure optimum operation of the instrument, perform the following preventive maintenance procedures as necessary:

- Open the detection cartridge transport only when removing or installing detection cartridges.
- When the detection cartridge is not in use, always keep the red cap in place and store in the detection cartridge box.
- Follow appropriate decontamination procedures as instructed by the laboratory safety officer.
- Contact a Beckman Coulter service engineer to inspect the instrument every two years.
- Use a Multimode Check Plate to regularly verify the performance of the cartridge. Contact Beckman Coulter sales or service for more details.
- Respond appropriately to any error messages displayed by Multimode Software.

Verifying Performance with Check Plate

The Multimode Check Plate is used to verify the performance of absorbance, fluorescence, and luminescence measurements performed on the PARADIGM Detection Platform. Verification runs performed with the check plate only verify detection cartridge performance; factory- and user-defined settings are not changed. The tests which need to be performed to verify performance differ between detection cartridges.

To verify the measurement performance of the Dual Color Luminescence Detection Cartridge, run all check plate test suites with the detection cartridge short name LUM appended to the end of the test name.

NOTE

Refer to the *Multimode Check Plate User's Manual* for information on running tests with the check plate.

Troubleshooting

WARNING

Only officially trained service engineers may perform service procedures on the instrument. Contact a Beckman Coulter service engineer when service is required.

Perform the following troubleshooting techniques when necessary. Also refer to *Performing Basic Maintenance and Troubleshooting* on page 2-1 of the *PARADIGM™ Detection Platform User's Manual.*

Table 1.3 Troubleshooting the PARADIGM Detection Platform

If	Then
Functional failure	Install the cartridge in a different detection cartridge slot. Contact Beckman Coulter Customer Technical Support.
Detection cartridge fails verification tests with check plate	Refer to the *Multimode Check Plate User's Manual*. Contact Beckman Coulter Customer Technical Support.

Calculations Appendix

Formulas used for the color separation are derived from the following set of equations:

$$L_{rf} = \text{Red} + \text{Green} \times CT_{RG} \quad \text{(equation 1)}$$

$$L_{gf} = \text{Red} \times CT_{GR} + \text{Green} \quad \text{(equation 2)}$$

where

L_{rf} is the luminescence of a sample as measured through the red filter, L_{gf} is the luminescence of a sample as measured through the green filter, Red quantifies sample content of luciferase inducing the red emission spectrum, Green quantifies sample content of luciferase inducing the green emission spectrum, CT_{RG} is the relative overlap (spectral cross talk) of green spectrum seen in the red emission channel, and CT_{GR} is the relative overlap (spectral cross talk) of red spectrum seen in the green emission channel.

The algebraic solutions to (equation 1) and (equation 2) are:

$$\text{Red} = (L_{rf} - [L_{gf} \times CT_{RG}])/(1 - CT_{GR} \times CT_{RG}) \quad \text{(equation 3)}$$

$$\text{Green} = (L_{gf} - [L_{rf} \times CT_{GR}])/(1 - CT_{RG} \times CT_{GR}) \quad \text{(equation 4)}$$

The unknown coefficients for the overlap (CT) are determined by measuring and inserting the red only and green only controls into (equation 1) and (equation 2), respectively, yielding

$$R_{rf} = Red_{Control} + 0 \times CT_{RG} \quad \text{(equation 5)}$$

$$R_{gf} = Red_{Control} \times CT_{GR} + 0 \quad \text{(equation 6)}$$

and

$$G_{rf} = 0 + Green_{Control} \times CT_{RG} \quad \text{(equation 7)}$$

$$G_{gf} = 0 \times CT_{GR} + Green_{Control} \quad \text{(equation 8)}$$

where

$Red_{Control}$ is the control inducing the red emission spectrum only, $Green_{Control}$ is the control inducing the green emission spectrum only, R_{rf} is the red control as measured through the red filter, R_{gf} is the red control as measured through the green filter, G_{rf} is the green control as measured through the red filter, and G_{gf} is the green control as measured through the green filter.

Paired equations (equation 5) and (equation 6) and (equation 7) and (equation 8) can be resolved into

$$CT_{GR} = R_{gf} / R_{rf} \quad \text{(equation 9)}$$

$$CT_{RG} = G_{rf} / G_{gf} \quad \text{(equation 10)}$$

Because (equation 9) and (equation 10) are ratiometric, the concentration of the red and green only controls may be undefined, though shall be considerable to yield accurate measurement values.

By inserting the solutions for the coefficients CT enumerated above in (equation 9) and (equation 10) into (equation 3) and (equation 4) above, it can be confirmed that the resulting equations comply with those provided by Promega, after normalization with the filter efficiencies $E_{rf} = (R_{rf} / R)$ and $E_{gf} = (G_{gf} / G)$, respectively. The values for filter efficiencies are stored in the cartridge, thus making a additional measurement to achieve R and G (without emission filter) unnecessary.

All the above equations in the appendix make no assumption on the nature of the signal, thus could be similarly applied to any other dual label detection with overlapping spectra, for example in absorbance or fluorescence reading.

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