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PARADIGM™ Glow Luminescence Detection Cartridge

User's Manual

PN A48439AB
June 2008

Beckman Coulter, Inc.
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Fullerton, CA 92835

BECKMAN COULTER®

PARADIGM™ Glow Luminescence Detection Cartridge
User's Manual
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All standard Beckman Coulter, Inc. policies governing returned goods apply to this product. Subject to the exceptions and upon the conditions stated below, the Company warrants that the products sold under this sales agreement shall be free from defects in workmanship and materials for one year after delivery of the products to the original Purchaser by the Company, and if any such product should prove to be defective within such one year period, the Company agrees, at its option, either (1) to correct by repair or at the Company's election by replacement, any such defective product provided that investigation and factory inspection discloses that such defect developed under normal and proper use, or (2) to refund the purchase price. The exceptions and conditions mentioned above are as follows:

- Components or accessories manufactured by the Company which by their nature are not intended to and will not function for one year are warranted only to reasonable service for a reasonable time. What constitutes a reasonable time and a reasonable service shall be determined solely by the Company. A complete list of such components and accessories is maintained at the factory.
- 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.
- Any product claimed to be defective must, if required by the Company, be returned to the factory, transportation charges prepaid, and will be returned to Purchaser with transportation charges collect unless the product is found to be defective, in which case the product must be properly decontaminated of any chemical, biological, or radioactive hazardous material.
- 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.

Representatives and warranties made by any person, including dealers and representatives of the Company, which are consistent or in conflict with the terms of this warranty, shall not be binding upon the Company unless reduced in writing and approved by an expressly authorized officer of the Company.

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:	650 nm
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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Glow Luminescence Detection Cartridges

Overview

The Glow Luminescence Detection Cartridges are for use with the PARADIGM™ Detection Platform. Installing a Glow Luminescence Detection Cartridge enables luminescence detection methods. Cartridges include (see *Measurement Specifications* on page 1-8) :

- PARADIGM™ Glow Luminescence Detection Cartridge - (PN A41584)
- PARADIGM™ Glow Luminescence Detection Cartridge (384) - (PN A56144)
- PARADIGM™ Glow Luminescence Detection Cartridge (96) - (PN A56143)

A glow 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 removable detection cartridges allow the detector to be configured for specific applications and easily expand the capabilities of the detector at any time.

The Glow 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 the *PARADIGM™ Detection Platform User's Manual* for installation instructions.

NOTE

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

About Glow 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.

The Glow 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.

CAUTION

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

NOTE

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

Applications of Glow Luminescence

Chemi- or bioluminescent reactions may be induced to measure the quantity of a particular compound in a sample. Examples of luminescent assays include:

- Reporter gene assays (the measurement of luciferase gene expression).
- Quantitation of adenosine triphosphate (ATP) as an indication of cell counts with cell-proliferation, cytotoxicity, and biomass assays.
- Enzyme measurements with luminescent substrates, such as immunoassays.

Analyzing Glow 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 per 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 1-4) .

Concentrations or qualitative results are derived from raw data by means of a standard curve or comparison with reference controls. A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of a reference label. The

raw data is normalized to counts per second by dividing the number of counts by the read time per well.

Analyzing and validating luminescence intensity data typically consists of the following:

- *Background Correction* (page 1-4)
- *Detection Limit* (page 1-4)
- *Sample Volumes and Concentration of Reactants* (page 1-5)
- *Data Optimization* (page 1-6)

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 the *Multimode Analysis Software User's Manual*).

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:

$$\text{det limit} = 3 \times \text{stdev(blank)} / (\text{standard-blank}) \times \text{conc}_{\text{std}} \qquad \text{(equation 1)}$$

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 an ATP/luciferin-luciferase system, when total volume is held constant and ATP is the limiting reagent, the blanked light output is proportional to the concentration of ATP in the sample. At very high concentrations of ATP, the excess requirement for the availability of substrate may become violated because all the substrate is 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.

Performing Measurements

When the Glow 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 Glow Luminescence Detection Cartridge enables several default and sample measurement protocols in Multimode Software for different detection modes, plate formats, and read times, which may be used as configured or as examples to create new protocols.

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

- For information on creating detection methods using the Glow Luminescence Detection Cartridge, refer to the *Multimode Analysis Software User's Manual*.

- For information on creating and running protocols using the Glow Luminescence Detection Cartridge, refer to 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 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
Glow 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 Glow Luminescence Detection Cartridge are shown in Table 1.2.

Table 1.2 Measurement Specifications

Parameter	Specification	Specification	Specification
Detection Cartridge Name	PARADIGM™ Glow Luminescence Detection Cartridge	PARADIGM™ Glow Luminescence Detection Cartridge (384)	PARADIGM™ Glow Luminescence Detection Cartridge (96)
Short Name	LUM	LUM 384	LUM 96
Part Number	A41584	A56144	A56143
Weight	1.5 lb (0.7 kg)	1.5 lb (0.7 kg)	1.5 lb (0.7 kg)

Table 1.2 Measurement Specifications (continued)

Parameter	Specification	Specification	Specification
Plate Formats	Compatible with 6 to 1536 well microtiter plate formats	Compatible with 6 to 384 well microtiter plate formats	Compatible with 6 to 96 well microtiter plate formats
Detection Techniques	Glow Luminescence	Glow Luminescence	Glow Luminescence
Type	Single emission	Single emission	Single emission
Number of Slots	1	1	1
Top/Bottom	Top or Bottom, except for rearmost slot	Top or Bottom, except for rearmost slot	Top or Bottom, except for rearmost slot
Light Source	NA	NA	NA
Wavelength Range	Visible to 800 nm	Visible to 800 nm	Visible to 800 nm
Labels/ Substrates	Any compatible with wavelength range	Any compatible with wavelength range	Any compatible with wavelength range
Detection Limit Top (ATP) and Plate Read Times	• 96-well (200 µL): 0.5 fmol/well (2.5 min read) • 384-well (50 µL): 0.5 fmol/well (4 min read) • 1536-well (8 µL): 0.2 fmol/well (10 min read)	• 96-well (200 µL): 0.4 fmol/well (2.5 min read) • 384-well (50 µL): 0.2 fmol/well (4 min read) • 1536-well (8 µL): NA	• 96-well (200 µL): 0.3 fmol/well (2.5 min read) • 384-well (50 µL): NA • 1536-well (8 µL): NA
Linear Dynamic Range Top	5 logs in a single plate read	5 logs in a single plate read	5 logs in a single plate read
Check Plate Tests	Test names ending with -LUM	Test names ending with -LUM 384	Test names ending with -LUM 96

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 that need to be performed to verify performance differ between detection cartridges.

To verify the measurement performance of the Glow Luminescence Detection Cartridge, run all check plate test suites with the detection

cartridge short name (see Table 1.2) 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 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.

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