ABS

PARADIGM™ Absorbance 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:	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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Absorbance Detection Cartridge

Overview

The Absorbance Detection Cartridge is a detection cartridge set for use with the PARADIGM™ Detection Platform. The Absorbance Detection Cartridge uses a monochromator to perform absorbance endpoint, kinetic, and scan measurements.

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 Absorbance Detection Cartridge consists of two components: a detection component which measures the absorbance, and an excitation component which sets the absorbance wavelength(s). The detection component is installed in the top read detection cartridge transport and occupies one (1) detection cartridge slot. The excitation component is installed in the bottom read detection cartridge transport and occupies two (2) detection cartridge slots. Both components must be installed to perform absorbance measurements with the PARADIGM Detection Platform. Refer to *Installing Detection Cartridges* on page 1-18 of the *PARADIGM™ Detection Platform User's Manual*, for installation instructions.

About Absorbance

An absorbance detection method measures the absorbance of light through a sample—that is, the relative intensity of light at a specific transmitted wavelength after it passes through a sample (equation 1).

$$a = \log_{10} (I_0 / I) \qquad \text{(equation 1)}$$

where I_0 is the intensity of the incident light before it enters the sample, I is the intensity of light after passing through the sample, and a is the measured absorbance.

The Absorbance Detection Cartridge uses a monochromator with wavelength range of 230 nm to 1000 nm and a 4-nm bandwidth to measure the absorbance of samples.

Applications of Absorbance

Absorbance measurements are used to quantify the concentration of an absorbing species. It must be known at what wavelength a species absorbs light. For example, the concentration of nucleic acids can be quantified by measuring the absorbance at 260 nm.

Analyzing Absorbance Data

Absorbance is proportional to the thickness of the sample and the concentration of the absorbing species in the sample according to the Beer-Lambert Law (equation 2).

$$a - b = c * d * e + f \qquad \text{(equation 2)}$$

where a is the measured absorbance at a given wavelength, b is the blank at the given wavelength, c is the concentration of the absorbing species, d is the depth of the sample layer (or the path length through which the light passes), e is the specific extinction of the absorbing species at the given wavelength, and f is a correction factor for other

items, such as interference from another dye, sample turbidity, or other deviations from the Beer-Lambert Law.

Since e is constant for a given absorbing species, and the path length for a given volume is constant, the absorbance is linearly proportional to concentration when samples have a constant volume. Creating a standard curve using samples of known concentration of the absorbing species allows raw absorbance data to be related to concentrations for unknown samples, as long as samples are of the same volume or normalized for the path length (see *Path Length Effects* on page 1-4) .

At high concentrations, the absorbance may deviate from the Beer-Lambert Law and may not be linear with concentration. As a result, it should be targeted to run samples that fall within the linear range. Using multiple samples across a wide range of concentrations to generate the standard curve enables quantification of concentrations outside of the linear range when the standard curve is still climbing with increasing concentration.

Analyzing and validating absorbance data typically consists of the following:

- *Background Correction* (page 1-3)
- *Path Length Effects* (page 1-4)

Background Correction

At lower wavelengths, some microplates may have well-to-well variability. To correct for this background variability, pre-reading the plate with reference solvent or water in the wells to determine background is recommended. These background measurements can then be subtracted from the sample measurements. The Multimode Software automatically performs this background reduction using the pre-read feature.

For improved results for microplates with low well-to-well variability, it is recommended to run duplicates 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).*

Sample turbidity is another factor which must sometimes be accounted for in absorbance measurements. In turbid samples, particles suspended in the sample scatter light, which results in higher absorbance values. Measuring with a second reference wavelength that is greater than the measurement wavelength helps to correct for some of the background turbidity.

Path Length Effects

As described above by the Beer-Lambert Law (equation 2), the path length is a major contributor to the absorbance measurements of a sample. In a microplate, samples are measured vertically, so the path length is dependent on the volume of sample as well as the meniscus curvature and symmetry. The meniscus curvature is dependent on several factors as well, such as the composition of the sample and the surface treatment of the microplate. Variable meniscus formation across a plate or plates can result in well-to-well or plate-to-plate variability. Irregular meniscuses due to uneven wetting of the well can be improved by microplate shaking, or waiting for a minimum evaporation that lowers the liquid surface.

An asymmetrical meniscus curvature can have negative effects on the absorbance of a sample caused by irregular path lengths and refractive effects which deflect light away from the detector, resulting in higher absorbance values.

The well geometry of the microplate can also influence the path length. Measurements are typically made in the center of a well. This means that a round bottom well has a greater path length than a flat bottom well for the same volume of sample; therefore, the raw

absorbance values for the same sample would differ between these two plates. It is recommended to always use flat bottom microplates and perform a labware optimization (refer to *Optimizing Labware* on page 6-128 of the *Multimode Analysis Software User's Manual).*

When not using a standard within the microplate assay, it is necessary to normalize raw absorbance values for the path length or sample depth. To determine the sample depth, the absorbance of a standard from a 1-cm cuvette is compared to the absorbance of the same standard in a microplate under assay conditions (equation 3).

$$A - B = (a-b)/d = c \times e \qquad \text{(equation 3)}$$

where A is the absorbance of the standard measured in a 1 cm cuvette, B is the absorbance of a blank measured in a 1-cm cuvette, a is the absorbance of the same standard in a microplate, b is the absorbance of the same blank in the microplate, d is the sample depth, c is the concentration of the absorbing species, and e is the specific extinction of the absorbing species.

To determine the sample depth d:

1. Measure the absorbance of a standard in a 1-cm cuvette using a blank cuvette read.
2. Dispense the blank and the standard in replicates into a microplate as if used for the assay.
3. Measure the absorbance of the microplate.
4. Divide the blanked absorbance of the microplate (a-b) by the blanked absorbance of the cuvette (A-B) (equation 4).

$$d = (a-b)/(A-B) \qquad \text{(equation 4)}$$

Once the sample depth is determined, the concentration of samples can be determined by correcting the blanked absorbance for the sample depth and multiplying by the reciprocal of the specific extinction (1/e). This can be derived from (equation 3) by solving for c. For dsDNA, 1/e is about 50 (µg/mL)/(OD/cm). Multimode Software offers such quantification features in the Quantitation protocol editor.

Performing Measurements

When the Absorbance Detection Cartridge is installed in the PARADIGM Detection Platform, detection methods and protocols to perform absorbance measurements are enabled in Multimode Software.

When both components of the Absorbance Detection Cartridge are installed, the Absorbance Detection Cartridge enables the following detection methods:

- Absorbance

The Absorbance Detection Cartridge enables several default and sample measurement protocols in Multimode Software for different plate formats which may be used as configured or as examples to create new protocols.

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

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

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 *Optimizing Labware* on page 6-128 of the *Multimode Analysis Software User's Manual)*.

Plate handling guidelines include:

- Never touch the clear well bottom of plates.
- Visually inspect the bottom and rim of the plate before use to make sure it is free of dirt and contaminants.
- Keep unused plates clean and dry.
- Make sure the strips on strip plates are inserted correctly and level with the frame.
- Do not use U-bottom or V-bottom plates, unless performance has been tested and verified with this instrument. Undefined sample depth and irregular plastic density in the tip of the well may cause inaccurate measurements.

Table 1.1 Microplate selection guidelines

Measurement Technique	Microplate Type	Additional Considerations
Absorbance	all clear, clear bottom, UV clear, flat bottom	When an application specifies a surface treatment, only use plates with the correct treatment.

Measurement Specifications

The specifications for measurements using the Absorbance Detection Cartridge are shown in Table 1.2.

Table 1.2 Measurement specifications

Parameter	Specification
Detection Cartridge Name	PARADIGM™ Absorbance Detection Cartridge
Short Name	ABS-MONO, ABS-DET
Part Number	A41575
Weight	ABS-MONO: 2.4 lbs. (1.1 kg) ABS-DET: 0.9 lbs. (0.4 kg)
Detection Technique	Absorbance
Type	NA
Number of Slots/Top or Bottom	ABS-DET: 1 top ABS-MONO: 2 bottom
Filter Set	230-1000 nm Bandwidth: 4 nm
Wavelength Scan Speed	35 nm/s
Wavelength Accuracy	± 1 nm
Wavelength Repeatability	± 0.5 nm
Photometric Range	0 - 3.5 OD
Photometric Resolution	0.0001 OD
Photometric Accuracy @ 2 OD, 405 nm	± 2% + 0.010 OD
Photometric Precision @ 2 OD, 405 nm	± 1% + 0.005 OD

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 Absorbance Detection Cartridge, run all check plate test suites with the detection cartridge short name ABS-MONO or ABS-DET 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.

PARADIGM™ Absorbance Detection Cartridge User's Manual
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