*Pico*fluor™

Handheld Fluorometer

Operating Manual

Part Number: 998-0853
Version: 1.5

TURNER BIOSYSTEMS

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1 Introduction

1.1 Description

The *Pico*fluor™ is a dual-channel fluorometer designed for fast, reliable and accurate fluorescence measurements. When properly calibrated with a known standard, the *Pico*fluor™ can be used to determine concentrations of unknown samples.

1.2 General Information and Precautions

- The *Pico*fluor™ readily accepts methacrylate or polystyrene cuvettes. Do not use glass or quartz cuvettes.
- The Sample Compartment cannot accept cuvettes with an outer diameter over 12 mm. The 10 mm size refers to the internal diameter of the cuvette so some plastic cuvettes may also be too large.
- Do not force oversized cuvettes into the Sample Compartment. This can damage the Sample Compartment. If the cuvette does not easily fit into the Sample Compartment, use a different cuvette.
- Use caution around solvents as they may corrode the plastic case of the *Pico*fluor™.
- If a liquid is accidentally spilled inside the Sample Compartment, invert the *Pico*fluor™ to drain the excess liquid. Then wipe the inside area dry with a clean soft towel or tissue.
- If extra cleaning is needed, use a mild detergent to dampen the towel and wipe.
- Do not submerge the *Pico*fluor™ in water.
- Do not expose the *Pico*fluor™ to temperatures outside the specified range of 5 ^{o}C to 40 ^{o}C. Damage may occur to the unit and will not be covered under warranty.
- A minimum volume of 2 ml in a 10 x 10 mm cuvette is required for best results.
- The Minicell Adapter requires a minimum volume of 75 ml and a maximum volume of 250 ml.
- Avoid having any air bubbles in your sample as they can significantly affect the fluorescence reading.

1.3 Inspection

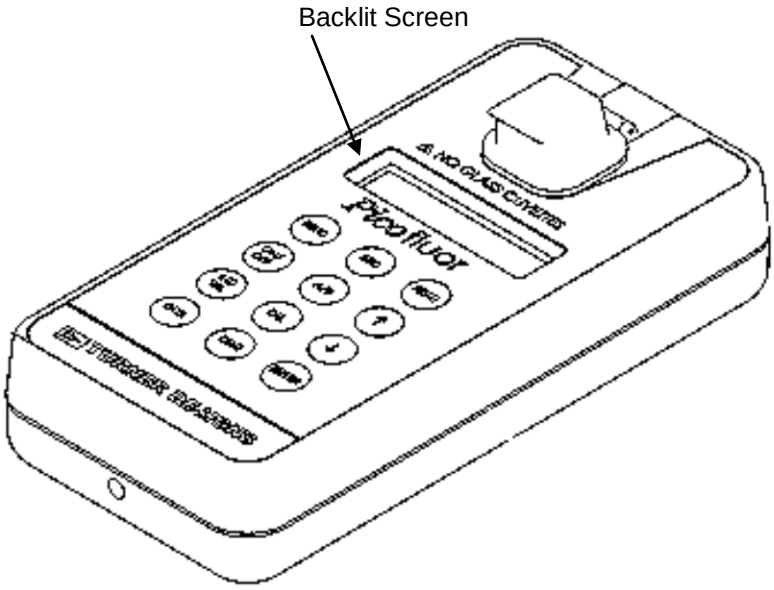
Upon receiving the instrument, inspect everything carefully to make sure all accessories are present. All shipments include:

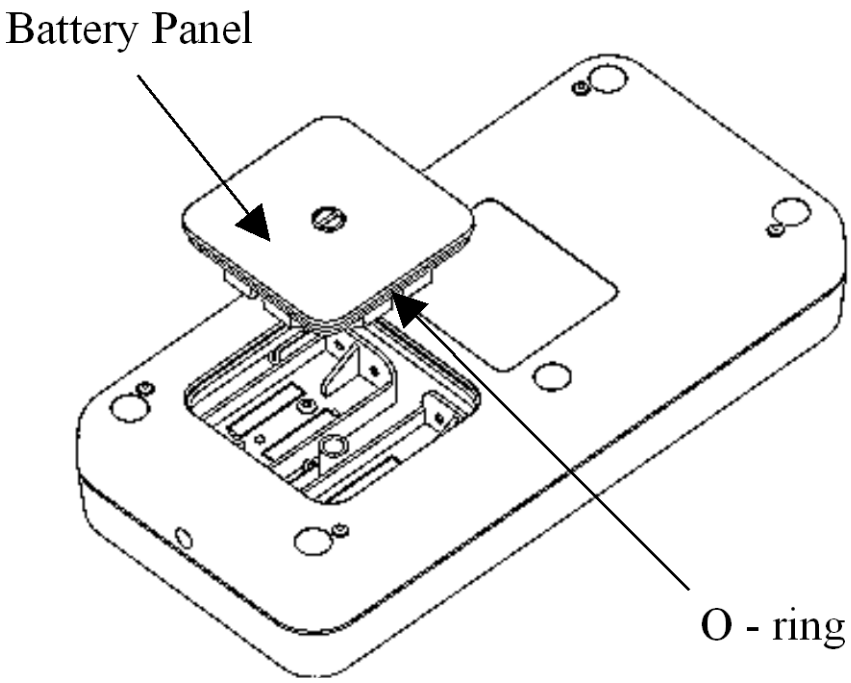
- The *Pico*fluor™
- 4 AAA Batteries
- 4 Plastic Cuvettes
- Internal Data Logging (IDL) package
- PC Interface Cable and Software Instruction
- Spreadsheet Interface Software (1CD)
- This Operating Manual

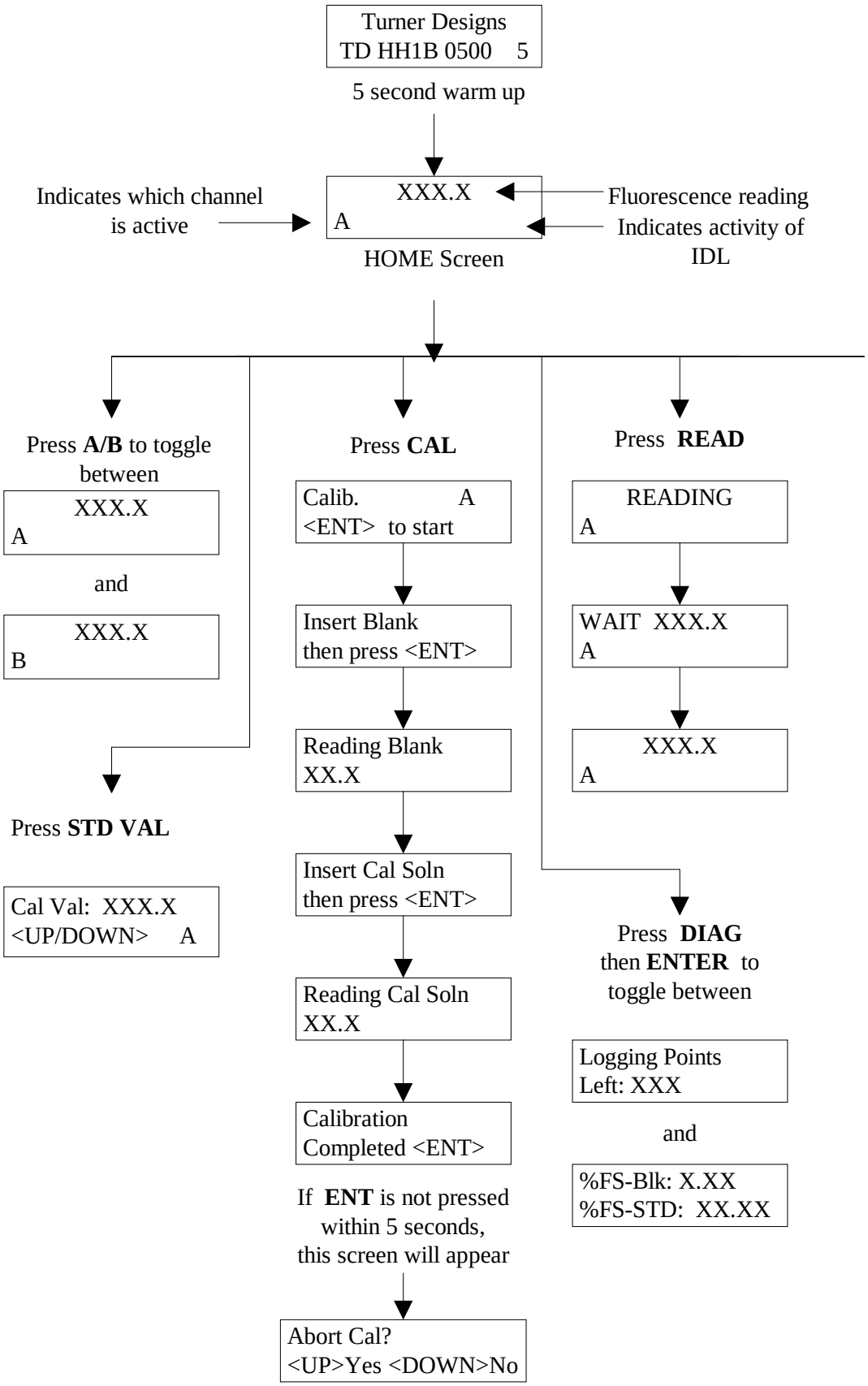
1.4 Setup

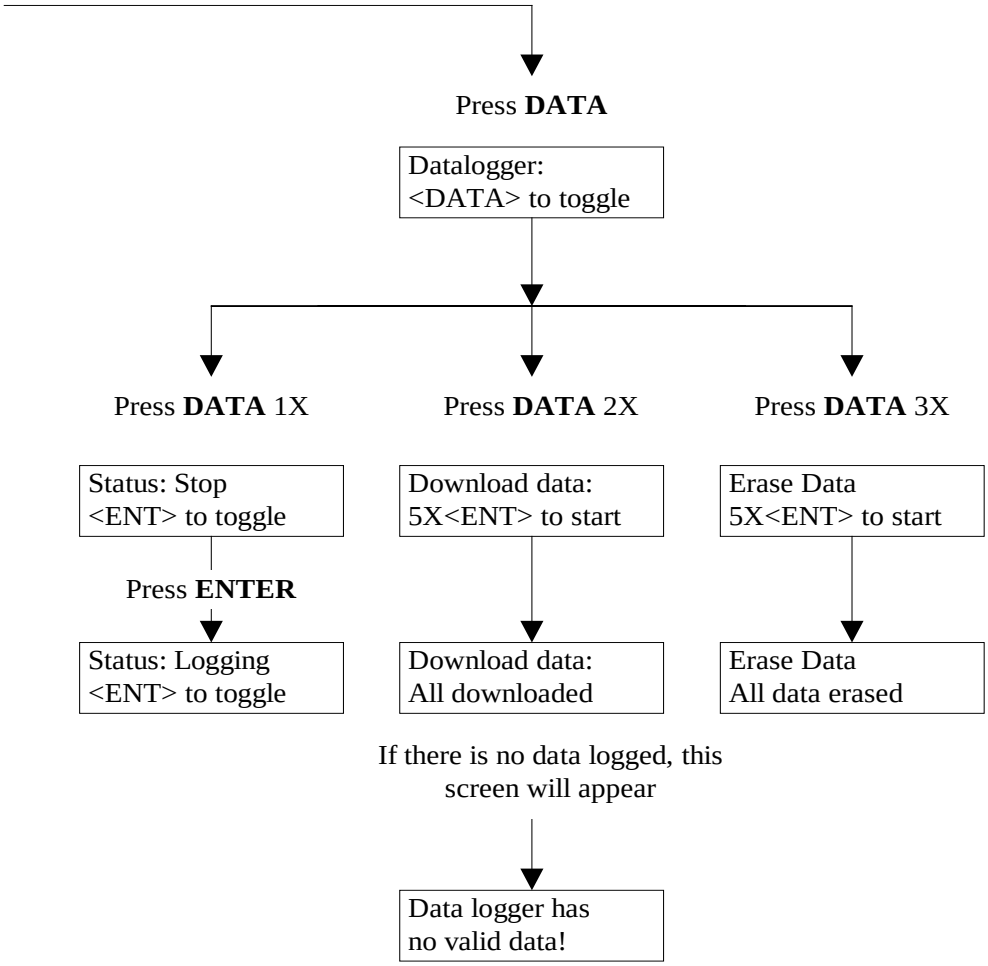
Before the *Pico*fluor™ can be used; the supplied batteries must be installed.

1. On the back of the instrument, loosen the screw and pull the screw up to remove the battery panel *See Section 2 for a diagram.*
2. Install the 4 AAA batteries into the appropriate spaces.
3. Replace the battery panel and tighten the screw. The panel has an o-ring, which creates a watertight seal. The battery panel may be difficult to install if there is no lubrication on the o-ring. Use a silicon based grease to lightly lubricate the o-ring, if necessary.


Backlit Screen
Picofluor


Battery Panel
O - ring


Turner Designs
TD HH1B 0500 5
5 second warm up
XXX.X
A
HOME Screen
Indicates which channel is active
Fluorescence reading
Indicates activity of IDL
Press A/B to toggle between
XXX.X
A
and
XXX.X
B
Press STD VAL
Cal Val: XXX.X
<UP/DOWN> A
Press CAL
Calib. A
<ENT> to start
Insert Blank
then press <ENT>
Reading Blank
XX.X
Insert Cal Soln
then press <ENT>
Reading Cal Soln
XX.X
Calibration
Completed <ENT>
If ENT is not pressed within 5 seconds, this screen will appear
Abort Cal?
<UP>Yes <DOWN>No
Press READ
READING
A
WAIT XXX.X
A
XXX.X
A
Press DIAG
then ENTER to
toggle between
Logging Points
Left: XXX
and
%FS-Blk: X.XX
%FS-STD: XX.XX


Press **DATA**
Datalogger:
<DATA> to toggle
Press **DATA** 1X
Press **DATA** 2X
Press **DATA** 3X
Status: Stop
<ENT> to toggle
Download data:
5X<ENT> to start
Erase Data
5X<ENT> to start
Press **ENTER**
Status: Logging
<ENT> to toggle
Download data:
All downloaded
Erase Data
All data erased
If there is no data logged, this screen will appear
Data logger has
no valid data!

4 Instrument Calibration and Operation

4.1 Instrument Power Up

To turn on the *Pico*fluor™, press the **ON/OFF** button. The instrument takes 5 seconds to warm up. After the warm-up, the *Pico*fluor™ is ready for use.

Pressing the **ON/OFF** button again will turn off the unit or, if left idle for 3 minutes, the unit will automatically turn off to save battery power.

A set of new batteries can last over 1,000 sample readings. If the batteries have low power or are not positioned properly, the following warning message will appear: “Batt Level < 20% !! Caution !!”

4.2 LCD

The *Pico*Fluor™ has a backlit, adjustable brightness LCD (Liquid Crystal Display) for easier viewing. The contrast may change with temperature and may get lighter as the unit gets colder. While on the HOME screen, press the **UP ARROW** button to darken the contrast and press the **DOWN ARROW** button to lighten it.

4.3 Detection Channel Configuration

The *Pico*fluor™ has 2 fluorescence detection channels. Identify the configuration by looking at the label on the back side of the *Pico*fluor™. See the table in Appendix A to confirm the correct configuration for your application.

Select the appropriate channel by pressing the **A/B** button to toggle between the 2 channels. The display will show a label in the lower left corner of the HOME screen to identify which channel is activated.

Model 8000-003
Channel A UV = Ultra Violet excitation
Channel B Blue = Blue LED excitation

Model 8000-004
Channel A RHOD = Green LED excitation
Channel B Blue = Blue LED excitation

See Appendix A for optical (wavelength) specifications.

4.4 Calibration Overview

The *Pico*fluor™ should be calibrated using a Primary Standard. A Primary Standard contains the same fluorescent material that is measured in the unknown samples. The *Pico*fluor™ will give an actual quantitative concentration reading when a Primary Standard of known concentration is used for calibration. The Primary Standard is either made to a known concentration, typically in ppb or ug/L units, or to a known dilution factor. Ideally, the Primary Standard and Blank samples, used for calibration, are made with the same water in which the tests are performed. The standard and samples must be in the linear detection range to achieve accurate quantitative results. *See Section 6.2 for more details about the linear range and sample quenching.*

4.5 Calibration

For best accuracy, always calibrate prior to a sample analysis. The *Pico*fluor™ will save the calibration settings for each channel until a new calibration is performed.

If the temperature of the sample or the *Pico*fluor™ changes significantly, the readings may show a small shift. In this case, consider recalibrating. The solid Secondary Standard is useful for checking the reading stability over time and can also be used to recalibrate if needed.

Cuvette orientation and cleanliness can have an impact on the result accuracy.

See Section4.8 for sample analysis guidelines to help insure the best results for the analysis.

4.6 Setting Calibration Standard Value

This defines the numeric value for the standard to be read. For example, if a Primary Standard has a 50 ug/L concentration, then set the value to 50 and the implied unit of measure will be in ug/L.

1. Press the **STD VAL** button.
2. Press the **UP** and **DOWN ARROW** buttons to set the standard value. Holding down either arrow buttons will result in fast scrolling of the value.
3. When finished, press the **ENTER** or **ESC** button to accept the value and return to the HOME screen.

4.7 Performing the Calibration

1. Press the **CAL** button.
2. Press the **ENTER** button to start the calibration.
3. Insert the blank sample and press the **ENTER** button. The *Pico*fluor™ will average the reading for 10 seconds and set the blanking zero point.
4. Insert the standard sample and press the **ENTER** button.
5. The reading is averaged for 10 seconds and the Standard Calibration value is set.
6. Press the **ENTER** button when calibration is complete to accept the calibration. If the **ENTER** button is not pressed within 10 seconds, a dialog will appear asking the user if aborting the calibration is desired. Press the **UP** or **DOWN ARROW** button to abort or accept the calibration, respectively.

At anytime during steps 1-4, press the **ESC** button to stop the calibration. The display will return to the HOME screen and will default the instrument to the previous calibration.

4.8 Sample Analysis

1. Insert a minimum of 2 mL of the sample using a plastic cuvette only.
2. Press one of the **READ** buttons. The instrument will measure and average the fluorescence signal over a 5-second interval.
3. The result will be displayed at the top and center of the HOME screen.
4. The top left corner will display “WAIT” for 5 seconds. Once the message “WAIT” disappears, the *Pico*fluor™ is ready to measure another sample.

4.9 Diagnostic Information

1. Press the **DIAG** button to access the diagnostic screens.
2. The first screen shows the number of available data points for internal data logging.
3. Press the **ENTER** button to toggle to the RFU (Relative Fluorescence Unit) values from the calibration blank and standard.
4. Press the **ESC** button when finished to return to the HOME screen.

If the instrument is calibrated using the highest standard concentration and the RFU-Std value is too close to the RFU-Blk value, the sample reading may exceed the instrument's detection limit.

Ideally:

$$\frac{\text{RFU-Std value}}{\text{RFU-Blk value}} \geq 3$$

5 Internal Data Logging (IDL)

The *Pico*fluor™ can log up to 1,000 data points. The DATA screens control logging, downloading, and erasing of data.

5.1 To activate the IDL

1. Press the **DATA** button 2 times.
2. Press the **ENTER** button to toggle between logging and stopping.
3. Press the **ESC** button when finished to return to the HOME screen.

5.2 Download Data

1. Connect the Picofluor™ to the PC's serial port using the provided interface cable.
2. Open the Spreadsheet Interface Software. *See Appendix B for computer requirements and installation.*
3. Press the **DATA** button 3 times.
4. Press the **ENTER** button 5 times to start the data download.
5. Press the **ESC** button when finished to return to the HOME screen.

5.3 Erase Data

1. Press the **DATA** button 4 times.
2. Press the **ENTER** button 5 times to erase all logged data.
3. Press the **ESC** button when finished to return to the HOME screen.

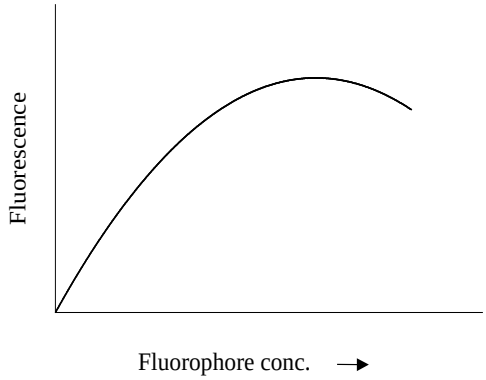
6 General Considerations for Analysis

6.1 Handling Samples

1. Take care not to spill liquid into the Sample Compartment. Promptly wipe any spills.
2. The *Pico*fluor™ is very sensitive and even small amounts of material from a previous sample may result in errors. Use a clean cuvette for each reading. Thorough and proper cleaning of cuvettes between sample readings is essential, and is especially important if the same cuvette is used for both the sample and the blank.
3. Fill the cuvette at least 50% full. Significant error will result if it is not adequately filled.
4. Ensure that the cuvette is clean and dry on the outside when taking readings. Moisture and condensation on the outside can result in error.
5. Minute bubbles in samples will cause errors in the readings. Be sure not to introduce bubbles into samples. Particular care must be taken with the Minicell Adapter. Slight tapping on the outside cuvette wall will often help dissipate bubbles.

6.2 Linear Range and Quenching

The linear range is the concentration range in which the *Pico*fluor™ readout is directly proportional to the concentration of the fluorophore. The linear range begins with the smallest detectable concentration and continues to an upper concentration limit that depends upon the properties of the fluorescent material, the filters used, and the path length. See the diagram below.



A nonlinear relationship occurs at higher concentrations where the fluorescence signal increases at decreasing rates in relation to the concentration change. At even higher concentrations, readings begin to decrease despite increasing sample concentrations. This effect is known as "signal quenching."

Linearity may be checked by diluting a sample 1:1 or some other appropriate ratio (be sure to use a corresponding buffer for the dilutions). If the sample is within the linear range, the reading will decrease in direct proportion to the dilution. If the reading does not decrease in direct proportion to the dilution or if the reading increases, the sample is beyond the linear range of the fluorophore.

6.3 Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. For greatest accuracy, read the blank, standard, and samples at the same temperature.

6.4 Positioning Samples

For low concentration samples, cuvettes often give slightly different measurements depending upon their orientation in the sample compartment. This is due to defects in the shape of the cuvette that are not visible to the human eye. It is recommended that the cuvette be marked at the top and positioned in the Sample Compartment the same way each time to minimize error.

6.5 Data Quality

The *Pico*fluor™ is only as accurate as the standards that are used to calibrate it. It is important to take care when preparing standards, samples, and blank. Follow the appropriate laboratory practices when preparing all solutions and reagents.

7 Warranty

7.1 Terms

Turner BioSystems warrants the *Pico*fluor™ Fluorometer and any accessories to be free from defects in materials and workmanship under normal use for a period of one year from the time of initial purchase under the following restrictions:

- The instrument and accessories must be installed, powered, and operated in compliance with the directions in this *Operating Manual* and the directions accompanying the accessories.
- Damage incurred during shipping is not covered by warranty.
- Damage resulting from measuring incompatible samples is not covered by warranty.
- Damage resulting from reagent spills is not covered by warranty.
- Damage resulting from contact with corrosive materials or atmosphere is not covered by warranty.
- Damage from salts, proteins and other moderately corrosive materials that are not promptly removed from the instrument are not covered by warranty.
- Damage caused by user modification of the instrument is not covered by warranty.

7.2 Warranty Service

To obtain service during the warranty period, take the following steps:

1. Write or call the Turner BioSystems Service Department and describe as precisely as possible the nature of the problem.
2. Carry out minor adjustments or tests as suggested by the Technical Support Department.
3. If the instrument is still not functioning properly, you must obtain an RMA number from the Turner BioSystems Service Department before shipping the instrument. This number is necessary for tracking and repair under warranty.
4. Reference the RMA number on the outside of the package. Pack the instrument well as damage incurred during shipping due to improper packing is not covered by warranty. Purchase shipping insurance for the package and ship it to the Turner BioSystems Service Department. All shipments must be prepaid.

The instrument will be repaired and returned free of charge for any customer in the United States. Turner BioSystems will pay for return shipment and include a reimbursement check for the cost of surface shipment to the Service Department.

Turner BioSystems cannot pay shipping, duties, or documentation costs outside the continental United States. Contact Turner BioSystems for instructions if you are an international customer who made a purchase directly from Turner BioSystems (not from a third party distributor). Customers outside of the United States who have purchased our equipment from an authorized distributor should contact their distributor directly for further instructions. The instrument will be repaired at no charge if it is under warranty.

IMPORTANT: Under no circumstances should the instrument or accessories be returned without prior authorization from Turner BioSystems or our authorized distributor.

Prior correspondence is needed to eliminate minor problems easily handled on-site in your laboratory in order to save money and minimize equipment downtime. Prior correspondence can also help determine the exact nature of the problem so that repairs can be tailored accordingly.

7.3 Out-of-Warranty Service

Follow the same steps as for Warranty Service. Our Service Department is happy to assist you by telephone or correspondence at no charge. Repair service will be billed at a flat rate. Your invoice will include freight charges.

Mailing Address:

Turner BioSystems
645 North Mary Avenue
Sunnyvale, CA 94085
USA

Phone: 1(408) 636-2400
Toll-Free: 1(888) 636-2401 (US/Canada)
Fax: 1(408) 737-7919

APPENDIX A Instrument Specifications

A.1 General Specifications

Specification	Description
Size	1.75” x 3.5” x 7.25” (4.45 cm x 8.9 cm x 18.4 cm)
Weight	14 oz (0.4 kg)
Dynamic range	4 orders of magnitude
Resolution	12 bits
LCD Display	2 x 16 characters
Case	Meets IP 67 Standard; dustproof and waterproof
Temperature	41 - 104 ^{o}F; 5 - 40 ^{o}C
Detectors	Photodiodes: measurement capability from 300—1000 nm
Calibration Type	Single-point
Alarms	Low battery, circuit failure, high blank
Cuvette Type	10 x 10 mm plastic
Warm-Up Time	5 seconds
Automatic Power Down	After 90 seconds of inactivity

A.2 Optical Specifications

Light Source	UV LED	Blue LED	Green LED
Excitation Optics	365 nm	460 nm	525 nm
Emission Optics	440 - 470 nm	515 - 575 nm	>570 nm
Limit of Detection		<50 pM Fluorescein	<200 pM Rhodamine

APPENDIX B Internal Datalogging

B.1 Shipping Checklist

The Internal Data Logging Package includes:
- Interface cable
- Spreadsheet Interface Software (1 CD)

Both of these items are necessary for retrieving the stored data from the *Pico*fluor™.

B.2 Hardware Requirements

- PC with Windows 95 or later
- MS Excel 5.0 or later
- At least 1 available serial port

B.3 Installation

1. Exit all Windows programs.
2. Insert the CD and run the setup program.
3. The setup wizard will install the necessary files. When the setup is complete, a SPREADSHEET INTERFACE SOFTWARE icon will appear in the PROGRAMS menu.
4. Restart the computer.

B.4 Connecting

1. Using the provided cable, connect the 9-pin adapter of the cable into the available serial port of the computer.
2. Plug the opposite end of the cable into the base of the *Pico*fluor™.
3. Open the Spreadsheet Interface Software.
4. Click on the box to the right of the COM port icon to select the appropriate COM port. This is usually COM port 1.
5. Click on the **START** button. The program will open MS Excel and should be ready for data transfer. The boxes left of the words COM port and MS Excel should both be green.
6. Follow the directions from Section 5.2 for collecting and downloading data from the *Pico*fluor™. Data will automatically appear in the Excel spreadsheet. Be sure to save this data before closing the software.

B.5 Troubleshooting

Difficulties can arise when parameters are set incorrectly or when cable connections are not tight enough. Here are some common problems.

1. The box to the left of the COM port is red. This means that the COM port is not available. Causes:
   - Another instrument or program (such as palm pilot/ hot sync) could be occupying the port, making it unavailable. Make sure to close all programs of this type before downloading data
   - The port selected is incorrect. Follow step 4 of connecting to choose another COM port.

2. All the lights are green, but no data is transferred, even though the instrument says “All data downloaded.”
   - The connection between the instrument and the computer is bad. Check and tighten the cable connections. Make sure both ends of the cable are plugged in tightly.

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