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PARADIGM™ Fluorescence Intensity Detection Cartridge

User's Manual

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Beckman Coulter, Inc.
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Fullerton, CA 92835

BECKMAN COULTER®

PARADIGM™ Fluorescence Intensity Detection Cartridge
User's Manual
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Warranty and Returned Goods Requirements

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.
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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.

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. 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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Fluorescence Intensity (FI) Detection Cartridge

Overview

The Fluorescence Intensity (FI) Detection Cartridge is a detection cartridge for use with the PARADIGM™ Detection Platform. The Fluorescence Intensity (FI) Detection Cartridge uses an ultra high power LED for the excitation of various fluorescent labels (see *Measurement Specifications*), enabling fluorescence intensity detection methods. Fluorescence Intensity (FI) Detection Cartridges are available for the following label sets (see *Measurement Specifications*):

- Coumarin-Fluorescein (PN A41577)
- Fluorescein-Rhodamine (PN A41578)
- Cy3-Cy5 (PN A56150)
- CFP-YFP (PN A70568)

A detection cartridge contains the light source, 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 Fluorescence Intensity (FI) Detection Cartridge is a single-position detection cartridge and occupies one (1) slot in the detection read transport. It may be installed in the top read detection cartridge transport for top reading, or in the bottom read detection cartridge transport for bottom reading. Refer to the *PARADIGM™ Detection Platform User's Manual* for installation instructions.

About Fluorescence Intensity

In a fluorescence intensity method, the source light is directed through an excitation filter and then focused by an objective lens from either above or below the plate, depending on whether the cartridge is installed in the top or bottom read detection cartridge transport. The filter passes only the wavelength band necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by one or two photo multiplier tubes.

The Fluorescence Intensity (FI) Detection Cartridge uses an ultra high power LED and an optimized filter set together with the PARADIGM Detection Platform's photon counting detection capabilities, resulting in superior detection limits and linear dynamic range at short read times.

Applications of Fluorescence Intensity

Most assays use a fluorescent label to selectively attach to certain compounds. The amount or concentration of the compound can then be quantified by measuring the fluorescence intensity of the label, which is attached to the compound, and comparing with a label of known concentration (standard). Such methods are often used to quantify low concentrations of DNA or RNA, for example.

Other assays dedicated to binding studies require two types of labels designed for use of the fluorescent resonance energy transfer (FRET) effect. The dual-photomultiplier tube (PMT) design of the PARADIGM Detection Platform enables such dual emission assays to be performed with faster read times and improved precision.

Analyzing Fluorescence Intensity Data

Fluorescence intensity data is dependent on a number of variables. As a result, raw data from the same plate may appear significantly different from one instrument to the next (for example, a PARADIGM Detection Platform versus a DTX Multimode Detector). Raw data is compared to a standard curve with known concentrations of a reference label.

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. When selecting a fast read mode, the raw data becomes slightly lower because during the continuous plate movement, some signal is collected under non-optimum focusing conditions.

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

- *Background Correction and Quantification*
- *Detection Limit*
- *Linearity and the Linear Dynamic Range*

Background Correction and Quantification

A blank is a well that contains everything used with the sample wells except for the label and sample-specific compounds. Using assay buffer only or an empty well may be some approximation of a blank, but should be verified for each assay.

The blank sample reveals the offset underlying each raw data sample. This offset does not carry information on the label and is therefore subtracted before any data reduction is performed.The blanked raw data are proportional to the amount of label in a sample such that the label concentration is quantified by (equation 1).

$$conc_{label} = (sample - blank) / (standard - blank) \times conc_{std} \qquad \text{(equation 1)}$$

where $conc_{std}$ is the concentration of the standard, and sample, blank and standard are average values of any replicates for the sample, blank and standard wells. In the general case where the standard curve covers a concentration range of more than a few linear logs, (standard - blank) / $conc_{std}$ is equivalent to the slope of the standard curve, and the concentration of the label is determined by (sample - blank) / (slope of standard curve).

Background is caused by multiple effects of the assay, microplate, and detector optics. Low background is an indicator for a suitable plate type and match between cartridge (filter set) and fluorophore. Background is quantified using the blank and standard, and can be expressed by the following equation:

$$background = (blank) / (standard - blank) \times conc_{std} \qquad \text{(equation 2)}$$

where $conc_{std}$ is the concentration of the standard, and blank and standard are average values of any replicates for the blank and standard wells.

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

After blank subtraction from the raw data, some wells may be unexpectedly lower (or higher) than zero due to noise, blank contamination, blank replicate non-uniformity, or an unsuitable blank. Because of this, there is a lower limit on the concentration that may be quantified. Technically, this detection limit is defined by the non-uniformity of blank replicates (standard deviation), quantified by (equation 3), which is analogous to (equation 1):

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

where conc_{std} is the concentration of the standard, stdev(blank) is the standard deviation of the blank replicates, and blank and standard are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks and the calculation of standard deviation. Due to the invocation of noise (by the use of the standard deviation), the detection limit typically depends on read time, and is thus a measure for sensitivity at a given read time.

Linearity and the Linear Dynamic Range

Within a wide range at moderately high concentrations, blanked raw data is proportional to the amount of label in a sample. When the standard curve after blank reduction is not linear in concentration at the lower end, there may be an inappropriate or contaminated blank.

At very high concentrations, saturation (that is, when the relationship between concentration and raw data is no longer linear) may be seen in raw data from products other than PARADIGM Detection Platform. The PARADIGM Detection Platform and the Fluorescence Intensity (FI) Detection Cartridge prevent saturation by adapting the excitation light intensity to each sample and scaling the raw data accordingly, resulting in a larger linear dynamic range.

The linear dynamic range (LDR) is defined by:

$$LDR = \log_{10}[(\text{max conc lin}) / (\text{detection limit})] \qquad \text{(equation 4)}$$

where max conc lin is the highest concentration in the linear range that may be quantified.

Performing Measurements

When the Fluorescence Intensity (FI) Detection Cartridge is installed in the PARADIGM Detection Platform, detection methods and protocols to perform fluorescence intensity measurements are enabled in Multimode Software.

When installed in the top read detection cartridge transport, the Fluorescence Intensity (FI) Detection Cartridge enables the following detection methods for the wavelengths and labels supported by the detection cartridge (see *Measurement Specifications*):

- Fluorescence Intensity Top
- Fluorescence Resonance Energy Transfer (FRET)

When installed in the bottom read detection cartridge transport, the Fluorescence Intensity (FI) Detection Cartridge enables the following detection methods:

- Fluorescence Intensity Bottom
- Fluorescence Resonance Energy Transfer (FRET)

The Fluorescence Intensity (FI) 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 Fluorescence Intensity (FI) Detection Cartridge may be created using Multimode Software.

- For information on creating detection methods using the Fluorescence Intensity (FI) Detection Cartridge, refer to the *Multimode Analysis Software User's Manual*.
- For information on creating and running protocols using the Fluorescence Intensity (FI) 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 for each supported measurement type.

NOTE

For each type of assay (defined by plate type and sample volume), it is recommended to optimize the labware and reading height (refer to the *Multimode Analysis Software User's Manual*).

Plate handling guidelines include:

- Keep unused plates clean and dry.
- Visually inspect the bottom and rim of the plate before use to make sure it is free of dirt and contaminants.

- 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
Fluorescence Intensity Top Read	solid black	When an application specifies a surface treatment, only use plates with the correct treatment.
Fluorescence Intensity Bottom Read	solid black with clear bottom	When an application specifies a surface treatment, only use plates with the correct treatment.

Measurement Specifications

The specifications for measurements using the Fluorescence Intensity (FI) Detection Cartridge are shown in Table 1.2.

Table 1.2 Cartridge Specifications

Parameter	Specification	Specification	Specification	Specification
Detection Cartridge Name	PARADIGM™ Fluorescence Intensity (coum-fluor) Detection Cartridge	PARADIGM™ Fluorescence Intensity (CFP-YFP) Detection Cartridge	PARADIGM™ Fluorescence Intensity (fluor-rhod) Detection Cartridge	PARADIGM™ Fluorescence Intensity (Cy3-Cy5) Detection Cartridge
Short Name	FI-COFL	FI-CFP-YFP	FI-FLRH	FI-CY3CY5
Part Number	A41577	A70568	A41578	A56150
Weight	1.5 lb (0.7 kg)	1.5 lb (0.7 kg)	1.5 lb (0.7 kg)	1.5 lb (0.7 kg)
Detection Technique	Fluorescence Intensity	Fluorescence Intensity	Fluorescence Intensity	Fluorescence Intensity
Type	Dual emission	Dual emission	Dual emission	Dual emission
Number of Slots	1	1	1	1
Top/Bottom	Top or Bottom	Top or Bottom	Top or Bottom	Top or Bottom

Table 1.3 Measurement Specifications

Parameter	Specification	Specification	Specification	Specification
Detection Cartridge Name	FI-COFL	FI-CFP-YFP	FI-FLRH	FI-CY3CY5
Light Source	LED, ultra high power	LED, ultra high power	LED, ultra high power	LED, ultra high power
Filter Set	EX: 360-35 EM1: 465-35 EM2: 535-25	EX: 445-20 EM1: 485-20 EM2: 535-25	EX: 485-20 EM1: 535-25 EM2: 595-35	EX: 535-25 EM1: 595-35 EM2: LP655
Labels*	Coumarin FRET: Coumarin-Fluorescein	AlexaFluor 430 Thioflavin FRET: CFP-YFP	Fluorescein FRET: Fluorescein-Rhodamine	Rhodamine FRET: Cy3-Cy5
Detection Limit Top and Plate Read Times	384-well plate (75 µL): • 10 fmol/well (1 min read) • 10 fmol/well (2 min read) 1536-well plate (8 µL): • 3 fmol/well (1.5 min read) • 3 fmol/well (4 min read)	384-well plate (75 µL): • 10 fmol/well (1 min read) • 5 fmol/well (2 min read) 1536-well plate (8 µL): • 5 fmol/well (1.5 min read) • 5 fmol/well (4 min read)	384-well plate (75 µL): • 0.20 fmol/well (1 min read) • 0.10 fmol/well (2 min read) 1536-well plate (8 µL): • 0.05 fmol/well (1.5 min read) • 0.03 fmol/well (4 min read)	384-well plate (75 µL): • 0.30 fmol/well (1 min read) • 0.15 fmol/well (2 min read) 1536-well plate (8 µL): • 0.12 fmol/well (1.5 min read) • 0.06 fmol/well (4 min read)
Linear Dynamic Range Top	4 logs in a single plate read	5 logs in a single plate read	6 logs in a single plate read	6 logs in a single plate read
Check Plate Tests	test names ending with -FI-COFL	test names ending with -FI-CFP-YFP	test names ending with -FI-FLRH	test names ending with -FI-CY3CY5

* Additional labels compatible with the excitation and emission wavelengths of the cartridge may also be used. All trademarks are the property of their respective owner.

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 Fluorescence Intensity (FI) 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 1.4 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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