HTRF

PARADIGM™ Homogeneous Time Resolved Fluorescence (HTRF*) Detection Cartridge

User's Manual

Beckman Coulter PN A48436
Revision AA
October 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.

Table of Contents

Warranty and Returned Goods Requirements, iii

Safety Information, v

Warning and Caution Definitions, v

Electrical Safety, vi

High Voltage, vi

Disposal of Electronic Equipment, vi

Laser Light, vii

Laser Specifications, vii

Chemical and Biological Safety, viii

Moving Parts, ix

Cleaning, ix

Maintenance, x

Homogeneous Time-Resolved Fluorescence (HTRF*) Detection Cartridge, 1

Overview, 1

About Homogeneous Time-Resolved Fluorescence, 2

Applications of Homogeneous Time-Resolved Fluorescence, 3

Analyzing Homogeneous Time-Resolved Fluorescence Data, 3

Performing Measurements, 8

Microplate Recommendations, 8

Measurement Specifications, 10

Preventive Maintenance and Troubleshooting, 11

Verifying Performance with Check Plate, 12

Troubleshooting, 13

Homogeneous Time-Resolved Fluorescence (HTRF*) Detection Cartridge

Overview

The Homogeneous Time-Resolved Fluorescence (HTRF*) Detection Cartridge is a detection cartridge for use with the PARADIGM™ Detection Platform. Installing the HTRF Detection Cartridge enables time-resolved fluorescence detection methods for europium cryptate and europium acceptors.

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 HTRF Detection Cartridge is a dual emission detection cartridge and occupies two (2) slots. It may be installed in the top read detection cartridge transport only. Refer to *Installing Detection Cartridges* on page 1-18 of the *PARADIGM™ Detection Platform User's Manual*, for installation instructions.

About Homogeneous Time-Resolved Fluorescence

Homogeneous time-resolved fluorescence (HTRF) is a measurement technique based on fluorescence resonance energy transfer (FRET) using the advantages of time-resolved fluorescence (TRF) reading.

FRET is a radiationless transfer of excitation energy from a donor fluorophor to an acceptor fluorophor. The acceptor is chosen such that its excitation spectrum overlaps the emission spectrum of the donor fluorophor. The energy transfer can only occur when the donor and acceptor fluorophors are close in proximity. Because of this fact, signal measured at the emission wavelength of the acceptor fluorophor indicates binding. The signal is normalized with the emission of the donor, making the results independent from the amount of label in a well. This improves replicate uniformity and the overall precision of the results, as well as compensates for potential absorption of assay compounds.

HTRF utilizes a donor fluorophor with a long fluorescence lifetime (LT), such as Europium. The acceptor fluorophor acts as if it also has a long fluorescence lifetime. This allows the time-gating principle of time-resolved fluorescence to be applied to the acceptor emission to separate specific signal from background and signal caused by compound interference.

Time-gating electronics introduce a delay between the flashes and the start of signal accumulation. During the delay, the unspecific fluorescence caused by test compounds, assay reagents, and the microplate vanishes while only a small portion of the specific fluorescence from the acceptor fluorophor is sacrificed. Enough of the specific signal remains, with the benefit of reduced background.

NOTE

HTRF is a registered trademark of Cisbio International, France. For more information about HTRF, refer to http://www.htrf.com.

The HTRF Detection Cartridge utilizes a Xenon flash lamp. The excitation filter selects wavelengths suitable for the excitation of Europium cryptates below 330 nm. Emission filters detect signal for Europium cryptate (616 nm) and typical Europium acceptor labels (665 nm). The PARADIGM Detection Platform incorporates a dual emission design to measure both emission channels simultaneously, improving precision and reducing read times.

Applications of Homogeneous Time-Resolved Fluorescence

Homogeneous time-resolved fluorescence (HTRF) is used in competitive assays to quantify the binding between two labeled molecules, or the disintegration of a bound complex. Binding partners may have similar molecular weights as opposed to fluorescence polarization methods. HTRF is a homogeneous assay that requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

Analyzing Homogeneous Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of flashes. Each flash consists of triggering the Xenon flash lamp, pausing

for a specifed length of time, and measuring the fluorescence intensity of the sample. These flashes are repeated several times, as specified in the measurement protocol timing parameters (see Table 1.1 for suggested timing parameters).

Analyzing and interpreting TRF data typically consists of the following:

- *Data Reduction* (page 1-4)
- *Data Optimization* (page 1-4)

Data Reduction

Data reduction for HTRF methods consists of two steps.

First, a ratio of the signal measured by the emission from the acceptor label at 665 nm to the signal measured by the emission of the donor label at 616 nm is calculated and multiplied by a factor of 10,000. This generates what is called the HTRF signal.

In the second step, ratios are calculated that represent the relative change in the HTRF signal compared to that of the assay background (represented by assay controls potentially named negative or Standard 0). This value is called the Delta F and is formatted as a percentage, though values greater than 100 may be achieved.

The default and sample protocols enabled by the HTRF Detection Cartridge calculate the HTRF signal using the data reduction function Ratio Cisbio. Delta F is also calculated if negative controls are defined in the sample plate layout. For easier comparison with data from other instruments, Delta F may be normalized using further data reduction.

Data Optimization

The measurement noise is dependent on the read time per sample, or the number of flashes applied. In particular, the detection limit improves when the read time is increased. Therefore, it is important to

specify the read time when comparing measurements. Defining the number of flashes (pulses) cannot be used for comparative purposes because the flash rate varies from system to system.

Measurement results may be further optimized by optimizing the timing parameters. Table 1.1 may be used as a guideline for the selection of timing parameters.

Table 1.1 HTRF timing parameters

Parameter	Value	Comment
Pulse length	50 µs	suggested value for HTRF cartridge; time interval for flash monitoring
Measurement delay	30 µs	suggested value for HTRF cartridge; delay to ensure flash has vanished
Integration time per cycle (flash)	500 µs	suggested value for HTRF cartridge; time window accumulating signal

There are two timing parameters that can be optimized to adjust the performance of the measurement as desired: time per plate (or time per well) and integration time per flash.

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.

As the number of flashes (read time per well) is increased, several aspects of the data improve:

- Delta F values show less variability (better CVs)
- Small Delta F values are better distinguished from noise; noise of background is reduced

The second timing parameter which may be optimized is the Integration time per cycle. Care must be taken in optimizing the integration time to take into account noise. Delta F is higher at low integration times, but noise is also high at low integration times. The optimum integration time is where noise is minimized while maximizing Delta F.

In the example shown below, the optimum integration time (read time per cycle) appears to be in the 500 to 1000 µs range, as noise is minimized and Delta F is still relatively high. Going beyond 1000 µs shows sharp decline in Delta F without any apparent improvement in noise.

Figure 1.1 Relationship between integration time, noise, and Delta F

NOTE

Z′ is a qualification parameter that can be used to validate the results of a measurement. It is a function of assay resolution (equation 1), 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.

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

Performing Measurements

When the HTRF Detection Cartridge is installed in the PARADIGM Detection Platform, detection methods and protocols to perform time-resolved fluorescence measurements are enabled in Multimode Software.

When installed in the top read detection cartridge transport, the HTRF Detection Cartridge enables the following detection methods:

- Time-Resolved Fluorescence (TRF)

The HTRF 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 HTRF Detection Cartridge may be created using Multimode Software.

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

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 1.2 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 *Creating and Editing Labware* on page 6-115 of the *Multimode Analysis Software User's Manual)*.

Plate handling guidelines include:

- Keep unused plates clean and dry.
- 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 1.2 Microplate selection guidelines

Measurement Technique	Microplate Type	Additional Considerations
Time-Resolved Fluorescence (TRF)	solid white	When an application specifies a surface treatment, only use plates with the correct treatment.

Measurement Specifications

The specifications for measurements using the HTRF Detection Cartridge are shown in Table 1.3.

Table 1.3 Measurement specifications

Parameter	Specification
Detection Cartridge Name	PARADIGM™ HTRF Detection Cartridge
Short Name	HTRF
Part Number	A41583
Weight	2.6 lbs (1.2 kg)
Detection Technique	Time-Resolved Fluorescence
Type	Dual emission
Number of Slots	2
Top/Bottom	Top
Light Source	Xenon Flash Lamp, 8 ms pulse period
Filter Set	EX: HTRF EM1: 616-10 EM2: 665-10
Labels	Europium cryptate, Europium acceptors
Applications	HTRF certified by Cisbio International
Check Plate Tests	test names ending with -HTRF

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 HTRF Detection Cartridge, run all check plate test suites with the detection cartridge short name HTRF 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,* Section , *Performing Basic Maintenance and Troubleshooting*.

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.

PARADIGM™ HTRF Detection Cartridge User's Manual

PN A48436 AA (October 2007)

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