FP

PARADIGM™ Fluorescence Polarization Detection Cartridge

User's Manual

Beckman Coulter PN A48434
Revision AA
May 2007

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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:	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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Fluorescence Polarization (FP) Detection Cartridge

Overview

The Fluorescence Polarization (FP) Detection Cartridge is a detection cartridge for use with the PARADIGM™ Detection Platform. Installing the Fluorescence Polarization (FP) Detection Cartridge enables fluorescence polarization detection methods for specific labels, depending on the cartridge. Fluorescence Polarization (FP) Detection Cartridges are available for the following labels:

- Fluorescein (PN A41581)
- Rhodamine (PN A41582)

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

The Fluorescence Polarization (FP) Detection Cartridge is a single slot detection cartridge and occupies one (1) slot. It may be installed in the top or bottom read 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 Fluorescence Polarization (FP) Detection Cartridge in the top read detection cartridge transport.

About Fluorescence Polarization

Fluorescence polarization (FP) is a measurement technique that uses linearly polarized excitation light. The fluorescence emission is measured behind polarizing optics that filter emission light parallel and perpendicular to the polarization plane of the excitation light.

The PARADIGM Detection Platform incorporates a dual emission design to measure both emission channels (parallel and perpendicular) simultaneously, improving precision and reducing read times when measuring two different emission wavelengths.

Figure 1.1 depicts an overview of FP. The process can be described as follows:

1. Excitation light is linearly polarized (EX polar.) and reflected into the sample by a beamsplitter (B). Labeled ligands oriented as shown by the dipole (represented in the figure as an "I") in sample para. are most easily excited.
2. Nanoseconds after excitation, the fluorophores emit energy with a wavelength greater than the excitation wavelength which is measured as emission. The emission may be in any direction, but can be summarized by three extreme cases as shown in Figure 1.1:
    - sample para displays the case in which the labeled ligand does not move at all. In this case, the emission is in the same direction as, or parallel to, the excitation. Most likely indicates that the ligand is bound to a larger, less mobile molecule. This leads to high polarization data.
    - sample perp displays the case in which the labeled ligand is highly mobile. In the short time between excitation and emission, the fluorophore dipole has rotated in the horizontal plane by 90 degrees. As a result, the emission is perpendicular to the excitation, and is most likely caused by unbound ligand. This leads to low polarization data.

- sample undetected displays the case in which the labeled ligand is highly mobile, but rotates 90 degrees out of the measurement plane. The fluorescence emission in this case is undetected. The probability of occurrence is the same as for sample perp.

Figure 1.1 Simplified diagram of fluorescence polarization measurement

The cases shown above are for illustrative purposes. In an FP assay, having signal in only one channel (parallel or perpendicular) does not occur for the following reasons:

- The decay of a fluorophor label follows exponential decay, but the emission for some individual fluorophores may occur earlier or later than the fluorescence lifetime, which is a statistical average.
- The fluorophores are randomly oriented in solution. Thus, any dipole orientation is possible. The fluorophore in these cases contributes to both the parallel and perpendicular emissions proportionally based on its orientation.

Applications of Fluorescence Polarization

Fluorescence polarization (FP) measurements provide information on molecular orientation and mobility, and is typically used to quantify the success of a binding reaction between a smaller labeled ligand and a binding site at a much larger or immobilized molecule. FP can also be used to quantify the dissociation or cleavage of the labeled ligand from a binding site.

FP is a homogeneous microplate assay technique and requires only mixing and measuring—no wash steps are required as in an ELISA. It can also be miniaturized, which makes it useful for high-throughput screening applications.

Analyzing Fluorescence Polarization Data

Fluorescence polarization (FP) results in two sets of fluorescence raw data which are reduced to a single set of FP values.

FP assays in microplates are typically designed with two control samples:

- LOW control sample—minimal polarization value resulting from unbound labeled ligand only
- HIGH control sample—maximum polarization value resulting from bound labeled ligand only

The FP data for a sample is evaluated based on its relative position between the low and high control values. Total intensity may also be determined from the raw data and is proportional to the amount of label in a sample.

Analyzing and interpreting FP data typically consists of the following:

- *Blank Correction* (page 1-7)
- *Data Reduction* (page 1-7)
- *Data Qualification and Validation* (page 1-9)

Blank Correction

Many FP assays use small fluorescent label concentrations in the lower nM range. In this range, blank controls become significant when compared to samples. In order to make FP values independent from label concentration and instrument variations, raw data for parallel and perpendicular values are corrected for blanks prior to data reduction:

- $I_{para} = raw_{para} - blank_{para}$
- $I_{perp} = raw_{perp} - blank_{perp}$

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*).

Data Reduction

The FP data reduction receives two sets of fluorescence raw data as an input and generates a single set of FP data output. There are two alternative scales commonly used to measure FP. Both scales can be uniquely transformed into each other:

- Milli Polarization units (typically ranging from 0 to 500mP)
- Anisotropy units (ranging from 0 to 0.400, accordingly).

The formula for converting raw data to polarization data (in mP units) is as follows:

$$P[mP] = \frac{I[para] - GI[perp]}{I[para] + GI[perp]} \times 1000 \qquad \text{(equation 1)}$$

Anisotropy units may also be determined using the relationship between mP and anisotropy:

$$Anisotropy = \frac{\frac{2P[mp]}{1000}}{3 - \frac{P[mp]}{1000}} \qquad \text{(equation 2)}$$

The G-Factor in (equation 1) corrects blanked raw data for differences in the detection efficiencies for parallel and perpendicular emission from the sample. Main contributions to the G-factor are optical specifications of beamsplitters and mirrors between the sample and the PMTs. The G-factor may also be affected by the emission spectrums of the fluorophores and the emission filters used. For example, using fluorescein or the Pan Vera Green low polarization standard (Invitrogen P-3088) results in different G-Factors. The G-Factor is on the order of one. Deviations from unity (G = 1) do not affect the quality of the resulting FP data.

The value of the G-factor is determined by solving (equation 1) for G and applying the formula to the low control. The G-factor can then be described by the equation below:

$$G = \frac{1 - \frac{P[low]}{1000}}{1 + \frac{P[low]}{1000}} \times \frac{I[para[low]]]}{I[perp[low]]]} \qquad \text{(equation 3)}$$

NOTE

The G-Factor is automatically calculated in the **G-factor_FP Fluorescein** and **G-factor_FP Rhodamine** template protocols enabled by the Fluorescence Polarization (FP) Detection Cartridge.

The low polarization control may be addressed with a value determined on a calibrated cuvette-based fluorometer. The value may be also found in the literature, or provided by an assay manufacturer. In principle, an arbitrary value could be defined. Using the above equation (equation 3) for the G-factor is equivalent to fitting the G-factor value such that the low polarization control attains the desired value. When ignoring the actual G-factor (assuming G=1), the polarization values are shifted (values may be less than zero, or much greater than expected). Normally the low polarization control is used for this calibration step, because its polarization value is often close to zero FP units, while the assay window (difference from the low to the high polarization value) depends on the assay (mainly the relative size of the bound complex to the unbound ligand).

For monitoring the assay preparation, the total intensity can be retrieved from the raw data. Total intensity is proportional to the amount of label in a sample. Total intensity does not give information on binding success.

The total intensity of the samples can be calculated using the following formula:

$$TotalIntensity = I[para] + 2GI[perp] \qquad \text{(equation 4)}$$

The factor of two compensates for the undetected sample which is not measured by either the parallel or perpendicular emission channels (Figure 1.1 on page 4).

Data Qualification and Validation

When validating the data of an FP measurement and the assay, there are two factors to look at: the precision value, and the Z' parameter.

The FP precision value is a measure of replicate uniformity determined by the standard deviation of replicates at a label concentration of 1 nM. Since the precision of a measured signal also depends on the read time,

the read time must also be specified. A longer read time leads to a lower (better) precision value.

Z' is a qualification parameter that can be used to validate the results of a measurement. It is a function of assay resolution, which is a ratio of the average standard deviation of high and low control replicates (S) to the assay window (W)—the difference between the average value of the high controls and the average value of the low controls.

$$Z' = 1 - 6\frac{S}{W} \qquad \text{(equation 5)}$$

The assay window is dependent on the fluorophore lifetime and relative size of the receptor to the ligand. Precision values are better (lower) at higher signals, which normally come from higher label concentrations.

For a given assay window W, Z' is a downward sloping linear function. That is, as precision values get higher (worse), the Z' value gets lower (worse). Thus, to get a better Z' value, the precision must be improved.

As was discussed earlier, precision is dependent upon assay characteristics (sample volume, label concentration) and read time. In many cases, the assay characteristics are defined and cannot be changed. In this case, the only way to improve precision is to increase the read time per well. For this reason, a Z' value of around 0.6 is typically sufficient for high throughput primary screening applications; higher values may be desired when results are more critical.

NOTE

The Z' qualification factor is automatically calculated in the **Z'-Factor determination (FP Fluorescein)** template protocol enabled by the Fluorescence Polarization (FP) Detection Cartridge.

Performing Measurements

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

When installed in the top read detection cartridge transport, the Fluorescence Polarization (FP) Detection Cartridge enables the following detection methods:

- Fluorescence Polarization

The Fluorescence Polarization (FP) Detection Cartridge enables several default and sample measurement protocols in Multimode Software for different 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 Polarization (FP) Detection Cartridge may be created using Multimode Software.

For information on creating detection methods using the Fluorescence Polarization (FP) 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 Fluorescence Polarization (FP) 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 for each supported measurement type.

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 Polarization	solid black	When an application specifies a surface treatment, only use plates with the correct treatment.

Measurement Specifications

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

Table 1.2 Measurement specifications

Parameter	Specification	Specification
Detection Cartridge Name	PARADIGM™ Fluorescence Polarization (Fluorescein) Detection Cartridge	PARADIGM™ Fluorescence Polarization (Rhodamine) Detection Cartridge
Short Name	FP-FLUO	FP-RHOD
Part Number	A41581	A41582
Weight	1.5 lbs. (0.7 kg)	1.5 lbs. (0.7 kg)
DetectionTechnique	Fluorescence Polarization	Fluorescence Polarization
Type	Dual emission	Dual emission
Number of Slots	1	1
Top/Bottom	Top or Bottom	Top or Bottom
Light Source	LED, ultra high power	LED, ultra high power
Filter Set	EXP: 485-20 EMP1: 535-25 EMP2: 535-25	EXP: 535-25 EMP1: 595-35 EMP2: 595-35
Labels*	Fluorescein 1 nM	Rhodamine 4 nM
Detection Limit Top and Plate Read Times†	**384-well plate (75 µL):** 6 mP (1 min read) 3 mP (2 min read) **1536-well plate (8 µL):** 12 mP (1.5 min read) 6 mP (4 min read)	**384-well plate (75 µL):** 8 mP (1 min read) 4 mP (2 min read) **1536-well plate (8 µL):** 12 mP (3 min read) 6 mP (5 min read)
Check Plate Tests	test names that end with -FP-FLUO	test names that end with -FP-RHOD

*. Additional labels compatible with the excitation and emission wavelengths of the cartridge may also be used.
†. Replicate standard deviation at the label concentration specified under Labels.

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 verify detection cartridge performance only; 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 Polarization (FP) 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 *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.
The Z' value is too low	• Increase the amount of label in the sample, if acceptable for the assay. • Increase the read time per well.
Detection cartridge fails verification tests with check plate	Contact Beckman Coulter Customer Technical Support.

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PN A48434 AA (May 2007)

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