VersaFluor™ Fluorometer System

Instruction Manual

Catalog Numbers

**170-2402
through
170-2404**

BIO·RAD

Warranty

The VersaFluor Fluorometer and accessories are warranted against defects in materials and workmanship for one year. If any defects occur in the instruments or accessories during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.

2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.

3. Damage caused by substituting alternative parts.

4. Use of fittings or spare parts supplied by anyone other than Bio-Rad Laboratories.

5. Damage caused by accident or misuse.

6. Damage caused by disaster.

7. Corrosion caused by improper solvent or sample.

This warranty does not apply to parts listed below:

1. Fuses

2. Lamps

For any inquiry or request for repair service, contact Bio-Rad Laboratories. Inform Bio-Rad of the model and serial number of your instrument.

Important: This Bio-Rad instrument is designed and certified to meet EN61010-1* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

> Void the manufacturer's warranty

> Void the EN61010-1 safety certification

> Create a potential safety hazard

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent.

The VersaFluor Fluorometer is not compatible with chlorinated hydrocarbons (e.*g.*, chloroform), aromatic hydrocarbons (*e.g.*, toluene, benzene), or acetone. Use of organic solvents voids all warranties.

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* I.E.C. 1010 is an internationally accepted electrical safety standard for laboratory instruments.

Table of Contents

For technical service call your local Bio-Rad office. In the U.S., call 1-800-4BIORAD (1-800-424-6723).

Section 1
General Safety Information

1.1 Caution Symbol

Read the manual before using the VersaFluor Fluorometer. For technical assistance, contact your local Bio-Rad Office or, in the U.S., call technical services at 1-800-4BIORAD (1-800-424-6723).

Definition of Symbols

Caution (refer to accompanying documents).

Caution, hot surface.

This instrument is intended for laboratory use only.

This product conforms to the "Class A" standards for electromagnetic emissions intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

1.2 Precautions

1. Do not use near flammable materials.

2. Always inspect the VersaFluor Fluorometer for damaged components before use.

3. Always connect the system to the correct AC power source.

4. Always connect the correct instrument (printer or computer only) via the serial port connector.

5. Do not pour liquid into the sample chamber. Thorough clean-up is needed after each spill (Section 7.3).

6. Do not place objects on the VersaFluor Fluorometer.

7. Do not look directly at the lamp when on.

8. When replacing the lamp, turn the instrument off and let the lamp cool 5 minutes. The lamp gets very hot and may cause burns if touched before it has cooled.

9. Do not spill liquids directly on the lamp. The lamp may explode under liquid contact.

Section 2
Product Description

2.1 Packing List

The VersaFluor Fluorometer (catalog number 170-2402) is shipped with the following components. Upon receiving your instrument, please check that all items listed below were shipped. If any items are missing or damaged, contact your local Bio-Rad office.

Item	Quantity
Instruction manual	1
Warranty card (please complete and return)	1
VersaFluor Fluorometer	1
Power cable	1
Cuvette holder	1
Spare fuses (1.6 amp, slow-blow, T-type)	2
Disposable cuvettes, 12.5 x 12.5 mm (outside dimensions)	100
Excitation filter	1
Emission filter	1

2.2 Identification of System Components
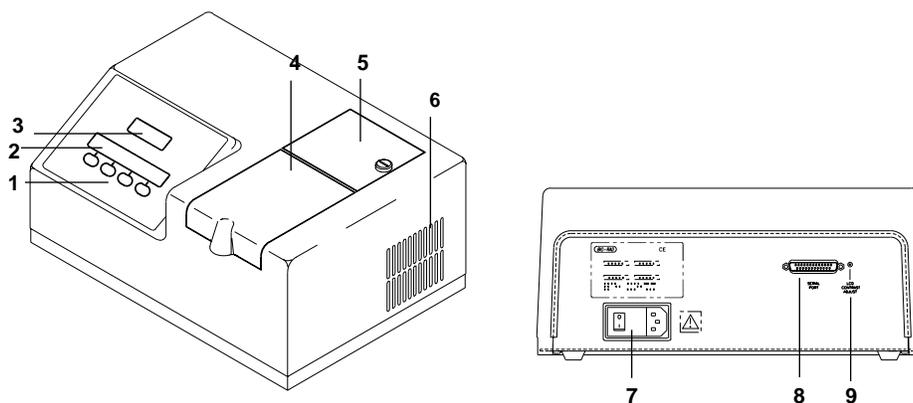
Fig. 2.1. View of VersaFluor Fluorometer System.

Instrument

1. Push Buttons — Change menu and select options by pressing buttons. Press a single button to perform the action described on the menu display. The function of each push button changes as the menu changes.

2. Menu Display — Displays various menus and options available.

3. Fluorescence Units Display — Displays relative fluorescence units (RFU).

4. Sample Compartment Door — Lift door to insert sample cuvette into the cuvette holder.

5. Lamp Housing Door — Lift door to replace the quartz halogen lamp.

6.	Fan Guard	Exhaust air leaves cabinet through this opening.
7.	Power Input Module and ON/OFF Switch.	Power cord is connected to the power input module. Instrument power switch.
8.	Serial Output Port	RS-232 connection to serial printer or computer.
9.	Menu LCD Contrast Adjust	Carefully adjust the menu contrast using a small screwdriver.

Sample Compartment
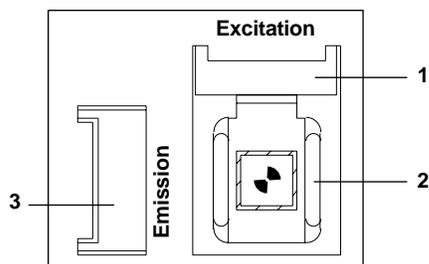
Fig. 2.2. Sample compartment top view.

1.	Excitation Filter Holder	Holds the excitation filter in place. The excitation filter selects the wavelength of the light which will illuminate the sample and excite fluorescence.
2.	Cuvette Holder	Holds cuvette in place. The cuvette holder can be removed by pressing the two tabs inward and pulling upward.
3.	Emission Filter Holder	Holds the emission filter in place. The emission filter selects the emitted fluorescent light that is detected by the fluorescence detector circuitry.

Section 3
Installation

3.1 Environmental Requirements

To insure correct operation and stable performance over an extended period of time, install the VersaFluor Fluorometer in a location which meets the following conditions:

- Room temperature between 20 and 35 °C. Not recommended for cold room use (4 °C).

- Not exposed to direct sunlight.

- Not subject to direct or continuous vibration.

- Not subject to intense magnetic or electromagnetic fields.

- Relative humidity between 0–95%.

- Area free from corrosive gases or other corrosive substances.

- Area with very little dust or other airborne particles.

- Allow a 10 cm minimum space around the instrument for proper air flow.

3.2 Power On and Initial Display

The VersaFluor Fluorometer is designed to operate at universal line voltage conditions of 100–240 VAC with an AC frequency of 50–60 Hz. With the power switch in the OFF position, plug the power cable into the power input module on the back of the instrument. To insure positive grounding, plug the VersaFluor Fluorometer instrument into a three-prong wire receptacle.

Turn on the power switch located on the power input module in the back lower right corner of the instrument. The menu display will show the following messages:

```
BIO-RAD
VERSAFLUOR™
```

```
BIO-RAD VER. 1.30

```

```
01/01/97            12:00:00
SAMPLE START 00001
```

```
        RANGE  SET
PRINT  00000 ZERO  SETUP
```

The first three screens are part of the instrument initialization and the fourth screen is the main menu display. The VersaFluor Fluorometer instrument must warm-up for 20 minutes prior to use. Measurements read prior to the warm-up time may cause irreproducible fluorescence readings.

3.3 Adjusting the LCD Contrast

The menu display LCD is factory adjusted for normal viewing. To change the contrast of the menu display, locate the adjusting screw accessible from the back of the instrument (Section 2.2). Inside the round hole above the label "LCD CONTRAST ADJUST" is the adjusting potentiometer. Insert a small flat-bladed screwdriver into the round hole and carefully turn the adjusting screw to increase or decrease the menu display contrast.

3.4 Setting Up the VersaFluor Thermal Printer

The VersaFluor Thermal Printer, Model DPU-414 is ready for use with the VersaFluor Fluorometer. The procedure for setting up the printer for the first time is described below. Please refer to the Thermal Printer manual for more information.

Thermal Printer

1. Turn off the printer power.

2. Insert the DC plug on the AC adaptor into the power supply jack on the printer.

3. Plug the AC adaptor into an outlet.

4. Attach the serial cable adaptor to the serial cable.

5. Connect the 9-pin end of the serial cable to the serial port located on the back printer. Connect the 25-pin end of the serial cable to the serial port located on the back of the VersaFluor Fluorometer.

6. Load the thermal paper into the printer (Refer to the Printer manual).

7. The printer DIP switches must be set in the printer so that the VersaFluor Fluorometer can communicate with the printer. Slide the power switch to ON while pressing the ONLINE button. Release the ONLINE button and a list of the current settings will be printed.

8. The print out of the current settings is followed by the prompt:

    "Continue? : Push On-line SW"

    "Write? : Push paper feed SW"

    Push the ONLINE button to continue.

9. Dip SW1 is printed, prompting the input of new settings for switch numbers 1–SW1. ON can be set by pushing the ONLINE button once. OFF can be set by pushing the FEED button once.

    Note: Always input either ON or OFF for every setting in order for switch number 1 through 8 because DIP SW Set Mode can not be canceled once it is initiated.

    The setting is printed after the ONLINE or FEED button is pushed, confirming the new setting. As soon as switch number 8 is set, the printer will prompt with "Continue?" or "Write?" and steps 8 and 9 are repeated for DIP SW2 and DIP SW3. When switch number 8 of DIP SW3 is set, the printer writes the settings to memory regardless of which button is pushed.

    Caution: Never turn the printer off while it is writing the new settings to memory.

5

Below are the DIP switch settings to use with the VersaFluor Fluorometer. Please refer to the printer manual for full descriptions of the DIP switch settings.

DIP SW1

Switch No.	Action	Setting
1	Press FEED	(OFF)
2	Press ONLINE	(ON)
3	Press ONLINE	(ON)
4	Press FEED	(OFF)
5	Press ONLINE	(ON)
6	Press FEED	(OFF)
7	Press ONLINE	(ON)
8	Press ONLINE	(ON)

DIP SW2

Switch No.	Action	Setting
1	Press ONLINE	(ON)
2	Press ONLINE	(ON)
3	Press ONLINE	(ON)
4	Press ONLINE	(ON)
5	Press ONLINE	(ON)
6	Press ONLINE	(ON)
7	Press ONLINE	(ON)
8	Press FEED	(OFF)

DIP SW3

| Switch No.	Action	Setting
1	Press ONLINE	(ON)
2	Press ONLINE	(ON)
3	Press ONLINE	(ON)
4	Press FEED	(OFF)
5	Press FEED	(OFF)
6	Press ONLINE	(ON)
7	Press ONLINE	(ON)
8	Press ONLINE	(ON)

VersaFluor Fluorometer

The baud rate and flow control parameters is set on the VersaFluor Fluorometer in order to communicate with the thermal printer. Set the baud rate to 9600 and the flow control to XON. See Section 4.8 for serial port function and parameters.

Section 4
Introduction

4.1 Fluorometer System

The VersaFluor Fluorometer measures the concentration of various samples (analytes) by fluorescence. By labeling a molecule with a fluorescent dye, the molecule can be accurately quantitated by measuring the amount of fluorescence. A fluorescent molecule has the ability to absorb energy at particular wavelengths and emit this energy at longer wavelengths. Fluorescent dyes are classified by their excitation and emission wavelengths. Figure 4.1 shows a typical excitation and emission spectrum. The excitation curve is at a shorter wavelength than the emission curve. It is desirable to have no overlap between the two curves, insuring that all the energy in the emission spectrum is a result of fluorescence.

The excitation and emission spectrums vary from dye to dye. The VersaFluor Fluorometer has the capability to work with various dyes by using removable optical filters which select for the specific excitation and emission wavelengths of a particular dye.
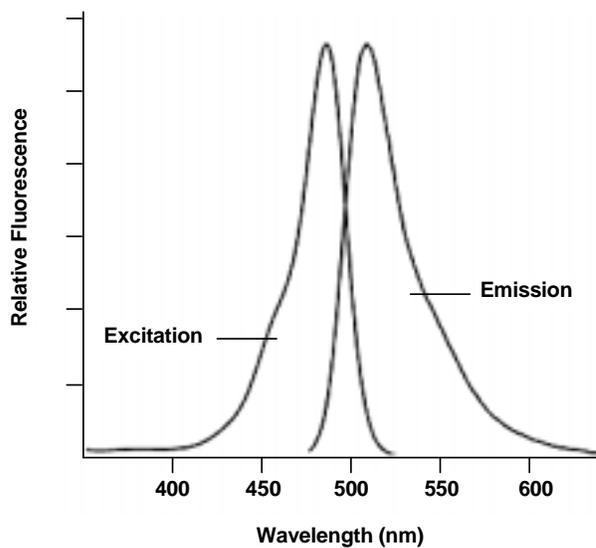
Fig. 4.1. Excitation and emission spectra of fluorescein.

For example, fluorescein has an excitation maxima at 490 nm and an emission maxima at 520 nm (Figure 4.1). The EX490/10 excitation filter and the EM520/10 emission filter, both of which have a 10 nm band pass, are used with fluorescein. The excitation filter allows only light between 485 and 495 nm to pass through the sample. The emission filter removes the excitation light and allows only the emitted light with a wavelength between 515 and 525 nm to reach the detector.

The optical system of the VersaFluor Fluorometer is diagrammed in Figure 4.2. The quartz halogen lamp emits light in the 350 nm to 900 nm range. Light emitted by the quartz halogen lamp passes through a small aperture in the lamp housing before passing through the excitation filter. The excitation filter allows only specific wavelengths of light to pass through.
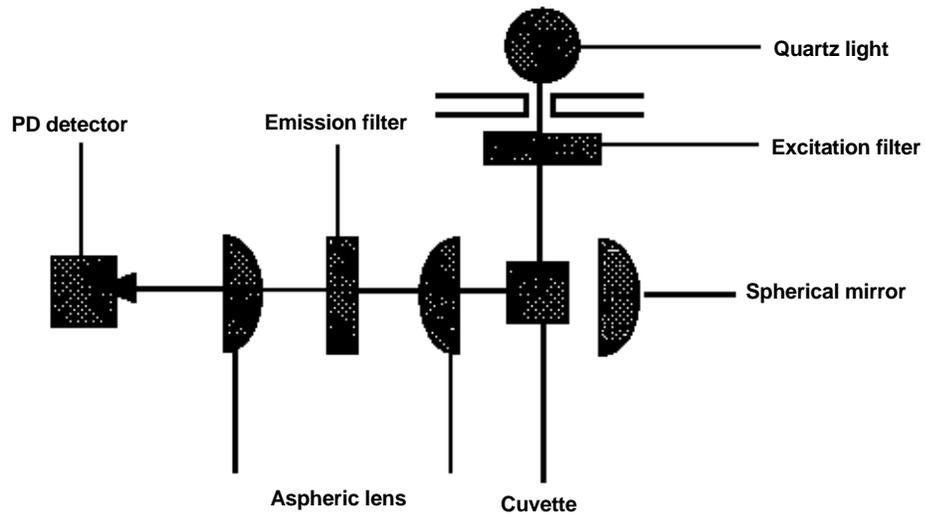
Fig. 4.2. Optical system.

Emitted fluorescent light from the cuvette is focused by the spherical mirror and passes through the first aspheric lens. The emission filter allows light specific to the fluorescent compound to reach a second aspheric lens which directs the fluorescent light to the photodiode detector. The detector system is a broad spectrum photodiode with analog circuitry to amplify the signal. The amplified analog signal is converted into a digital signal and processed by the controlling microprocessor before being sent to the fluorescence units display. The number in the Fluorescence Units Display (Section 2.2) represents the relative fluorescent units (RFU).

4.2 Operation Menu Overview

The VersaFluor Fluorometer is operated by selecting the menu of choice and pressing one of the push buttons to perform a desired action. Each menu gives the user a different set of choices. Figure 4.3 shows the menu flow diagram of the instrument.

```
                                    ┌──────────────────────────────┐
                                    │        RANGE  SET            │
                                    │ PRINT 00000   ZERO   SETUP   │
                                    └──────────────────────────────┘

┌──────────────────────────────┐
│ SET FULL RANGE:      00000    │
│  UP    DOWN    —>    EXIT      │
└──────────────────────────────┘

                                    ┌──────────────────────────────┐
                                    │ AVRG    GAIN                 │
                                    │ 1 SEC   LOW    MORE   EXIT   │
                                    └──────────────────────────────┘

                                    ┌──────────────────────────────┐
                                    │ PRINT   TIMED                │
                                    │ SETUP   SETUP   MORE   EXIT  │
                                    └──────────────────────────────┘

┌──────────────────────────────┐
│ BAUD  CNTL   PRINT            │
│ 9600  X-ON   SPACES   EXIT    │
└──────────────────────────────┘

                                    ┌──────────────────────────────┐
                                    │ CLOCK   LAMP  SAMPLE         │
                                    │ SETUP   ON    SET#1   EXIT   │
                                    └──────────────────────────────┘

                                    ┌──────────────────────────────┐
                                    │ 07/01/97            09:50:39 │
                                    │ UP     DOWN    —>     EXIT    │
                                    └──────────────────────────────┘

                                    ┌──────────────────────────────┐
                                    │ TIMED    SET                 │
                                    │ START    INTVL        EXIT   │
                                    └──────────────────────────────┘

┌──────────────────────────────┐
│ TIMED            COUNT 001    │
│ STOP                          │
└──────────────────────────────┘

                                    ┌──────────────────────────────┐
                                    │ INTVL  01 SEC   COUNT 001    │
                                    │ UP     DOWN    —>     EXIT    │
                                    └──────────────────────────────┘
```
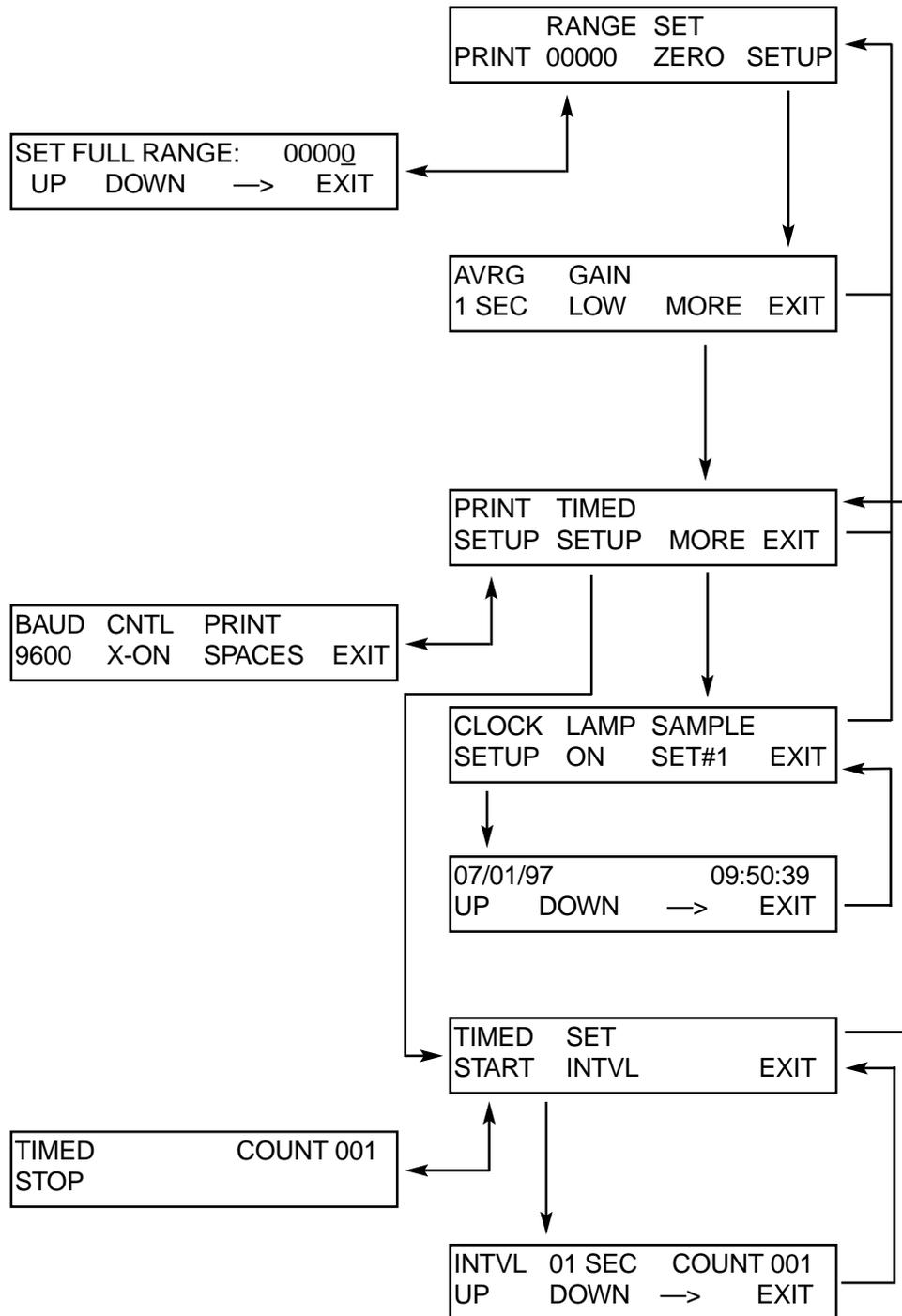
Fig. 4.3. Menu flow diagram.

4.3 Gain Function

Three gain settings are available in the VersaFluor Fluorometer instrument: LOW, MED, and HIGH gain. There is a ten-fold difference between the gain settings. For example, if the fluorometer shows a reading of 10,000 RFU on high gain, it will show approximately 1,000 RFU on medium gain and approximately 100 RFU on low gain. The reading range of each gain setting is between 0 and 19,999 RFU. If the reading is beyond the range of the instrument, the fluorescence display will show 19,999.

A quick method for determining which gain setting to use is to first set the gain to MED with the range at 00000. Place a sample with the highest concentration into the instrument and read the fluorescence display. If the value is below 19,999, then use MED or HIGH gain. If the value is 19,999, use LOW gain. Some trial and error work may be necessary to determine the correct gain setting to use.

The user can change gain setting at any time during the sample measurement procedure. It is recommended to check the zero of the instrument and re-zero if necessary, if the gain is changed while reading samples. If the samples are read from the LOW gain setting to the MED gain setting, the MED gain relative fluorescence units should be divided by 10 when compared to the LOW gain setting units.

To change the gain setting, press the SETUP button in the main menu. Press the GAIN button to select the gain setting (Figure 4.4). The gain can be toggled between LOW, MED, and HIGH.
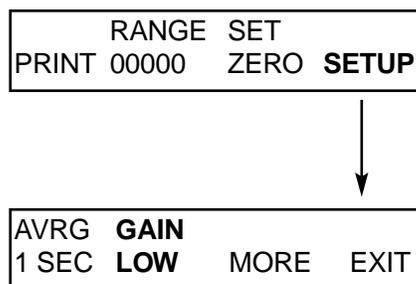
```
          RANGE  SET
PRINT  00000     ZERO  SETUP
```

```
AVRG   GAIN
1 SEC  LOW     MORE   EXIT
```

Figure 4.4 Gain setting menus.

4.4 Range Function

The fluorescence units shown on the fluorescence display can be set to a desired value. The maximum value for the instrument is 19,999 RFU. Adjusting the range will change the current maximum instrument reading to a different value set by the user. For example, a sample shows a reading of 4,000 RFU on MED gain, but the user would prefer to see 2,000. Setting the range to 2,000 will change the current maximum reading to 2,000, and all future values will also be scaled to the new range. It is important to set a new range value whenever a new fluorescent assay is being performed, because the fluorescent values will be scaled according to the last sample used to set the range.

Setting the range higher than the amount of fluorescence detected by the fluorometer may cause some fluctuation in the fluorescence units display. For example, a sample displays a reading of 1,000 RFU on MED gain, but the range is set to 10,000. There could be a fluctuation in the display by as much as 10 units (background instrument noise times the range factor of 10).

To set the range of the instrument to a desired value, place the highest concentration sample cuvette into the cuvette holder. Press RANGE on the main menu to access the range menu (Figure 4.5). Use the arrow button (—>) to move the cursor along the five digit number. Use the UP and DOWN buttons to raise and lower the value. Press the EXIT button to return to the main menu. The fluorescence display will read the set value.
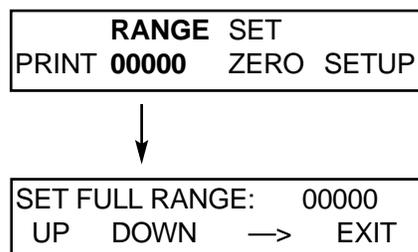
```
┌─────────────────────────────────┐
│          RANGE  SET             │
│  PRINT  00000    ZERO  SETUP    │
└─────────────────────────────────┘
              │
              ▼
┌─────────────────────────────────┐
│  SET FULL RANGE:    00000       │
│    UP    DOWN    —>    EXIT      │
└─────────────────────────────────┘
```

Figure 4.5 Setting the Range menus.

4.5 Set Zero Function

The VersaFluor Fluorometer can be zeroed by placing the baseline cuvette (cuvette with baseline fluid) into the cuvette holder, closing the sample compartment lid, and pressing the SET ZERO button on the main menu (Figure 4.6). The fluorescence display should read 0 ± 5 units after zeroing. Always zero the instrument with a range setting of 00000 before setting the range value with the highest concentration sample.

```
┌─────────────────────────────────┐
│          RANGE  SET             │
│  PRINT  00000    ZERO  SETUP    │
└─────────────────────────────────┘
```

Fig. 4.6. Set Zero menu.

4.6 Print Averaging Function

The menu item AVRG is used to apply averaging to the samples before printing. When the PRINT button is pressed, the instrument takes sample readings for a specified period of time and averages them. The menu item AVRG determines the duration of the averaging. The average value is sent to the printer. When AVRG is set to 1 second, the instrument reads 1 second worth of samples and averages it. Changing the AVRG to 2 and 4 seconds changes the length of time over which samples are averaged. The averaging function does not affect the instrument's internal sample filtering process which automatically averages the data.

To access print averaging, press the SETUP button in the main menu (Figure 4.7). Press the AVRG button to select the sample averaging time. Print averaging time can be toggled between 1, 2, or 4 seconds.
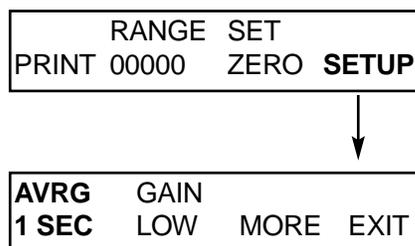
```
        RANGE  SET
PRINT 00000   ZERO  SETUP
```

↓

```
AVRG    GAIN
1 SEC   LOW    MORE  EXIT
```

Fig. 4.7. Print averaging menus.

4.7 Setting the Clock

The date and time information is sent to the printer or computer when the fluorometer is turned on. This information is also sent to the serial port during timed or kinetic reading. Press the CLOCK SETUP button from the third menu to access the clock setup parameters (Figure 4.8). Use the arrow (—>) to scroll between the date and time. Use the UP and DOWN buttons to raise and lower the values. Press EXIT to leave this menu.

```
CLOCK  LAMP  SAMPLE
SETUP  ON    SET#1   EXIT
```

↓

```
07/01/97           09:50:39
UP    DOWN     —>      EXIT
```
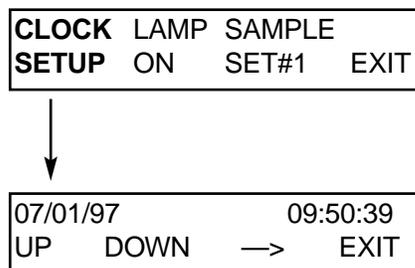
Fig. 4.8. The date and time menus.

4.8 Serial Port Functions

The VersaFluor Fluorometer has the ability to communicate with an external printer or computer via the serial port located at the back of the instrument. The VersaFluor Fluorometer sends the data to these peripherals as ASCII text. The VersaFluor Thermal Printer, Model DPU-414 can be used with the VersaFluor Fluorometer (see Section 3.4 for printer set-up). When connecting the VersaFluor to an external printer (not the VersaFluor printer) or computer, a "null-modem" cable should be used. The correct pin-out for this cable is described on the next page. Check the printer or computer for the correct serial port connector end (male or female).

DB25F Connector to VersaFluor (Male)	Signal and Direction	DB25M Connector to serial device
2	TX - - >	3
3	< - - RX	2
4	RTS - - >	5
5	< - - CTS	4
7	Common	7
20	DTR - - >	20
(other pins are open in VersaFluor)		

The serial port can be reconfigured by using print setup command (Figure 4.9). The three parameters that can be configured are the baud rate, flow control, and the data spacing. To access the print parameters, press SETUP in the main menu, then press MORE in the second menu, and finally press PRINT SETUP in the third menu (Figure 4.3). The serial port baud rate can be changed by pressing the BAUD button. The baud rates settings are NONE, 1200, 2400, 4800, or 9600. The data flow control can be set to XON or R/CTS by pressing the CNTL button. The print spacing can be set to NONE, SPACES, or TABS. Refer to Section 3.4 for setting up the VersaFluor thermal printer. For other printers or computers, please refer to the instrument's instruction manual for correct settings.
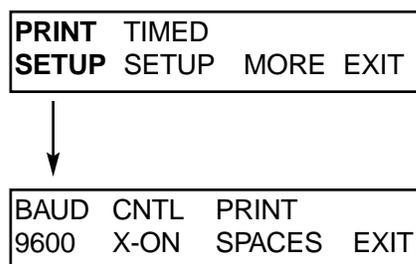
```
PRINT  TIMED
SETUP  SETUP   MORE  EXIT
```

```
BAUD  CNTL   PRINT
9600  X-ON   SPACES   EXIT
```

Fig. 4.9. Serial port menus.

4.9 Kinetic Readings

The VersaFluor Fluorometer supports kinetic studies via timed data sampling. The user can set the number of readings and the intervals between readings. Each time a reading is taken, the value is shown on the fluorescence display. The value and date/time information is also sent to the serial port for printing or downloading to a computer database.

To access the kinetic or timed reading parameters, press SETUP in the main menu, then press MORE in the second menu, and finally press TIMED SETUP (Figure 4.3). To set the timed reading parameters press the SET INTVL button. The timed interval can be in seconds (SEC) or minutes (MIN). The number of readings can be set in the COUNT area. Use the arrow button (—>) to scroll between time and counts. Use the UP and DOWN buttons to raise or lower each value. The example in Figure 4.10 shows that readings will occur every 5 seconds, 15 times (a total of 75 seconds). Press the EXIT button when the timed reading parameters are set.
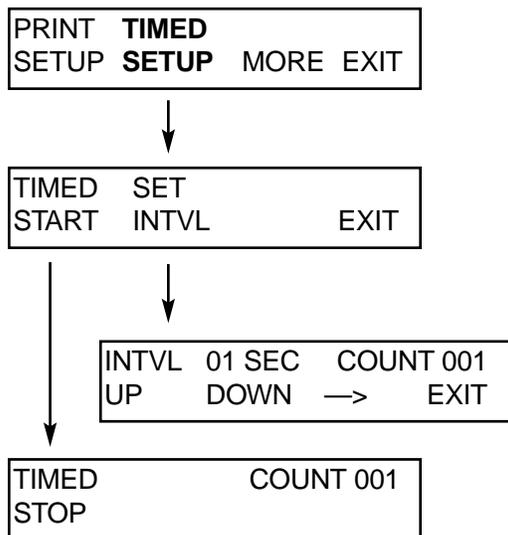
```
┌──────────────────────────────────────┐
│PRINT   TIMED                          │
│SETUP   SETUP    MORE   EXIT           │
└──────────────────────────────────────┘
                  │
                  ▼
┌──────────────────────────────────────┐
│TIMED   SET                            │
│START   INTVL              EXIT        │
└──────────────────────────────────────┘
        │         │
        │         ▼
        │  ┌──────────────────────────────────┐
        │  │INTVL  01 SEC    COUNT 001         │
        │  │UP     DOWN    —>      EXIT        │
        │  └──────────────────────────────────┘
        ▼
┌──────────────────────────────────────┐
│TIMED               COUNT 001          │
│STOP                                   │
└──────────────────────────────────────┘
```

Fig. 4.10. Timed reading menus.

To start the timed readings, press the TIMED START button. An active timed reading menu will appear. To stop the readings at any time, press the TIMED STOP button. This screen will remain active until all the readings are finished. The COUNT value will increase sequentially as the readings progress. Each time a reading is taken, the relative fluorescence units will be displayed and sent to the serial port. When the last reading is taken, the menu display will automatically return to the TIMED START and SET INTVL screen.

4.10 Lamp ON/OFF

The lamp on the fluorometer can be turned on or off. To access this function, press SETUP in the main menu, then press MORE in the second menu, and finally press MORE in the third menu (Figure 4.3). Press the LAMP button to turn the lamp ON or OFF (Figure 4.11). Press EXIT to leave this menu. After turning the lamp back on, let the instrument warm up for at least 10 minutes.

```
┌──────────────────────────────────────┐
│CLOCK   LAMP  SAMPLE                   │
│SETUP   ON    SET#1    EXIT            │
└──────────────────────────────────────┘
```

Fig. 4.11. Lamp ON/OFF menu.

4.11 Resetting the Print Sample Number

When the PRINT button is pushed, the fluorometer sends the fluorescence data and a sample number to the printer. This sample number increases sequentially every time the PRINT button is pushed. The SAMPLE SET#1 button must be pushed to reset the sample number to one. To access this function, press SETUP in the main menu, then press MORE in the second menu, and finally press MORE in the third menu (Figure 4.3). Press the SAM-PLE SET#1 button to reset the sample number (Figure 4.12). The menu will display 00001, indicating the sample number has been reset. Press EXIT to leave this menu.

```
CLOCK  LAMP  SAMPLE
SETUP  ON    SET#1   EXIT
```

Fig. 4.12. Sample number reset menu.

Section 5
Operating Instructions

5.1 Quick Guide Procedure

This section describes the typical procedure for taking fluorescence measurements. Menu options are described in Section 4. A typical sample measurement requires five steps:

Note: The instrument should warm up for at least 20 minutes before taking any readings.

1. **Selection and insertion of two optical filters.**

   Insert the emission and excitation filters into the VersaFluor Fluorometer.

2. **Set the gain.**

   Press the SETUP button on the main menu and set the gain to LOW, MED, or HIGH.

3. **Zero the instrument.**

   Set the range to 00000 by pressing the RANGE button. Adjust the range to 00000. Press EXIT. Place the baseline sample cuvette in the cuvette chamber and press the SET ZERO button. When blinking stops the fluorescence display should read 0 ± 5. If not, re-zero the instrument by pressing the SET ZERO button.

4. **Set the range.**

   Set the range by placing a cuvette containing the highest concentration standard in the cuvette holder and closing the sample compartment lid. Wait approximately 10 seconds for the detector to adjust to the light conditions. Press the RANGE button. Adjust the range to the desired setting. The maximum setting is 19,999 RFU. Press EXIT.

5. **Measure the sample.**

   Place a sample cuvette in the cuvette holder and close sample compartment lid. Wait approximately 10 seconds for the detector to adjust to the light conditions. Record or print the RFU value in the fluorescence display. Read all remaining samples.

5.2 Instrument Parameter Set-Up

1. The VersaFluor Fluorometer instrument must warm up for 20 minutes prior to use. If the instrument is not fully warmed up, the reading may be irreproducible.

2. Select an excitation and emission filter which are to be inserted in the filter holders. Orient the filters so that the notched groove on the filter holder faces the cuvette holder.

    Caution: Use filters which are tailored to the sample being measured. Using incorrect filters will greatly degrade the accuracy of the fluorescence measurement.

3. Press the SETUP button on the main menu to access the print averaging and gain settings.

4. Press the AVRG to select the print averaging time (1, 2, or 4 seconds).

5. Press GAIN to select the gain setting (LOW, MED, or HIGH). Then press EXIT to return to the main menu.

6. Add the standards, unknown samples, and blanks to the disposable cuvettes.

7. Follow the sample measurement guidelines below to insure accurate results:

    • Warm up the instrument for at least 20 minutes.

    • Always use calibrated pipets to insure accurate pipetting.

    • Mix standards and sample completely by using a disposable transfer pipet.

    • Remove any air bubbles in the cuvettes.

    • Hold fluorometer cuvettes by the upper edges since the cuvettes have four optically clear sides.

    • Clean the cuvette sides with a lint-free tissue.

    • Read all standards and samples at ambient temperature.

    • While taking a reading, keep the samples in the fluorometer only. This helps reduce sample photobleaching.

5.3 Setting Up a Standard Curve

The range should be set on the fluorometer with a known standard and a fluorescent dye. Depending on the protocol used, a single-point or multi-point standard curve is required.

1. Set the range of the instrument to 00000 by pressing the RANGE button. Adjust the range to 00000. Press EXIT.

2. Zero the instrument by placing the baseline cuvette (blank) in the cuvette holder and closing sample compartment lid. Wait approximately 10 seconds for the detector to adjust to the light conditions. Press the SET ZERO button; when blinking stops, read the fluorescence display. If fluorescence display is 0 ± 5, the instrument is zeroed. If not, re-zero the instrument by pressing the SET ZERO button.

3. Set the range of the instrument by placing the highest concentration standard cuvette in the cuvette holder and closing the sample compartment lid. Press the RANGE button. Adjust the range to the desired setting. The maximum setting is 19,999 RFU. Press EXIT. The fluorescence display should read the set range value. Read or print the RFU number in the fluorescence display. Remove the highest concentration standard cuvette.

Optional: The zero of the instrument can be rechecked by placing the baseline cuvette (blank) in the cuvette holder. Wait approximately 10 seconds for the detector to adjust to the light conditions. If the instrument needs to be re-zeroed, press the SET ZERO button. When blinking stops, the fluorescence display should read 0 ± 5.

4. For multiple standard samples, read the remaining samples. Wait approximately 10 seconds for the detector to adjust to the light conditions. Read or print the RFU number in the fluorescence display. The value shown on the fluorescence display is a measure of the difference between the baseline sample and the highest concentration standard.

   Note: Fluorescence measurements can be made continuously. It is not necessary to zero the instrument before each measurement unless the baseline conditions change. Check the zero of the instrument with the baseline fluid (blank) when switching between gain settings.

5.4 Reading Unknown Samples

1. Place a cuvette with an unknown sample in the cuvette holder and close the sample compartment lid. Wait approximately 10 seconds for the detector to adjust to the light conditions. Read or print the RFU number in the fluorescence display.

2. Repeat for all remaining unknown samples.

   Note: Fluorescence measurements can be made continuously. It is not necessary to zero the instrument before each measurement unless the baseline conditions change. Check the zero of the instrument with the baseline fluid (blank) when switching between gain settings.

3. The sample concentrations can be determined by comparing its RFU values with the standard(s).

Section 6
Troubleshooting

6.1 Troubleshooting Table

Problem	Cause	Solution
No display with power on	Burned out fuse	Replace fuse.
No RFU values with samples	Lamp OFF	Insure that the lamp is ON.
	Lamp burned out	Replace lamp.
	Thermal fuse tripped	Call Bio-Rad Service.
Lamp OFF with power ON	Lamp OFF	Insure that the lamp is ON.
	Thermal fuse tripped	Call Bio-Rad Service.
Negative values	Readings below set zero (blank)	1. Zero the instrument with the range set to 00000. Set the range with the highest concentration standard.
		2. Zero between gain settings.
Readings fluctuate	Interference in light path	1. Check that there are no scratches on the cuvette.
		2. Check that there are no particulates floating in the cuvette.
		3. Wipe the cuvette walls with a lint-free tissue.
		4. The instrument reading may fluctuate somewhat at high gain setting with low concentration samples.
		5. Insure that the gain and range have been set properly (Sections 4.3 and 4.4).

6.2 Performance Check—Internal Diagnostics

Under normal conditions, the VersaFluor Fluorometer will operate for many years with minimal maintenance. If the instrument is operating, but a problem is suspected, run the performance check.

1. The VersaFluor Fluorometer instrument can be tested by internal diagnostics which verify correct operation of the electronics. With the sample compartment door closed, the internal diagnostics are accessed by holding down any of the four buttons while turning on the instrument. The diagnostic display below will be shown.

```
       DIAGNOSTICS
*****  MED    .06FS  LAMP
```

2. The three right-hand push buttons are used to operate the diagnostics. Starting from the right, the first push button turns the lamp on or off, the second selects the voltage to be inserted into the instrument, and the third selects the amplifier gain. The diagnostics are restricted to testing the analog electronics and digital processing, which includes post amplification, gain changes, A/D conversion, digital filtering, and the LCD display. One of three fixed voltages is inserted into the analog amplifiers just following the photodiode (PD) detector and preamplifier.

3. Two different values can be changed through the diagnostic routines: the voltage setting and the gain setting of the analog amplifier. The voltage can be set to either 0, .006FS, or .06FS. The analog amplifier gain can be set to LOW, MED, or HIGH.

4. All diagnostic testing is performed with the lamp turned off. The lamp can be turned on or off by pressing the button marked LAMP. To determine if the lamp is on or off, inspect the cuvette holder. If the lamp is on, light will be visible inside the cuvette holder.

5. With the voltage set to 0, the reading on the LCD is the background noise present in the analog electronics, it will be different for each gain setting. The other two voltages (.006FS and .06FS V) are typical of what the electronics would experience during normal operation.

6. To determine correct instrument operation, record all nine values and subtract the respective noise (0 reading) from the .006FS and the .06FS readings. Use the table below to record the values.

	0	.006FS	.06FS
LOW Gain			
		-	-
		=	=
MED Gain			
		-	-
		=	=
HIGH Gain			
		-	-
		=	=

7. The resulting six numbers will be used to determine if the instrument electronics and digital processing are working correctly. The following table lists the expected values and the tolerance of each value.

Gain	.006FS	.06FS
LOW	12 ± 5	125 ± 50
MED	125 ± 25	$1,250 \pm 250$
HIGH	$1,250 \pm 200$	$12,500 \pm 2,000$

8. If the calculated values are within the ranges given above, the analog electronics and digital processing is operating correctly. If the instrument fails this test, contact your local Bio-Rad service representative.

Section 7
Maintenance

7.1 Fuse Replacement

1. The fuses for the VersaFluor Fluorometer instrument are located inside the power input module on the back of the instrument (Figure 2.1). Turn the instrument off and unplug the power cord before attempting to replace the fuses.
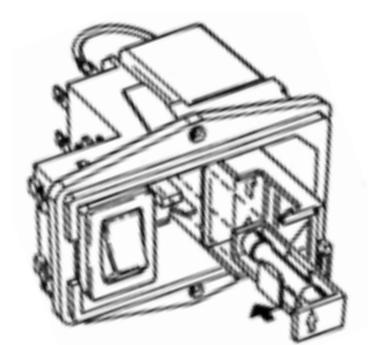

Fig. 7.1. Fuse replacement.

2. To remove the fuses; carefully pry the cover open using a small screwdriver. Inside the power input module, there are two fuse carriers each containing a 5 x 20 mm fuse. Carefully slide the fuse carriers out and remove the burned-out fuses. Replace with new fuses.

   Warning: Replace fuses with the current rating of 1.6 ampere, slow blow, T-type.

3. With new fuses in place, slide the fuse carriers into the power input module and snap the cover shut.

7.2 Lamp Replacement

1. Turn the instrument off and remove the power cord. Allow the lamp to cool for 5–10 minutes.
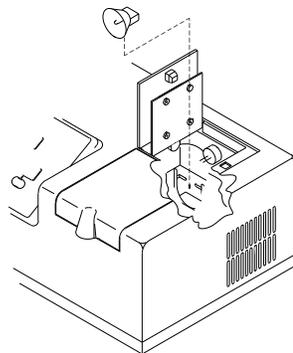
Fig. 7.2. Lamp replacement.

2. Locate the fastener on the lamp cover. Carefully rotate the fastener 1/4 turn using a coin or screwdriver. Open the lamp cover. The lamp is located at the bottom of the lamp housing.

3. The lamp base has two prongs which push into a socket at the back of the lamp housing. To remove the lamp, grasp it firmly at the base and pull it straight up.

   Caution: Never touch the lamp with bare fingers. Quartz halogen lamps can be destroyed by the oil from skin. Use a tissue or soft cloth to remove the lamp. If the lamp is contaminated with oil or dirt, clean with a dry soft cloth.

4. To install a new lamp, slide the new lamp into the socket on the base of the lamp housing. Close the lamp cover and push the fastener to lock.

5. Turn the instrument on and inspect the sample compartment to ensure that the lamp is lit.

7.3 Cleaning Up a Fluid Spill

The fluid handling system, an integral part of the VersaFluor Fluorometer, provides easy cleanup in case of spills. By using a special purpose optical bench and a drain pan, the VersaFluor Fluorometer is able to minimize the delay caused by fluid spills (< 2 ml). If a spill is apparent, no electrical hazard is created because the fluid is contained by the fluid handling system, a collection of components designed to hold and manage fluid spills.

1. Turn the instrument off and unplug the power cord.

2. To clean up spills, open the sample compartment and remove the cuvette holder by pressing the two tabs inward and pulling upward. Remove the two filters.

3. Using a clean cotton swab or cloth, dry all three cavities: the excitation filter slot, the emission filter slot, and the cuvette holder cavity. Insure the glass lenses and mirror are dry. Do not apply excessive pressure on the lenses and mirror while cleaning. Dry the entire cuvette holder and filters.

4. Replace the cuvette holder and filters into the sample compartment.

5. Since fluid can accumulate in the drain pan, two drainage tubes are provided to allow overflow fluid to exit the instrument. The overflow fluid will drain out the bottom of the instrument. Clean up any fluid under the instrument. A few drops of fluid may continue to leak out the bottom of the instrument after a spill. Check the area under the instrument periodically for any signs of spills.

Section 8
Filters for Fluorescent Protocols

Application	Excitation Filters	Catalog Number	Emission Filters	Catalog Number
DNA Quant (Hoechst 33258)	EX 360/40 (340–380 nm)	170-2420	EM 460/10 (455–465 nm)	170-2421
ß-Galactosidase (MUG)	EX 360/40 (340–380 nm)	170-2420	EM 460/10 (455–465 nm)	170-2421
ß-Glucuronidase (MUGluc)	EX 360/40 (340–380 nm)	170-2420	EM 460/10 (455–465 nm)	170-2421
Apopain (Z-DEVD-AFC)	EX 360/40 (340–380 nm)	170-2420	EM 520/10 (515–525 nm)	170-2424
pH indicator (BCECF)	EX 490/10 (485–495 nm)	170-2422	EM 520/10 (515–525 nm)	170-2424
Calcium indicator (Fluo-3)	EX 490/10 (485–495 nm)	170-2422	EM 520/10 (515–525 nm)	170-2424
DNA Quant (PicoGreen™)	EX 480/20 (470–490 nm)	170-2427	EM 520/10 (515–525 nm)	170-2424
Oligo Quant (OliGreen™)	EX 480/20 (470–490 nm)	170-2427	EM 520/10 (515–525 nm)	170-2424
Protein Quant (NanoOrange™)	EX 480/20 (470–490 nm)	170-2427	EM 590/10 (585–595 nm)	170-2425
Fluorescein	EX 490/10 (485–495 nm)	170-2422	EM 520/10 (515–525 nm)	170-2424
Ethidium Bromide	EX 510/10 (505–515 nm)	170-2423	EM 620/10 (615–625 nm)	170-2426
RNA Quant (RiboGreen™)	EX 490/10 (485–495 nm)	170-2422	EM 520/10 (515–525 nm)	170-2424

Section 9
Specifications

Instrument

Light source	20 watts incandescent quartz halogen 350 nm to 900 nm. The quartz halogen lamp is rated to operate for 2000 hours.
Sensing device	Photodiode
Optical filters	Excitation 1" diameter with holder
	Emission 1" diameter with holder
Sample source	Cuvette: 12.5 mm x 12.5 mm (outside dimension)
Sensitivity selection	Three settings: LOW, MED, and HIGH. Approximate factor of 10 between each setting.
Dynamic range	Greater than 5 orders using all three sensitivity selections.
Printer output	RS-232, Serial

AC Power Requirement

AC power input	100–240 VAC 50–60 Hz, 1.6 A slow-blow fuse

Size and Weight

Overall size	23 cm (L) x 33 cm (W) x 15 cm (H)
Shipping weight	4 kg

Environmental Requirements

Storage environment	0 to 70 °C, humidity 0–95% (non-condensing)
Operating environment	20 to 35 °C, humidity 0–95%

Regulatory

Meets the requirements of:	EN61010-1, EN50082-1, EN55011/03

Section 10
Systems, Accessories, and Reagents for VersaFluor Fluorometer

Catalog Number	Product Description
VersaFluor Fluorometer	
170-2402	**VersaFluor Fluorometer 100/120/220**, includes a standard cuvette holder, 100 standard disposable cuvettes, and one excitation and one emission filter
170-2403	**VersaFluor Fluorometer 120V**, includes everything listed in 170-2402 plus a 120V thermal printer and cable
170-2404	**VersaFluor Fluorometer 100V**, includes everything listed in 170-2402 plus a 100V thermal printer and cable
Optical Excitation Filters	
170-2420	**EX360/40 (340–380 nm)**
170-2427	**EX480/20 (470–490 nm)**
170-2422	**EX490/10 (485–495 nm)**
170-2423	**EX510/10 (505–515 nm)**
Optical Emission Filters	
170-2421	**EM460/10 (455–465 nm)**
170-2424	**EM520/10 (515–525 nm)**
170-2425	**EM590/10 (585–595 nm)**
170-2426	**EM620/10 (615–625 nm)**
VersaFluor Thermal Printer	
170-2409	**100V VersaFluor Thermal Printer,** with cable and 100V AC adaptor
170-2410	**120V VersaFluor Thermal Printer,** with cable and 120V AC adaptor
170-2411	**220V VersaFluor Thermal Printer,** with cable and 220V AC adaptor
170-2412	**Thermal Printer Paper,** 10 rolls
170-2413	**Thermal Printer Universal Battery pack**
VersaFluor Disposable Cuvettes	
170-2415	**Standard Cuvette**, 12.5 x 12.5 mm (outside dimension) 4-sided optically clear disposable, polycarbonate, 3.5 ml, 100
Accessories	
170-2406	**Standard Cuvette Holder**
170-2430	**Quartz Halogen Replacement Light Bulb**
170-2450	**Optical Filter Storage Case**

Catalog Number	Product Description
Fluorescent Reagents	
170-2480	**Fluorescent DNA Quantitation Kit,** Hoechst 33258
170-2481	**Hoechst 33258 (bisbenzimide) Dye**
170-3130	**FluorAce™ Apopain Assay Kit**
170-3150	**FluorAce™ ß-Galactosidase Reporter Assay Kit**
170-3151	**FluorAce™ ß-Glucuronidase Reporter Assay Kit**
170-3141	**Fluo-3 Fluorescent Calcium Indicator Kit**
170-3142	**Fluo-3 AM**
170-4143	**Fluo-3 K+ Salt**
170-4134	**4-Br-A23187**
170-3145	**Calcium Calibration Buffer**
170-3146	**pH Indicator (BCECF-AM)**
170-3147	**pH Indicator (BCECF Acid)**

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