TRF

PARADIGM™ Time-Resolved Fluorescence Detection Cartridge

User's Manual

Beckman Coulter PN A48435
Revision AB
August 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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Time-Resolved Fluorescence (TRF) Detection Cartridge

Overview

The Time-Resolved Fluorescence (TRF) Detection Cartridge is a detection cartridge for use with the PARADIGM™ Detection Platform. Installing the Time-Resolved Fluorescence (TRF) Detection Cartridge enables time-resolved fluorescence detection methods for Europium chelate and Samarium chelate.

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 Time-Resolved Fluorescence (TRF) 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 Time-Resolved Fluorescence (TRF) Detection Cartridge in the top read detection cartridge transport.

About Time-Resolved Fluorescence

Time-resolved fluorescence (TRF) is a measurement technique that depends on three characteristics which lead to better discrimination between the specific signal (proportional to the amount of label) and the unspecific fluorescence resulting from background and compound interference:

- pulsed excitation light sources.
- time-gated electronics faster than the fluorescence lifetime (LT).
- labels with prolonged fluorescence LT.

The time-gating electronics introduce a delay between the light pulses cut-off and the start of signal accumulation, as shown in Figure 1.1 on page 6. During the delay (t_2), the unspecific fluorescence caused by test compounds, assay reagents, and the microplate vanishes while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains under the decay curve during period t_3 with the benefit of reduced background.

The Time-Resolved Fluorescence (TRF) Detection Cartridge and PARADIGM Detection Platform provide the pulsed excitation light sources and time-gated electronics; the label is an assay characteristic, and can often be found in dyes based on lanthanides (rare earth metals) such as europium (LT of about 700 µs), samarium (LT of about 70 µs), or terbium (LT of about 1000 µs).

The Time-Resolved Fluorescence (TRF) Detection Cartridge utilizes an ultraviolet LED that emits in the range between 350-380 nm for excitation of Europium chelates, and comes equipped with emission filters for both europium and samarium. This enables single label europium and samarium assays as well as dual label assays involving both europium and samarium to be run. The PARADIGM Detection Platform incorporates a dual emission design to measure both emission channels simultaneously, improving precision and reducing read times.

NOTE

Europium cryptate and terbium require excitation wavelengths below 330 nm and cannot be measured with this Time-Resolved Fluorescence (TRF) Detection Cartridge.

Applications of Time-Resolved Fluorescence

Time-resolved fluorescence (TRF) is useful in some fluorescence immunoassays, such as DELFIA (dissociation-enhanced enzyme linked fluorescence immunoassay). TRF is also useful in some assay variants of TR-FRET (time-resolved fluorescence resonance energy transfer) in which the FRET acceptor label acts as a quencher only and does not emit fluorescence. The proximity between donor label and acceptor (quencher) is then quantified by the intensity decrease of the donor label.

DELFIA requires washing steps as in an ELISA, but the TR-FRET assay involving quenching is a homogeneous microplate assay technique and 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 Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of pulses. Each pulse consists of turning on the LED, turning off the LED, pausing for a specified length of time (t_2), and measuring the fluorescence intensity of the sample during period t_3. These pulses 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:

- *Blank Correction* (page 1-4)
- *Data Normalization* (page 1-5)
- *Data Optimization* (page 1-5)

Blank Correction

Although background is significantly lower than with fluorescence intensity measurements, it is recommended to use blank or assay controls.

A blank is a well that contains everything used with the sample wells except for the label and sample-specific compounds which may yield unwanted interference. 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.

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 Normalization

TRF raw data changes in magnitude when the timing parameters are changed. However, TRF data are normalized for a number of 1000 pulses. This means that the sample raw data does not change when only the number of pulses is changed.

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.

Data Optimization

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

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. For TRF, the read time per well increases with the selected number of pulses. The time between pulses (see Table 1.1), however, may be different on various systems. Therefore, it is important to specify the read time when comparing measurements.

All low light level detection devices have some measurement noise in common. In order to average out the measurement noise, optimization of the time per plate (or time per well) shall target for accumulating as many counts as possible. Within some range, the reduction of noise (CVs, detection limit) can be accomplished by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

Further optimizing measurement results is done by optimizing the timing parameters. Table 1.1 may be used as a guideline for the selection of timing parameters.

Table 1.1 Time-resolved fluorescence timing parameters

Parameter	Value	Comment
Pulse length	0.100 ms	suggested value for TRF cartridge; period for excitation of sample; t_1 in Figure 1.1
Measurement delay	0.010 ms	suggested value for TRF cartridge; delay to ensure excitation pulse has vanished; t_2 in Figure 1.1
Integration time per cycle (pulse)	1.890 ms	suggested value for TRF cartridge; time window accumulating signal; t_3 in Figure 1.1

Figure 1.1 Timing parameters for time-resolved fluorescence

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When neglecting the time delay t_2 compared to the integration time window t_3, the accumulated signal A can be approximated by the following equation:

$$A/A_{max} = (1-\exp(-M)) \times 100\% \qquad \text{(equation 1)}$$

In the equation above, M is the size of the time window (or integration time) divided by the exponential decay time constant (or the fluorescence LT of the label).

$$M = (\text{integration time})/(\text{fluorescence LT}) \qquad \text{(equation 2)}$$

For example, using Europium, which has a fluorescence lifetime of 700 µs, and the suggested integration time per cycle from Table 1.1 of 1.890 ms (or 1890 µs), M = 1890/700 = 2.7. Inserting this value of M into (equation 1) yields A/A_{max} = 93%.

To optimize the integration time per cycle (pulse), the integration time should be set such that the value of M enables the desired signal to be achieved (see Table 1.2). For example, to achieve more than 86% signal, select an integration time such that M is greater than 2.0. Using the Europium example and solving (equation 2) for the integration time, the integration time may be set to M (2.0) times the fluorescence lifetime (700 µs), or 1400 µs (1.4 ms).

Table 1.2 Achievable accumulated signal percentage versus M

M	0.25	0.5	0.75	1.00	1.25	1.50	2.00	3.00
A/A_{max} [%]	22	39	53	63	71	78	86	95

NOTE

M may be technically limited by the time between pulses. Further gain in signal beyond some value of M may be negligible to improve results.

When performing a dual label Europium-Samarium measurement, there are additional timing parameters. There is some residual cross-talk of the Samarium signal captured in the Europium emission channel. Samarium has a much shorter fluorescence lifetime, so to reduce the cross-talk of Samarium in the Europium channel, Europium is measured in a time window shifted away from the time window for Samarium. This allows the Europium to be quantified without any cross contamination from the Samarium. The known Europium concentration can then be used to remove the Europium cross-contamination in the Samarium channel.

Suggested timing parameters for a dual label Europium-Samarium measurement are listed in Table 1.3:

Table 1.3 Time-resolved fluorescence timing paramaters for dual label Europium-Samarium

Parameter	Value	Comment
Pulse length	0.100 ms	suggested value for TRF cartridge; time interval for flash monitoring
Measurement delay (first window)	0.010 ms	suggested value for TRF cartridge; delay to ensure flash has vanished
Integration time (first window)	0.100 ms	suggested value for TRF cartridge; time window accumulating Samarium signal
Measurement delay (second window)	0.140 ms	suggested value for TRF cartridge; read out of Samarium signal
Integration time (second window)	0.750 ms	suggested value for TRF cartridge; time window accumulating Europium signal

Performing Measurements

When the Time-Resolved Fluorescence (TRF) 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 Time-Resolved Fluorescence (TRF) Detection Cartridge enables the following detection methods:

- Time-Resolved Fluorescence

The Time-Resolved Fluorescence (TRF) 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 Time-Resolved Fluorescence (TRF) Detection Cartridge may be created using Multimode Software.

- For information on creating detection methods using the Time-Resolved Fluorescence (TRF) 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 Time-Resolved Fluorescence (TRF) 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 1.4 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 1.4 Microplate selection guidelines

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

Measurement Specifications

The specifications for measurements using the Time-Resolved Fluorescence (TRF) Detection Cartridge are shown in Table 1.5.

Table 1.5 Measurement specifications

Parameter	Specification
Detection Cartridge Name	PARADIGM™ Time-Resolved Fluorescence (TRF) Detection Cartridge
Short Name	TRF-EUSA
Part Number	A41579
Weight	1.5 lbs (0.7 kg)
Detection Technique	Time-Resolved Fluorescence
Type	Dual emission
Number of Slots	1
Top/Bottom	Top
Light Source	ultra high power LED, 3 ms pulse period
Filter Set	EX: 370-80 EM1: 616-10 EM2: 642-10
Labels	Europium chelate, Samarium chelate
Detection Limit Top and Plate Read Times	**384-well plate (75 µL):** 8 amol (1 min read) 5 amol (2 min read) **1536-well plate (8 µL):** 3 amol (1.5 min read) 1.5 amol (4 min read)
Linear Dynamic Range Top	6 logs in a single plate read
Check Plate Test	test names ending in -TRF-EUSA

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 Time-Resolved Fluorescence (TRF) Detection Cartridge, run all check plate test suites with the detection cartridge short name TRF-EUSA 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 1.6 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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PN A48435 AB (August 2007)

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