

Fluorophos® ALP Test System Model FLM300 User's Guide



Copyright

This user's guide is copyrighted by Advanced Instruments with all rights reserved. Under copyright laws, this guide may not be reproduced in any form, in whole or part, without the prior written consent of Advanced Instruments.

© 2017 by Advanced Instruments, Norwood, MA 02062.

Advanced Instruments has reviewed this guide thoroughly. All material contained within is believed reliable, but the accuracy and completeness are not guaranteed or warranted, and are not intended to be representations or warranties concerning the product described.

Microsoft® and Windows® are registered trademarks of Microsoft Corporation in the United States and other countries.

All other trademarks are the property of Advanced Instruments.

Hot-Line™ Service

If you have any questions regarding the proper operation of your instrument or would like to order replacement parts, please contact our Hot-Line™ Service department by calling one of the following numbers:

800-225-4034 (toll-free within the USA and Canada; after normal business hours, use extension 2191)

+1 781-320-9000 (elsewhere)

+1 781-320-0811 (fax)

Contents

Safe Use	5	Reagent storage/stability	19
Supplies, Parts & Accessories	7	Reagent handling	20
Reagents, Calibrators & Controls	7	Calibration	20
Foreword: Principles of Fluorescence	8	How to determine the need for different calibration curves: calibration ratio and product type	21
		Reconstituting reagents	21
		Daily instrument checks	21
		Sample preparation	22
		Determining the need for the high-turbidity test procedure	22
		Calibrating and testing high-turbidity samples	23
		Test procedure	23
		Interpretation of results	23
Chapter 1			
Installation and Setup	9	Chapter 4	
Step 1. Find a location for the instrument	9	Instrument Maintenance	25
Step 2. Unpack the instrument	9	Daily controls	25
Step 3. Obtain additional items	10	Cleaning Spills	25
Step 4. Check excitation filter	11	Reagent storage	26
Step 5. Load printer paper	11	Optical filter replacement	26
Step 6. Power-up and run diagnostic test	12	Optical filter care	26
Step 7. Set up heating block	12	Pipette maintenance	26
Step 8. Set date and time	12	Fuse replacement	26
Chapter 2		Chapter 5	
Instrument Operation	13	Troubleshooting and Service	27
Hazardous material caution	13	Service and maintenance cautions	27
Function of major components	13	Obtaining service	27
Using the 25- μ L or 75- μ L pipette	15	Troubleshooting checks	28
Using the 2-mL pipette	15	Internal diagnostics	28
Instrument start-up	15		
Calibration	15	Appendix A	
Preparing reagents	16	Additional Technical Information	30
Test procedure	16	Appendix B	
Changing operating settings	16	Troubleshooting Table	35
Chapter 3			
Fluorophos[®] ALP Milk Pasteurization Assay	18		
Overview	18		
Alkaline Phosphatase (ALP) background	18		
The Fluorophos [®] ALP reaction	19		
Materials required	19		

Contents

Appendix C	
Product Specifications	36
Appendix D	
Regulatory Notices	38
Appendix E	
Warranty & Warranty Duties	41
Appendix F	
Supplemental RS-232 Information	44
Appendix G	
Symbol Definitions	45
Appendix H	
Product Disposal and Recycling	46
Appendix I	
Service Log	48
Appendix J	
Index	50

WARNING: To reduce the risk of bodily injury, electric shock, fire, and damage to your instrument, please read and observe the precautions in this User's Guide.

- If the product is used in a manner not in accordance with the equipment design, operating instructions, or manufacturer's recommendations, the operation of the product may be impaired to the extent that a safety hazard is created.
- Do not attempt to perform electrical work if you are not fully qualified. This manual is not a substitute for electrical training.

Symbol Conventions

The list of symbols below is for reference only. Not all symbols shown may be used on this instrument or in the accompanying documentation.



The exclamation point within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance (servicing) instructions in the literature accompanying this product.



The lightning flash with an arrowhead symbol within an equilateral triangle is intended to alert the user to the presence of uninsulated dangerous voltage within the product enclosure that may be of sufficient magnitude to constitute a risk of electric shock to persons.



This symbol indicates the possible presence of biologically hazardous material.



This symbol indicates the possible presence of toxic material.



The static symbol is intended to alert the user to the presence of internal components that could be damaged by static electricity.



This symbol indicates the presence of alternating current (AC).



This symbol indicates the presence of a fuse.



This symbol indicates the presence of protective earth ground.



This symbol indicates the power is ON.



This symbol indicates the power is OFF.

Additional symbol definitions are available in Appendix G.

General Cautions

- This product should be operated only with the type of power source indicated on the product's electrical ratings label. Refer to the installation instructions included with the product.
- If the power cord provided is replaced for any reason or if an alternate cord is used, the cord must be approved for use in the local country. The power cord must be approved for the product's listed operating voltage and be rated at least 20% greater than the volt-ampere (VA) ratings marked on the product electrical ratings label. The cord end that connects to the product must have an IEC 60320 connector.
- Plug the product into an approved grounded electrical outlet.
- Do not disable the power cord grounding pin.
- If an extension cord or power strip is used, make sure that the cord or strip is rated for the product, and that the total ampere ratings of all products plugged into the extension cord or strip do not exceed 80% of the cord's or strip's rating limit.
- Route power cords so that they will not be walked on, tripped on, or pinched by items placed upon or against them. Pay particular attention to the plug, electrical outlet, and the point where the cord exits the product.
- Do not pull on cords and cables. When unplugging cords or cables, grasp the corresponding connector.
- Do not install or use this product in any area subject to extreme short-term temperature variations, or locations that exceed the specified operating environment temperatures.
- Never use this product in a wet area.
- To avoid injury or fire hazard, do not operate this product in an explosive atmosphere.
- Do not install or use the product on an unstable, non-level work surface.
- Do not operate this product with the covers removed or unsecured.

FCC Requirements

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Re-orient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio TV technician for help.

Supplies, Parts & Accessories

To order parts and accessories, contact your local Advanced Instruments Distributor or Advanced Instruments Customer Service Department:

- 800-225-4034 (toll free in the USA and Canada)
- +1 781-320-9000 (elsewhere)
- +1 781-320-3669 (fax)

Part number	Description
FLA880	Pipette Tips 25-75 μ L (100)
FLA020	Fluorophos® Cuvettes (250)
FLA835	Printer Paper (5 rolls)
FLA865	25- μ L Pipette
FLA885	75- μ L Pipette
FLA887	Plunger Wire for 75- μ L Pipette
FLA840	Cuvette Holder Cleaners (50 cleaners)
FLA810	Heating Block Thermometer
FL2176	20-Sample Heating Block
FL3008SM	Service Manual
FL3005UG	User's Guide
FL2333	Excitation Filter (ALP) Assembly
FL3028	Emission Filter Assembly
FLA888	2 mL Pipette
ACM205	Pipette Tips 2-mL (25)

Reagents, Calibrators, & Controls

To order calibrators and standards, contact your local Advanced Instruments Distributor or Advanced Instruments Customer Service Department:

- 800-225-4034 (toll free in the USA and Canada)
- +1 781-320-9000 (elsewhere)
- +1 781-320-3669 (fax)

Part number	Description
FLA005	Cheese Extraction Buffer
FLA225	225-Test ALP Reagent/Cuvettes Kit
FLA250	ALP Calibrator Set
FLA260	ALP PhosphaCheck® Pasteurization Controls
FLA280	ALP Daily Instrument Control

Foreword

Principles of Fluorescence

The technology of fluorescence has been used in analytical assays for over 100 years. Molecules can be excited to higher energy levels by the absorption of electromagnetic radiation such as visible light. The excited molecular state is short-lived, and certain molecules will emit light in the process of returning to the lower energy level. The emitted light is usually lower in energy than the excitation light. The emission of this lower-energy light is referred to as fluorescence. Light that is lower in energy is longer in wavelength. For example, light with a wavelength of 560 nm has less energy than light with a wavelength of 439 nm.

Many fluorometric assays take advantage of the ability to measure the conversion of non-fluorescent molecules to highly fluorescent molecules, such as enzymatic assays. Inorganic molecules can be measured with fluorescence by combining them with other non-fluorescent molecules that will fluoresce when attached to the inorganic ion.

One of the advantages of fluorescence assays is the improved sensitivity. Fluorescence assays can typically measure as little as 10^{-10} g/mL of analyte, compared to colorimetric assays which measure 10^{-7} g/mL.



In order to set up your instrument properly, it is important that you read and follow the steps in this chapter.

Please follow these steps carefully and be sure to read *Chapter 2—Instrument Operation* before attempting to run tests using your instrument.

Step 1. Find a location for the instrument

When choosing a location for your fluorometer, be sure to meet the following criteria.

Adequate space

- The dimensions of the instrument are 16.5 × 12.5 × 7 inches (42 × 32 × 18 cm). Be sure to keep your workplace free of debris, especially underneath and behind the instrument, where proper ventilation is needed.
- The heating block will perform optimally if placed in a draft-free location.
- Changes to the ambient temperature or airflow conditions around the instrument may require adjustment of the heating block's set point.

Electric outlet availability

Your instrument will need to operate within five feet of a properly grounded, three-prong electrical outlet capable of continuously supplying 1 ampere at 100-240V. If the instrument is not grounded properly, its operation may be impaired and a safety hazard may exist. Therefore, be sure to test the outlet and record the results before operating your instrument.

For proper instrument operation, you should also choose an outlet that is not subject to excessive electric interference or surges.

NOTE: If your laboratory has electrical fluctuations, we recommend using a line conditioner with your instrument.

Step 2. Unpack the instrument

To unpack your fluorometer, take the following steps.

- a. Carefully unpack your fluorometer, accessories, and supplies and inspect them for shipping damage. Use the enclosed packing list to verify that all items have been received.
- b. Save the instrument shipping boxes and packaging material in case future transport of the instrument becomes necessary.
- c. If any item on the packing list appears to be missing from your shipment, please search carefully through and under all packing materials. If the item is not found, notify your receiving department immediately. Advanced Instruments can only be responsible for items reported missing within 10 days of a shipment's arrival.
- d. If you receive any damaged items, save the cartons and packing material those items came in for inspection by the insurer. The carrier, dealer, and Advanced Instruments must be notified within 24 hours in order for your warranty and insurance to apply. Have the transportation company inspect items, fill out a "Report of Concealed Damage," and file your claim. Then, notify Advanced Instruments immediately for repair or replacement.
- e. Fill out the warranty card enclosed. Mark the appropriate boxes if you wish to receive additional information. You may mail the card (postage required outside of the United States) or fax to 781-320-8181.



Fig. 1: FLM300 Fluorophos® Test System and Supplies

The items included in your shipment are listed in the table below.

Part #	Description
FLM300	Fluorophos® ALP Test System
FL3050	Black Body Calibration Tool
FLA865	25µL Sample Pipette
FLA885	75µL Sample Pipette
FLA888	2mL Sample Pipette
ACM205	2mL Pipette Tips (25 count)
FLA880	25-75µL Pipette Tips (100 count)
FL3005UG	Fluorophos® ALP Test System User’s Guide (not pictured)
FL2176	Heating Block, shipped in accessory kit
FLA810	Heating Block Thermometer (not pictured)
FL0408	Paper Roll Holder
	Thermal Printer Paper (2 rolls, re-order package of 5 rolls as FLA835)
	Cuvette Holder Cleaners (One pack, re-order package of 50 as FLA840)
	AC Power Cord (as specified)
FLM5-6	Warranty Card (not pictured)

Table 1.1: Fluorophos® ALP Test System Packing List

The starter test kit contents are shown in Fig. 2 below.



Fig. 2: FLMA02 ALP Starter Test Kit

Items in the ALP Test Kit are listed in the table below.

Part #	Description
FLA224	Fluorophos® ALP 225 Test Reagent Set
FLA250	Fluorophos® ALP Calibration Set
FLA260	Fluorophos® ALP Pasteurization Controls
FLA280	Fluorophos® ALP Daily Instrument Control
FLA020	Fluorophos® ALP Cuvettes

Table 1.2: FLMA02 ALP Starter Test Kit Packing List

Step 3. Obtain additional items

You will also need these items that are not supplied by the manufacturer:

- A vortex mixer.
- Soft, lint-free wiping tissues.

Step 4. Check excitation filter



NOTE: Use extreme caution whenever you handle a filter or filter assembly. The filters scratch easily.

To clean your filter, use a piece of lint-free, non-abrasive tissue and a small amount of 70% isopropanol solution. Be sure the filter is completely dry before re-installing.

This excitation filter assembly (FL2333) should be replaced annually to maintain optimal performance.

Step 5. Load printer paper

A roll of thermal printer paper and a paper roll holder are supplied with the instrument. These must be installed in the printer as follows:

- a. Fully depress the printer cover release button located immediately in front of the printer cover, then lift up on the printer cover to open. Release the printer cover release button.
- b. Unroll approximately 6" (15 cm) of paper from the roll.
- c. Insert the plastic roller into the center of the paper roll. Place the paper roll and roller into the V-shaped roller supports as shown in Fig. 3. Make sure the paper roll is oriented as shown in Fig. 4, **feeding up from the bottom of the roll**. When both ends of the roller are in the proper position, the paper roll is level. If the paper roll is not level, carefully reseal the roller between the roller guides.
- d. Hold the paper down onto the surface of the instrument and center the paper on the printer tear bar. See Fig 5. When the paper is centered, gently close the printer cover. Press on the top of the printer cover to make sure it is firmly latched with the printer mechanism.
- e. The printer is now ready for operation. See Fig 6.

CAUTION: Do not attempt to manually pull the paper through the printer, as this may damage the mechanism. Either release the printer cover by fully depressing the printer cover release button and lifting up on the cover, or use the **FEED** button on the keypad to advance the paper.

Note: Red LED indicates the printer door is open. Yellow LED indicates there is no paper.



Fig. 1: Open paper compartment



Fig. 2: Paper in V-shaped roller supports

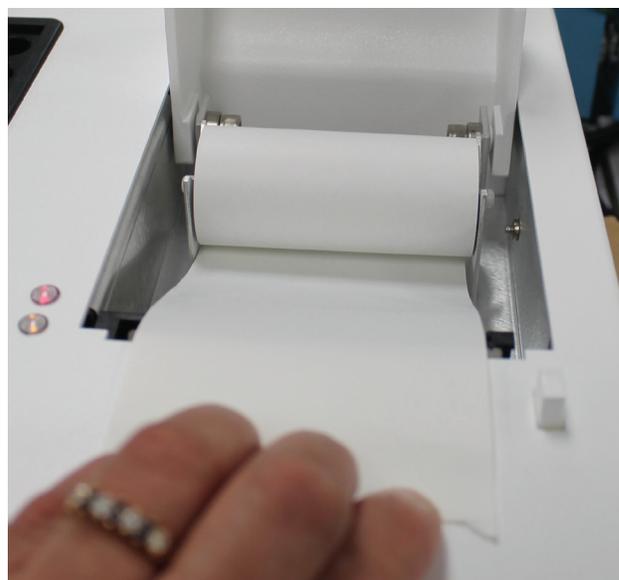


Fig. 3: Paper centered on tear bar

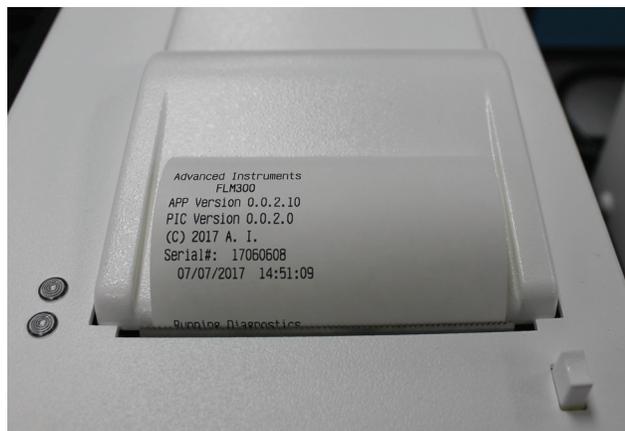


Fig. 4: Printer cover closed

Step 6. Power-up and run diagnostic test

- Plug the fluorometer power cord into a properly grounded outlet.
- Switch the fluorometer power switch into the **ON** position. The display will report its current status and configuration.
- The display will then begin a 15-minute countdown, during which the cuvette chamber will stabilize at the required operating temperature.
- During the countdown, some brief diagnostic tests run to ensure the instrument is functioning properly.
Note: See Appendix B (Troubleshooting Table) if any diagnostic test fails.
- After the countdown is completed, the fluorometer will display "[CALIB] Calibration/Product [TEST]," and will be ready for use.
- To verify the cuvette chamber temperature is 38 ± 1 °C, place a sample cuvette with 2 mL water into the chamber, and place the heating block thermometer in the cuvette. Allow the temperature to stabilize.
- If the temperature is not 38 ± 1 °C, use the **Chamber Temp Adj** function in the Setup menu to adjust the set point up or down in 0.1 °C increments. After allowing the temperature to stabilize to the new set point, verify the change or make another adjustment.

Step 7. Set up heating block

- Place the heating block in the recess on top of instrument.
- When the instrument power is switched to the **ON** position (Step 5), the red LED in front of the heating block illuminates, indicating that the heating block has

not yet reached its set point.

- Insert the heating block thermometer into the heating block thermometer well.
- Allow the heating block to warm up for at least 30 minutes to the set temperature and stabilize. When the block reaches the set temperature, the green LED in front of the heating block illuminates.
- Use the heating block thermometer to check the temperature of the heating block.
- If the temperature is not 38 ± 1 °C, use the **Heater Block Adj** function in the Setup menu to adjust the set point up or down in 0.1 °C increments. After allowing the temperature to stabilize to the new set point, verify the change or make another adjustment.
- When the heating block temperature is stable at 38 ± 1 °C, it is properly set up and ready to use.

Step 8. Set date and time

Now set the date and time on the instrument using the information below.

To display the current settings

- When the fluorometer display scrolls [CALIB] Calibration/ Product [TEST] press **SETUP** on the keypad, and then press **ENTER**. The display changes to **Daily Instrument Control Adj**.
- Press the < or > key to cycle through setup menu options to **Set Date/Time**.
- Press **START** to display the current date and time.

To change the current settings

- Use the < key to set the preferred date format (mm/dd/yy or dd/mm/yy).
- Use the > key to set the preferred time format: **24hr** or to display time in 12-hour format using **am** or **pm**, as appropriate.
- Press **CLEAR**.
- Now enter the current date and time (including zeros if needed) one digit at a time, from left to right, in 24-hour format, using the numeric keypad.

If you need to start over, press **CLEAR**.

- Either press **ENTER** to store the new setting or press **STOP** to restore the original setting.

NOTE: If you attempt to enter an invalid setting, you will hear three beeps when you press **ENTER**. The setting is rejected, and the process is cancelled. You must start over to set the date and time.



This chapter contains information on operating the fluorometer. We have provided additional information for the Fluorophos® ALP Milk Pasteurization Assay later in this user's guide. Read both this chapter and the test chapter before running any tests on the fluorometer.



Hazardous material caution

WARNING: The specimens used in this product may be hazardous to your health and to the health of others. Follow national and locally-recognized safety standards and/or protocols for handling and disposing of such specimens.

If a hazardous material is spilled on or inside the fluorometer, the user is responsible for decontamination. Appropriate steps are outlined by the user's National Safety Council or equivalent agency for hazardous materials, and the policies and procedures established within the user's place of business.

To avoid injury or fire hazard, do not operate this product in an explosive atmosphere.

Function of major components

Operation of the instrument will be quicker and easier if you become familiar with the locations and functions of the components, systems and controls described in the subsequent text before proceeding further.

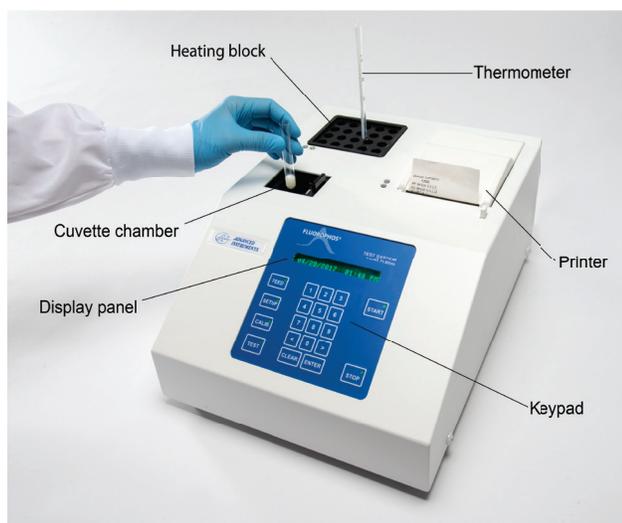


Fig. 7: Fluorometer components

Power panel

Contains the power plug, fuses and rocker-type power switch.

Serial port

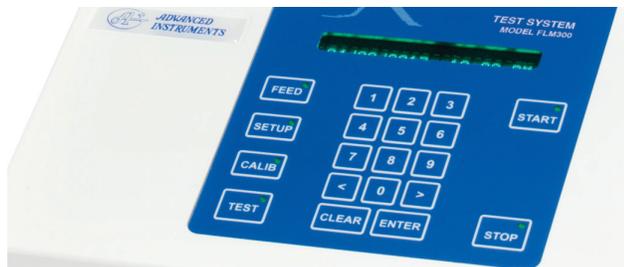
This instrument transmits a significant amount of information via the serial port. Almost every item of information displayed by the instrument is also transmitted over the serial port. This includes test results, all error messages, and most of the display data from the diagnostic menu.

Printer

The printer is located beneath the slotted door in the top right side of the fluorometer. Fully depress the printer cover release button located in front of the printer cover, then lift up on the printer cover and check that adequate thermal paper is properly loaded.

Display panel (Fig. 8 and Table 2.1)

The microprocessor communicates with the instrument operator by means of alphanumeric messages printed on paper tape and displayed on the illuminated display panel located at the top of the keypad.



Fluorometer panel display and keypad

Key	Function
FEED	Advances printer paper
SETUP	Activates setup & diagnostics menus
CALIB	Initiates calibration procedure
TEST	Activates diagnostic test menu
1 through 0	Allows numeric input, as required
< and >	Steps through menu items
CLEAR	Clears some data in setup mode and when using sample identification
ENTER	Confirms sample identification and/or setup menu items
START	Starts tests and setup procedures
STOP	Cancels the procedure in process

Table 2.1: Keypad layout and functions

Keypad

The keypad allows operator input to the microprocessor. (See Fig. 8 for the layout and Table 2.1 for the functions of the keypad switches.)

Cuvette door

The cuvette door, located to the left of the keypad, slides closed to protect the cuvette testing chamber.

Cuvette testing chamber

Illuminates the sample with the proper wavelength light, and measures fluorescent emission from the sample with a photomultiplier tube.

Measurement and control circuits (inside)

The microprocessor-controlled circuits automatically illuminate the sample, measure photo multiplier tube output, regulate temperatures, and process calibration data and sample test information. They also present calibrated test results on the digital display.

Electronic circuits (inside)

The main circuitry is contained on two printed circuit boards in a motherboard/daughter board configuration. More technical details are available in the instrument service manual.

Processor Board: The processor board is the smaller of the two printed circuit boards and contains the Intel 80C186EB central processor, two flash EPROMs, RAM, real-time clock, watchdog circuit, and other logic.

Application Board: The application board contains the circuits for controlling and interfacing with the other subsystems such as the keypad, display, LEDs, photomultiplier tube, thermistors, heaters, RS-232, printer and barcode ports, etc.

Pipettes

The Advanced® positive displacement pipettes are factory-set for precise fixed-volume delivery and shipped ready for use. If needed, calibration adjustment keys and detailed instructions are provided with each pipette. See Fig. 9 for components.

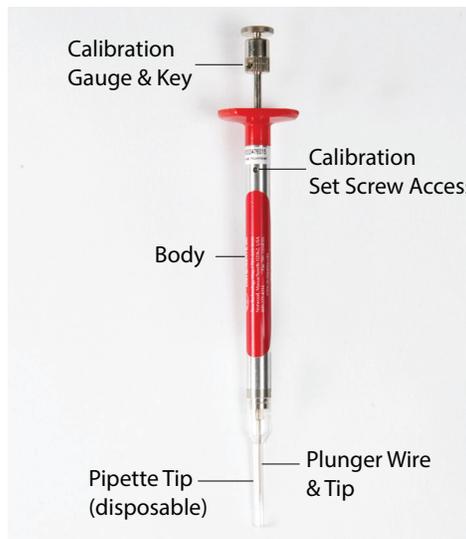


Fig. 8: Pipette for Fluorophos® ALP Test System

Using the 25- μ L or 75- μ L pipette

Operate the pipette as follows:

- a. Depress the plunger fully.
- b. Immerse the pipette tip about 1/4" or 6-7 mm into the liquid to be pipetted.
- c. Release the plunger smoothly and gently.
- d. Remove the pipette tip from the liquid and wipe the outside (do not wick out any fluid from the inside).
- e. Immerse the pipette tip about 1/4" or 6-7 mm into the liquid in the fluorometer cuvette and depress the pipette plunger fully several times.
- f. Depress the pipette plunger fully and while depressed, remove the pipette from the cuvette.

NOTE: To eliminate sample-to-sample contamination between pipetting sessions, remove and replace the disposable pipette tip after each sample.

Occasionally, the pipette plunger tip and wire assembly may require replacement. Calibration and component replacement instructions are supplied with each pipette.

Using the 2-mL pipette

The 2-mL pipette is a fixed-volume pipette, and is ready for use. It is used for dispensing the ALP Test Reagent, the ALP Calibrators, and the ALP Daily Instrument Control. Operate the pipette as follows:

- a. Place a clean tip firmly onto the pipette, making sure that there is a tight seal.
- b. Depress the plunger completely before placing the clean tip into the reagent to be dispensed.
- c. Immerse the tip approximately 1/8" down into the reagent to be dispensed.
- d. Allow the plunger to return to the release position. This must be done slowly.
- e. Remove the tip from the sample solution.
- f. To dispense the collected reagent, hold the point of the tip against the wall of the cuvette and depress the plunger slowly to the stop position.
- g. After the contents of the tip have been dispensed, move the tip away from the wall of the cuvette before allowing the pipette plunger to come to its release position.
- h. Dispose of the used pipette tip. Never reuse the same pipette tip, especially between different reagents.

Instrument start-up

To start up the test system, take the following steps:

- a. Turn on the fluorometer power switch.
- b. The instrument will run start-up diagnostics and count down 15 minutes while the cuvette chamber and heater block warm to 38°C. If the instrument cannot meet the required operating conditions for any reason, an error message will be displayed.
- c. When the instrument warm-up requirements have been met and the instrument is ready for operation, the display will begin to scroll "[CALIB] Calibration/Product [TEST]", at which time the operator may press **CALIB** to calibrate a fluorometer channel or **TEST** to begin testing a product. Pressing either of these keys presents the name of the product group and channel on which the last test or calibration was accomplished. At this point, either press **ENTER** to select that test or use the arrow keys to step through the menu of available choices and press **ENTER** to select the required test or calibration.

Calibration

Periodic calibration of the fluorometer is required to maintain instrument accuracy.

In general, each product type to be tested requires its own calibration curve. The calibration parameters for each calibrated product may be stored in a separate "channel" of the instrument memory and are used by the fluorometer for each test in that channel until changed by performing a new calibration in the same channel.

It is important to calibrate the instrument under the following conditions:

- When the fluorometer is initially installed.
- For each different product type that is analyzed.
- After significant service procedures are performed (such as replacing the sample illumination LED or optical filters).
- When adjustments are made to bring A-D Mode into specification.
- If a product name is changed.

The fluorometer will not allow tests to be performed using an uncalibrated channel; "**Calibration Required**" will be displayed when any new channel is defined. After calibration, if the calibration ratios of two product types are found to be within 5% of each other, they may both be run on the same channel.

Calibration ratios are determined by the instrument during calibration and the calibration-ratio differences between specific products are mainly due to differences in color and turbidity.

The fluorometer is designed to be able to run several different tests. Each test has its own specific calibration procedure. Please see the chapter detailing specific test instructions for further calibration information.

Preparing reagents

To run tests on the fluorometer, you will need to reconstitute the appropriate reagents. Please see information supplied with each reagent type for instructions on reconstitution.

Test procedure

The fluorometer is designed to verify proper pasteurization of dairy products. Some products have their own specific test procedure. Please see the chapter detailing specific test procedures and refer to the product inserts for further instructions.

Changing operating settings

The fluorometer has been individually set up at the factory for normal operation, but the setup procedure may be required for changing the date and time, customizing operating parameters, or testing certain instrument functions.

To change settings, use the following procedure.

- a. To access the Setup menu, press the **SETUP** key at "[CALIB] Calibration/Product [TEST]". The display changes to "[*] Setup/Diag [>]". Press the **ENTER** key to enter the Setup menu.
- b. The first Setup menu function is "**Daily Instrument Control Adj**", to select another function on the Setup menu, repeatedly press the > or < keys until the desired item is displayed.
- c. To activate a displayed function, press **START**. If the function enables modifying an operating parameter, the display will change to indicate the current setting, either as a numeric value or the symbol "*" next to the current setting. In most cases, the setting may be changed by pressing < or >.
- d. Press **ENTER** to save the new setting or **STOP** to cancel the new setting. The current setting will be printed for verification and the display will revert to the title of the item selected, indicating that the Setup menu is again available.

- e. Press **STOP** to exit the Setup menu.

The following menu choices allow you to change operating settings. Please note that other options are also available, and they are used in troubleshooting. Those options are discussed in the next chapter.

Daily Instrument Control Adj

This menu item reports the Daily Instrument Control value, then allows the user to make an adjustment to the PMT high voltage setting. Before starting, dispense 2.0 mL of the Daily Instrument Control into a clean, labeled cuvette. Place the cuvette in the $38 \pm 1^\circ\text{C}$ heating block and allow to warm for 15 minutes.

When the **START** key is pressed, **Insert DI. Control, Close Door: [ENTER]** displays. Insert the cuvette with 2mL Daily Instrument Control warmed to 38°C in the sample chamber, and close the cuvette door. Press **ENTER** to display the FLU reading. When **[YES]* ADJUST [NO]** displays, press **YES** to adjust the photomultiplier tube high voltage signal, and **NO** to keep the original setting and exit this function. YES causes the instrument to adjust the high voltage to FLU value 602, and display **[OK]* Adjust > [NO]**. Press **YES** to change the high voltage to this new setting and exit the function, or press **NO** to leave the high voltage at the original setting and exit the function.

Chamber Temp Adj

This menu item is used to adjust the temperature of the cuvette chamber. When **[-] 0.0 [+]** displays, use the < and > keys to choose how many $^\circ\text{C}$ down or up to adjust the cuvette chamber temperature. At the **[YES]* OK? [NO]** display, press **YES** to exit this function, or choose **NO** to make another adjustment.

Heater Block Adj

This menu item is used to adjust the temperature of the heater block. When **[-] 0.0 [+]** displays, use the < and > keys to choose how many $^\circ\text{C}$ down or up to adjust the heater block temperature. At the **[YES]* OK? [NO]** display, choose **YES** to exit this function, or choose **NO** to make another adjustment.

Set ID

This menu item enables a means of entering an identification number for each sample test result. When enabled, each test prompts for an identification number to be entered via the numeric keypad or optional barcode scanner. The identification numbers entered are displayed and sent to the printer port and the RS-232

port with the sample test results. Sample ID numbers may not exceed 13 characters.

Dis/Enable Beeper

By default, the fluorometer keypad has no audible indication that a key has been adequately pressed. The “**Dis/Enable Beeper**” function enables an audible indication.

Print Calib Ratios

This function will print the calibration points and ratio for all product channels that have been calibrated.

Output Avg F/min

This menu item enables or disables the output of the average rate of increase in fluorescence during a test. The value is displayed just before the test results.

Set Date/Time

See Chapter 1, Step 8 for instructions on setting the date and time on the instrument.

Set Serial Number

This function displays the instrument serial number. To change the instrument serial number, press the keys **2, 4** then **CLEAR** in sequence, then enter the new serial number. When you are finished, press the **ENTER** key. Press the **STOP** key to return to the Setup menu.

Set Serial Rate

This menu item enables changing the serial rate of the data sent to the RS-232 port. The available choices are 1200, 2400, 4800, 9600, and 19200 baud.

Set Product Names

This function is used to write product names to the various product channels using barcode reader input. The factory default product names are shown in Table 2.2. Press the < and > keys to display one of the three product categories: Milks (mU/L), Cheeses (mU/kg) and Other. Press the **ENTER** key when the desired category displays. Then use the < and > keys to display the desired channel from the 20 available channels in that category. Press the **ENTER** key to select the displayed channel. For category **OTHER**, you will be prompted to select a unit of measure (mU/L or mU/kg) after accepting the name change.

The pre-named channel names may be overwritten. At the **Scan New Name** display, use a barcode reader attached to the instrument to scan in an alternate name. The scanned

name displays briefly. When the [**OK**]* **Accept** >[**NO**] displays press **OK** to accept the scanned product channel name, or **NO** to leave the current name. Note that renaming a channel will erase any existing calibration for that channel.

The following table lists the product categories and 20 default channel names in each category.

Category	Milks (mU/L)	Cheeses (mU/kg)	Other
Channel			
1	Whole	Cheese A	Butter
2	Low Fat 1%	Cheese B	Buttermilk
3	Low Fat 2%	Cheese C	Cream
4	Skim	Cheese D	Light Cream
5	Chocolate	Cheese E	Heavy Cream
6	Powdered	Cheese F	Sour Cream
7	UHT	Cheese G	Ice Cream 1
8	Milk A	Cheese H	Ice Cream 2
9	Milk B	Cheese I	Other A
10	Milk C	Cheese J	Other B
11	Milk D	Cheese K	Other C
12	Milk E	Cheese L	Other D
13	Milk F	Cheese M	Other E
14	Milk G	Cheese N	Other F
15	Milk H	Cheese O	Other G
16	Milk I	Cheese P	Other H
17	Milk J	Cheese Q	Other I
18	Milk K	Cheese R	Other J
19	Milk L	Cheese S	Other K
20	Milk M	Cheese T	Other L

Table 2.2: Product categories and factory default product names

Data Capture

This menu item enables or disables the data capture feature which sends the FLU data to the serial port.

Product/Factory

Product/Factory is for factory use only and has no field function.

Chapter 3

Fluorophos® ALP Milk Pasteurization Assay

This chapter includes information specific to the Fluorophos® ALP milk assay. Please read this chapter and chapters 1 and 2 on instrument operation before running tests.

NOTE: All References cited in this chapter are assembled on page 24.

Overview

The Fluorophos® ALP Assay is a rapid test for verifying proper pasteurization of dairy products. Alkaline Phosphatase (ALP), found naturally in raw milk, is destroyed at temperatures slightly above the temperatures necessary to destroy microorganisms pathogenic to man. Reduced levels of ALP in finished dairy products ensure that the products have been heated to the proper temperature and have not been contaminated with raw milk.

The Fluorophos® ALP Assay allows you to measure the ALP activity in your dairy products, allowing you to monitor completeness of pasteurization and detect raw milk contamination.

Alkaline Phosphatase (ALP) background

Numerous fluorometric analytical methods have been developed for analytes of interest to the dairy industry, and Shipe has reviewed some of them¹.

In 1935, Kay and Graham developed an alkaline phosphatase (ALP) assay which could be used to detect the completeness of pasteurization in dairy products². They demonstrated that natural milk ALP was destroyed at temperatures slightly above those necessary to destroy micro-organisms pathogenic to man. Reduced levels of ALP in finished dairy products therefore ensured that the product had been heated to the required temperature or that it had not been contaminated with raw milk during manufacture. The significance of this discovery was appreciated immediately and the ALP test was soon universally accepted. To this day, measurement of ALP activity is the only chemistry test which can be used to confirm that dairy products have been correctly pasteurized.

The original Kay and Graham ALP assay used phenylphosphate as substrate and required a 24-hour incubation for quantitative analysis. Since then, many modifications and improvements have been introduced. Currently, all quantitative ALP assays use one of three colorimetric substrates. The Scharer³ and Cornell⁴

methods still use phenylphosphate as in the original Kay and Graham procedure; the Aschaffenburg and Mullen procedure uses p-nitrophenyl phosphate⁵; and the Rutgers assay uses phenolphthalein monophosphate⁶.

Quantitative methods using the original phenylphosphate substrate measure enzymatically liberated phenol by coupling it with a color reagent to form an indophenol. The blue indophenol is removed from interfering turbidity caused by the dairy product in one of two ways. Both the American Public Health Association (APHA)⁷ and the Association of Official Analytical Chemists (AOAC)⁸ have approved methods using butanol extraction followed by spectrophotometric measurement. In addition, both the APHA and the AOAC have approved methods in which the indophenol is read after protein precipitation with barium and zinc salts^{7,8}.

Phenolphthalein monophosphate is used as substrate in the quantitative Rutgers method. Liberated phenolphthalein is dialyzed into deionized water during a three-hour incubation followed by spectrophotometric reading of the dialysate⁸.

In late 1988, Advanced Instruments presented preliminary data on a new approach to ALP testing in dairy products¹¹. The method is based on a specially designed substrate called Fluorophos® which when acted upon by ALP, is converted to a highly fluorescent product, Fluoroyellow®.

The quantitative Fluorophos® assay is the first milk ALP test which permits the continuous and direct measurement of the released reaction product from a fluorogenic substrate. The use of this fluorometric substrate eliminates the interferences and nonspecificity encountered in colorimetric assays and avoids the need for dialysis, butanol extraction, or protein precipitation which are tedious and time consuming.

The Fluorophos® method has been collaboratively studied^{12,13} and received IMS/FDA, AOAC, SMEDP, IDF and ISO approval¹⁴.

The Fluorophos® method was correlated to Salmonella and Listeria inactivation by Eckner in 1992¹⁵. The application of the Fluorophos® method to the determination of both high and very low levels of ALP

- Fluorophos® ALP Cheese Extraction Buffer
 - Unopened/opened, store at 2° to 8°C (36° to 46°F) for up to 2 years

Reagent handling



The reagents used in the ALP Pasteurization Assay are irritating to skin, harmful if swallowed, and pose danger of serious damage to health by prolonged exposure if swallowed. Refer to the individual Safety Data Sheets for complete hazard information.



NOTE: Do not freeze any of the listed reagents. Make sure that all bottles and vials are sealed tightly after use to prevent evaporation. Store all bottles and vials upright to prevent spills and leakage.

Calibration

The FLA250 Fluorophos® ALP Calibrator Set consists of three calibrators labeled A, B, and C. The calibrators are ready to use and designed to be used directly from the bottle.

Calibrator set preparation

- Mix gently by inversion prior to each use to assure optimal results.
- Label two (2) cuvettes for each of the three (3) calibrators.
- Dispense 2.0 mL of the appropriate calibrator into each clean, labeled cuvette.
- Place the cuvette in the 38 ± 1°C heating block and allow to warm for fifteen (15) minutes.

Calibrating with a product sample

Samples should be prepared as they are for regular testing.

- Using a 75-µL pipette, add a 75-µL sample of the prepared product to each of the pre-warmed calibrator cuvettes.
- Cover with parafilm and mix gently by inversion or vortex mixer.
- Return each cuvette to the heating block. Calibration must be completed within ten (10) minutes after the addition of the sample.

d. Press **CALIB**.

e. Select the product category by pressing < or > to display **Milks (mU/L)**, **Cheeses (mU/kg)** or **Other**, and then press **ENTER**.

f. Select one of the 20 product channels to be calibrated by pressing < or >, or by using the numbered keys. When the desired product channel is displayed, select it by pressing **ENTER**.

If the channel has been previously calibrated, a printout of that calibration will be printed.

g. The fluorometer displays **Insert A STD: [START]**

h. Gently mix one (1) of the cuvettes containing Calibrator A and the sample.

i. Open the cuvette chamber door and place Calibrator A in the chamber. Close the door and press **START**.

j. After thirty (30) seconds, the fluorometer will make a final reading (approx. 200 FLU) and display **Insert A STD: [START]**. Repeat with a second sample of Calibrator A. If the measurement between the samples is too large, you may be asked for up to a total of four samples.

k. Follow the prompts for Calibrators B and C. If the calibration was successful, then the fluorometer display is **CALIBRATION COMPLETE** and the channel will be calibrated for that specific product.

The calibration data is stored in the fluorometer until a new calibration is performed for the selected product.

NOTES

- If the fluorometer displays **STANDARDS REVERSED?** instead of prompting you to proceed with the next calibrator level, check to make sure that both cuvettes contain the correct calibrator. Prepare two new calibrator samples and repeat the procedure until the instrument prompts you to advance to the next calibration level.
- Calibration should be completed within 10 minutes from addition of product to calibrator.
- If the calibration is not accepted by the fluorometer, prepare new calibrators with the selected product and repeat the entire calibration procedure.
- The calibration procedure can be interrupted at any point during the calibration process by pressing **STOP**. If no test is in progress, the entire calibration will be cancelled. To continue calibration from that point, you must re-enter the calibration mode by pressing **CALIB** and starting the calibration procedure from the beginning.

However, if a test is in progress when **STOP** is pressed, only the test in progress will be cancelled. The cancelled test may be re-run by inserting another standard of the same value and pressing **START**, or the entire calibration sequence may be cancelled by pressing **STOP** again. The fluorometer will discard the new calibration data and revert to the previous calibration status.

How to determine the need for different calibration curves: calibration ratio and product type

As a general rule, when the calibration ratios for different products are within 5% of each other, those products may be run on the same channel.

For example, if a 2% Low Fat milk has a calibration ratio of 127.2 and a 3.2% Whole Milk has a calibration ratio of 133.3, both products may be run on the same channel. The differences in calibration ratios between different products are due to differences in fat content (2% vs. 3.2% in the previous example) and differences in color or added flavor (white vs. chocolate milk).

In all cases, use controls for verification when running different products on the same channel.

The four general rules for determining whether separate calibration curves are required are:

- All colored (flavored) products will require separate calibration curves.
- The fat content of individual dairy products determines whether the products can be run on the same calibration curve. The closer the fat content, the more likely the products can be run on the same channel.
- When the calibration ratios of two different products are within 5% of each other, the two products may be run on the same calibration curve.
- In all cases: When two products are spiked with 0.1% (v/v) mixed-herd raw milk and run on their own calibration curves and give the same results within $\pm 10\%$, then both products may be run on the same channel.

Reconstituting reagents

Each ALP Reagent Set contains two bottles each of Fluorophos® ALP Substrate and ALP Substrate Buffer. These items must be used together; do not intermix bottles from different reagent sets.

- a. Allow one bottle of Fluorophos Substrate and one

bottle of ALP Substrate Buffer to come to room temperature.

- b. To reconstitute, carefully decant the entire contents of the Substrate Buffer into the Fluorophos Substrate bottle.
- c. Mix by gentle inversion and allow to sit at room temperature for at least 30 minutes, or until fully dissolved.

Daily instrument checks

- a. Dispense 2.0 mL of the Daily Instrument Control into a clean, labeled cuvette.
- b. Place the cuvette in the $38 \pm 1^\circ\text{C}$ heating block and allow to warm for fifteen (15) minutes.
- c. Press **SETUP** on the keypad. When **[*] Setup/Diag [>]** displays, press **>** and then **ENTER** to enter the Diagnostics menu. When **A/D TEST** displays, press **START** to enter A/D mode.
- d. With nothing in the cuvette chamber, the display should read **302 \pm 4**.
 - If the value is outside of the specified range, clean the excitation and emission filters and repeat the A/D Test.
- e. Insert the pre-warmed Daily Instrument Control cuvette into the cuvette chamber and close the door.
- f. Once the display has stabilized, it should read **602 \pm 12**. Record the value.
 - If the value is outside of the specified range, the value can be corrected to 602 ± 2 with the **Daily Instrument Control Adj** function in the Setup menu.
 - If the instrument is adjusted to 602 ± 2 or has experienced excessive drift, it should be recalibrated with the Calibrator Set (P/N FLA250).
- g. Dispense 2.0 mL of the reconstituted substrate into a clean, labeled cuvette.
- h. Place the cuvette in the $38 \pm 1^\circ\text{C}$ heating block and allow to warm for 15 minutes.
- i. After 15 minutes, insert the pre-warmed reconstituted substrate cuvette into the cuvette chamber and close the door.
- j. Once the display has stabilized, it should read **< 1200**. Record the value.
 - If the value is above 1200, do not use this substrate. Reconstitute a new bottle of substrate and retest. Being open for more than 60 days, light, contamination, or not being refrigerated when not in

use could cause the A/D result to be above 1200. Avoid this situation by keeping the reconstituted substrate at 2-8°C, protected from light, and pouring off the amount that is going to be used into a clean vessel for pipetting.

Using PhosphaCheck Pasteurization Controls

PhosphaCheck Pasteurization Controls are used to monitor the performance of the pasteurizer and the alkaline phosphatase test system. Use PhosphaCheck Positive, Negative, and Normal Controls to verify the test procedure and the reliability of reagents, and monitor the precision of the assay.

- a. Remove the metal cap and rubber stopper on the vial.
- b. Add three milliliters deionized water to the vial.
- c. Replace the rubber stopper and mix by gentle inversion for one minute. Do not shake the controls or allow them to foam. Allow to sit at room temperature for at least 15 minutes, or until it is visibly homogeneous.
- d. Mix gently prior to each use to assure optimal results.

NOTE: Calibrate an unused channel of the Fluorometer using the PhosphaCheck Negative Control. Use the standard calibration procedure, substituting the Negative Control for the milk sample.

Test the Positive, Negative, and Normal Controls on this calibrated channel. Use the fluid milk test procedure, substituting the PhosphaCheck Controls for the milk sample.

Control Type	Expected Values
PhosphaCheck Positive Control	500 ± 100 mU/L
PhosphaCheck Negative Control	< 10 mU/L
PhosphaCheck-N Normal Control	< 40 mU/L

Table 3.1: PhosphaCheck expected values

Sample preparation

The following sample preparation procedures have been used with the Advanced® ALP Fluorophos® fluorometric method:

- a. **Whole milk, skim milk, low fat milk, chocolate milk, half and half, and heavy cream:** Mix the sample thoroughly before testing.
- b. **Buttermilk, sour cream, and creamed cottage cheese:** Homogenize in a mechanical blender before testing.
- c. **Concentrated and dry-milk products:** Reconstitute the product with laboratory grade water as necessary and

test according to the procedure for the original product.

- d. **Butter:** To each labeled 16 x 100 mm screw cap glass test tube, add a 0.5-gram butter sample taken from the center of the block. Add 5.0 mL Advanced Instruments FLA005 Cheese Extraction Buffer. Warm about 10 minutes in a 38°C water bath to allow the butter to dissolve in the buffer. Remove the tube and gently mix by inversion over parafilm or use a vortex mixer. Test as for fluid dairy products except multiply the mU/L printout by 10 to correct for the tenfold dilution, as explained in Appendix B.
- e. **Cheese:** To each labeled 16 x 100 mm screw cap glass test tube, add a 0.5 gram cheese sample. Add 5.0 mL Advanced Instruments FLA005 Cheese Extraction Buffer and macerate with a glass rod. Centrifuge 10 minutes at 1000xg. Sample from the upper layer and test as for fluid dairy products except multiply the mU/L printout by 10 to correct for the tenfold dilution as explained in Appendix B.

Determining the need for the high-turbidity test procedure

Some dairy products, such as ice cream mixes, are so turbid (i.e. have such a high fat content), that a slight change in the calibration procedure is required. Samples that cause this issue usually have greater than 10% fat and/or are very highly-colored products, such as chocolate and blueberry ice cream mixes.

Reducing the sample volume to 25 µL will appreciably decrease turbidity and permit calibration and testing of these products.

The 25 µL procedure should be used when the following conditions exist:

- Results of controls and calibration are acceptable when used with other products.
- The suspect product has a very dark color and/or has a fat content over 10%.
- The calibration ratio when using the suspect product is less than 50.
- The instrument cannot be calibrated because the fluorescence of Calibrator B is too close to the fluorescence of Calibrator C.

If any of these conditions exist, follow the calibration procedure for high-turbidity products.

Calibrating and testing high-turbidity samples

To calibrate and test high-turbidity samples use the same technique described for calibration and testing of normal samples, but use a 25- μ L sample. You can use the specially designed 25- μ L pipette supplied with your instrument to handle a sample of this size. To account for the times three dilution for this procedure, you will need to multiply your printed result by three.

NOTE: If the cheese extraction buffer is also used, the total dilution factor is 30 (3 x 10).

Test procedure

When the fluorometer is ready for use, follow these instructions to run a test.

Note: The test must be started within 20 seconds after addition of the product to the working substrate.

- a. Dispense 2 mL of the reconstituted Fluorophos® ALP Substrate into labeled fluorometer cuvettes for each test.
- b. Place the cuvettes into the heating block and incubate for 15 minutes at $38 \pm 1^\circ\text{C}$.

Note: Reconstituted Fluorophos® ALP Substrate is stable for 6 hours in the heating block.
- c. Pipette 75 μ L of the product to be tested into one of the preheated cuvettes containing reconstituted Fluorophos® ALP Substrate. Do not allow the sample to sit in the heating block once the product to be tested has been added to the reagent.
- d. Mix well with the vortex mixer.
- e. Place the cuvette in the fluorometer cuvette chamber and close the door.
- f. If [CALIB] Calibration/Product [TEST] or Date and Time displays, press the TEST key.
- g. If necessary, use the < or > keys to display the appropriate product category from Milks (mU/L), Cheeses (mU/kg), or Other, and then press ENTER.
- h. As necessary, use the < or > keys or the number keys to change to an appropriate channel for the sample to be tested and then press ENTER again.
- i. If the Sample ID function is enabled, use the fluorometer keypad or barcode scanner to enter an identification number for the sample being tested and then press ENTER.
- j. Make sure the cuvette door is closed and then press START to begin the test. The display will count down 60 seconds while the substrate and sample are being stabilized at 38°C .

- k. After 60 seconds the fluorometer will begin to measure and display the fluorescence of the sample in fluorescence units (FLU).
- l. After three minutes, if the function is enabled, the fluorometer displays the average increase in fluorescence and the ALP activity in mU/L or mU/kg.
- m. Remove the cuvette from the cuvette holder.
- n. If desired, test another sample in the same manner.
- o. When finished with ALP testing, press STOP a second time to return to the [CALIB] Calibration/Product [TEST] display.

Interpretation of results

Any test that yields a test result under 350 is considered ALP NEGATIVE. No further action is required because a negative result indicates proper and complete pasteurization.

Any sample that yields a test result over 350 mU/L is considered POSITIVE for bovine, reactivated and/or microbial ALP. If a sample tests positive, you must perform confirmation tests to determine whether the sample is positive for bovine, reactivated and/or microbial ALP.

Note: Refer to Appendix A for confirmation tests: Differentiating bovine (residual) ALP from microbial and/or reactivated ALP.

Citations

1. Shipe, W.F. *Fluorometric Methods: Applications and Limitations in Challenges to Contemporary Dairy Analytical Techniques*. Royal Society of Chemistry, London, 1984.
2. Kay, H.D. and Graham, W.R., The phosphatase test for pasteurized milk. *J. Dairy Res.* 6:191-203 (1935).
3. Scharer, H., A rapid phosphomonoesterase test for control of dairy pasteurization. *J. Dairy Science* 21:21-34 (1938).
4. Kosikowski, F.V., A simple universal dairy products phosphatase test. *Science* 110:480-481 (1949).
5. Aschaffenburg, R. and Mullen, J.E.C., A rapid and simple phosphatase test for milk. *J. Dairy Res.* 16:58-67 (1949).
6. Kleyn, D.H. and Lin, S.H.C., Collaborative study of new alkaline phosphatase assay system for milk. *J.O.A.C.* 51:802-807 (1968).
7. *Standard Methods for the Examination of Dairy Products*, 17th Edition, 2004. American Public Health Association, Washington, DC.
8. *Official Methods of Analysis*, 20th Edition, 2016. Association of Official Analytical Chemists, Arlington, VA.
9. Milk - Determination of alkaline phosphatase, ISO 3356/IDF 435:2009. International Dairy Federation, Brussels, Belgium.
11. Rocco, R.M. and Bargoot, F.G., An improved assay for alkaline phosphatase in fluid milk products. Presented at the 102nd AOAC Annual Meeting, Palm Beach, FL, August 29, 1988.
12. Rocco, R.M., Fluorometric Determination of Alkaline Phosphatase in Fluid Dairy Products: Collaborative Study, *J. Assoc. Off. Anal. Chem.* 73, Nov-Dec. 1990.
13. Fluorimetric method for the determination of alkaline phosphatase activity in cow's milk cheese: Interlaboratory collaborative study. *Bulletin of the IDF* No. 482:2016. International Dairy Federation, Brussels, Belgium.
14. ISO 11816-1/IDF 155-1:2013 Milk and Milk Products - Determination of Alkaline Phosphatase Activity - Part 1: Fluorometric method for milk and milk based drinks.
15. Eckner, K.F., Fluorometric Analysis of Alkaline Phosphatase Inactivation Correlated to Salmonella and Listeria Inactivation, *J. Food Protection* 55:960-963 (1992).
16. Lechner, E., and Regensburger, V., *Alkaline Phosphatase Activity in Milk and Milk Products*, Translated from *Deutsche Milchwirtschaft* 44 (17) 815-819 (1993).
17. ISO 11816-2/IDF 155-2:2016 Milk and Milk Products - Determination of Alkaline Phosphatase Activity - Part 2; Fluorometric Method for Cheese.
18. Rocco, R.M., Fluorometric Analysis of Alkaline Phosphatase in Fluid Dairy Products, *J. Food Protection* 53:588-591(1990).

Instrument Maintenance



This chapter describes the procedure for maintaining your instrument. Make sure the Fluorometer is unplugged when you clean or maintain it.

Daily controls

Use the Advanced Instruments Daily Instrument Control to monitor the day-to-day drift of the fluorometer, and to help determine when the instrument should be re-calibrated. If values fall outside of the ranges indicated, then a fluorometer drift problem can be quickly identified and corrected.

To order Daily Instrument Control, contact Advanced Instruments or an authorized representative.

Cleaning Spills

If a liquid is spilled into the fluorometer, unplug the power cord immediately to avoid electrical accidents. Small spills may generally be cleaned up and wiped off with the aid of a mild non-abrasive detergent.

Do not immerse the fluorometer in water under any circumstance.

Heating block spill

If a liquid is spilled or glass is broken such that it falls into the heating block wells, immediately unplug the power cord, remove the thermometer and cuvettes from the heating block and clean them separately. Remove the heating block from the instrument, and use a test-tube brush to clean the heating block wells. Take care not to scratch the anodized finish.

If a liquid spills around the heating block into its compartment in the top of the instrument, immediately unplug the power cord and remove the heating block. Carefully remove the liquid with a sponge or other absorbent material, and then clean the affected surfaces. Do not replace the heating block until all parts are dry. Place the power switch in the OFF position before re-connecting the AC power cord.

Cuvette holder spill

Failure to clean liquid spills out of the cuvette holder may result in incorrect fluorometer response due to dirty optics. If a liquid is spilled or glass is broken such that it falls into the fluorometer cuvette chamber, take the following steps before drying occurs:

a. Unplug the power cord immediately.

b. Remove any cuvette from the cuvette holder.

c. Use tweezers, forceps, and other tools as necessary, to remove any large pieces of broken glass from the cuvette holder.

d. Remove any liquid from the cuvette holder by inserting a cuvette holder cleaner straight down, firmly to the bottom of the cuvette holder. Allow the cleaner to absorb liquid for about 20 seconds, then remove. Discard the used cleaner carefully, as it may have picked up sharp bits of broken glass from the cuvette. Repeat with new cuvette holder cleaners as necessary until the cuvette chamber is empty.

e. Once all liquid has been removed from the instrument, remove the optical filters. The excitation filter is located to the right of the cuvette chamber, and marked "ALP". It will slide up and out of its slot. The emission filter is to the rear of the cuvette chamber in a slot which is capped. Remove the two screws at either end of the filter cap, remove the cap, then slide the filter up and out of its slot. Excess liquid spilled in the cuvette chamber should have escaped the chamber through a relief channel before reaching the optical filters. Examine both filters for any liquid, and if present, obtain Hot-Line™ Service as described in Chapter 5, as a more thorough instrument cleaning and check is required.

f. With the optical filters still removed from the instrument, also remove the 20-sample heating block from the cover of the instrument. Tip or invert the instrument as necessary to remove any broken glass remaining in the cuvette holder.

g. Moisten a clean, dry cuvette chamber cleaner with a small amount of distilled water; using large amounts of water can damage the optical filters. Insert the cleaner all the way to the bottom of the cuvette holder and twist to clean the entire surface of the cuvette holder. Repeat with two or three new cuvette holder cleaners to dry the cuvette holder. Replace optical filters and the emission filter cap and screws.

h. With the power switch in the OFF position, connect the power cord to the instrument and the power outlet.

- i. Turn the instrument on, and allow it to warm up.
- j. Run controls to verify instrument performance. If problems are evident when running controls, obtain Hot-Line™ Service as described in Chapter 5.

Reagent storage

See information supplied with each reagent for storage instructions.

Optical filter replacement

Because filters age and can easily be degraded by spills, scratches, and other things, the excitation and emission filters should be replaced annually.

To replace filter assembly:

- a. Turn off the fluorometer power switch and unplug the power cord from the power outlet.
- b. Carefully remove any cuvette from the cuvette holder. If there is any evidence of broken glass or spills in the cuvette holder, follow the cleanup procedure in this chapter.
- c. Facing the front of the instrument, the excitation filter holder is located to the right of the cuvette holder. Slide the excitation filter holder straight up and out of the instrument. If the filter holder is too slippery to grip, use a piece of masking tape to improve your grip and provide a pull tab.
- d. Slide in the new filter holder.
- e. Facing the front of the instrument, the emission filter is located behind the cuvette holder. Remove the two screws at either end of the emission filter cap, lift this piece up and remove the filter holder.
- f. Slide in the new filter holder and replace the cap and screws.

Optical filter care

Use extreme caution whenever you handle a filter or filter assembly. Handle optical filters only by the edges and never on the optical surfaces. The filters scratch very easily.

To clean the filter, use a piece of lint-free, non-abrasive tissue and a small amount of 70% isopropanol solution. Be sure the filter is completely dry before re-installing.

Replace excitation and emission filters annually.

Pipette maintenance

The tips supplied with the pipette are stated by the manufacturer to be chemically resistant to most reagents except chlorinated hydrocarbons. If the pipette piston (plunger tip) becomes worn or otherwise unusable and a replacement piston is available, please follow the replacement instructions supplied with the pipette. Always re-calibrate the pipette after replacing the piston or other internal parts, referring to the calibration instructions supplied with the pipette.

To eliminate possible sample-to-sample contamination between pipetting sessions, remove and replace the pipette tip after each sample.

Fuse replacement

If you determine that the instrument is not functioning because of blown fuses, you will need to replace the fuses using the following procedure.

- a. Switch the power switch to the off position and disconnect the power cord.
- b. Use a small flat-bladed screwdriver or similar tool to pry open the fuse holder door. Remove the fuse holder.
- c. Double-check the values marked on the fuses. Use 5 x 20 mm, 250V, time delay (Type T) 1-Amp fuses.
- d. Reinstall the fuse holder into the back of the instrument and close the fuse holder door.
- e. Reconnect the power cord and switch the power switch to the ON position. The instrument should start up as normal.

Troubleshooting and Service

This chapter contains very basic information to help you solve problems that might arise with your fluorometer. Please read all instructions very carefully, and if a solution cannot be found in this guide, contact Advanced Instruments for Hot-Line™ Service, or your local distributor.

Service and maintenance cautions

- Do not perform any service or maintenance yourself, except as detailed in this User's Guide.
- Unplug the power cord prior to opening or removing the cover, or else you may be exposed to electric shock, excessive temperatures, or mechanical hazards.
- Performing service or maintenance not detailed in the User's Guide, with or without a Service Manual, should only be done by a qualified service technician.
- Never restrict airflow into or out of the product. Occasionally, check the air vents for blockage.
- Wipe the exterior of the product with a soft, damp cloth as needed. Using cleaning products other than those specified, may discolor or damage the finish.
- If the product requires service for any of the following reasons, unplug the product from the electrical outlet and refer service to a qualified service technician.
 - The power cord, extension cord, power strip, or power input module is damaged.
 - Liquid has been spilled into the interior of the product.
 - A foreign object has fallen into the product.
 - The product has been dropped or damaged by a falling object.
 - There are noticeable signs of overheating or a burning odor.
 - The product does not operate normally when you follow the operating procedures.
 - The main supply fuse(s) or any internal fuse(s) continually fail.



- A discharge of static electricity from contact with the human body or other conductor may damage system boards or static sensitive devices. Never perform internal maintenance without following recommended static protection procedures.

- The product is equipped with operator accessible fuses. If a fuse blows, it may be due to a power surge or failure of a component. Only replace the fuse once. If the fuse blows a second time, it is probably caused by failure of a component part. If this occurs, refer service to qualified service personnel. Always replace the fuse with one of the same rating, voltage, and type. Never replace the fuse with one of a higher current rating.
- When servicing the product, use only factory-specified parts.
- The product contains an integral lithium battery that is not user-serviceable.

WARNING: When returning this product for service, or shipping this product to a second location, remove all hazardous specimens and decontaminate the product before packaging for shipment. If the product cannot be decontaminated, consult with your shipping agent on appropriate packaging and marking.

Obtaining service

Before contacting Advanced Instruments for Hot-Line™ Service, be sure to read through this chapter. If this information does not solve your problem, call the appropriate number below.

800-225-4034 (toll-free within the USA and Canada, 8:00am-4:30pm EST; after normal business hours, follow the voicemail instructions)

+1 781-320-9000 (elsewhere)

+1 781-320-0811 (fax)

If you purchased your instrument outside of the U.S. or Canada, please contact your Advanced Instruments authorized dealer for service or repair.

When contacting our service personnel, please have ready the model and serial numbers from the label on the back of your instrument, your user's guide or service manual, and the symptoms of your problem.

You should use a telephone as close to your instrument as possible to facilitate making recommended diagnostic checks.

If you need to order parts or service, a purchase order from your purchasing agent will be necessary.

After Hot-Line™ diagnosis, a service technician may assist in making minor repairs over the phone, providing you with recommended parts, part numbers, or may issue an authorization to ship the instrument for factory repair.

To return an instrument for repair or replacement

- a. Notify our service department to obtain an RMA (Returned Material Authorization) number.
- b. Contact Advanced Instruments before shipping to avoid any delays.
- c. Carefully pack and send the instrument, using its own specially-designed carton and packaging material. If you did not save the carton and packing materials, please request one from Advanced Instruments or an authorized representative.
- d. Be sure to prepay for any shipment to the factory. Advanced Instruments cannot accept collect shipments without prior approval. Please insure the shipment or accept the damage risk.
- e. Do not tip, store, or ship the fluorometer without first removing any cuvette containing liquid from the cuvette chamber and heating block. Please use cuvette chamber cleaners to remove any liquid that may be in the cuvette chamber.

Troubleshooting checks

Check operational requirements. If you are experiencing difficulties with your instrument, first carefully review the operational requirements listed in the product specifications and the recommended setup and operating procedures.

Check fuses. You will find the power switch and fuse holder beside the power cord connector on the left side of the instrument. Move the power switch to the off position and disconnect the power cord. Use a small flat-bladed screwdriver or similar tool to pry open the fuse compartment door. Slide out each fuse holder. Visually check for a blown fuse. If there is any doubt, test the fuses with a continuity checker or ohmmeter or simply replace them with new fuses.

Check error messages. The software of your instrument is designed to display specific error messages, many self-explanatory, that will help you discover the source of your problem.

You can find all error messages and descriptions of what they mean in Appendix B.

Internal diagnostics

Your instrument has been equipped with a series of diagnostic tests to allow you to isolate any problem. To access diagnostic checks, use the following procedure.

- a. To access the Setup menu, press the **SETUP** key when the display is **[CALIB] Calibration/Product [TEST]**. The display changes to **"[*] Setup/Diag [>]"**. Press the **>** key and then the **ENTER** key to enter the Diagnostics menu.
- b. To select another test on the Diagnostics menu, repeatedly press **>** or **<** until the desired item displays.
- c. To activate a displayed test, press **START**.
- d. Press **STOP** to exit the menu.

The following menu choices allow you to change operating settings. Please note that other options are available, but are settings, and are discussed in Chapter 2 Instrument Operation.

A/D Test

The A/D test is used to check the accuracy of the A/D conversion channel or to monitor the A/D channel for drift over time or temperature.

Barcode Test

This test performs a continuous check of the barcode port. Press the **START** key to enter the test. At the **"[START] Test [STOP]"** display press the **START** key once the barcode scanner is connected, or press the **STOP** key to return to the main menu.

After you press **START** you can begin scanning barcodes. If your code will not scan, check the manufacturer's user's guide to verify correct setup of the scanner for your symbologies. Press **STOP** to exit to the test menu.



NOTE: This test will only work after power on diagnostics have been completed.

Display/Print Test

Display/print test is a simple check of the display, serial port, and printer. Press the **START** key to run the test. It consists of a series of characters that scroll across the display, and a single line of the same characters print on the printer and serial port. The characters displayed have been chosen to illuminate every dot in the character matrix. This makes it possible to distinguish any dots that no longer work on the display. Press **STOP** to end the test.

Beeper Test

This test exercises the beeper. At the **[ON] BEEPER [OFF]** display, press the < key to exercise the beeper, and press the > key to stop exercising the beeper. Press the **STOP** key to exit this function.

Keypad Test

The keypad test enables checking each key on the keypad except the STOP key, and the lamps of each illuminated key. Press **START** to begin the test, then press each individual key to be tested. As each key is pressed, its name should be displayed and the lamp of each illuminated key should light. Press **STOP** key twice to end the test.

Assistance

Provides the manufacturer's name, address, telephone, fax and internet information and the instrument model, software version, and serial number. After the display, this information prints for your records.

Event Record

This function recalls the last 200 instrument events and is used to aid service in reviewing what is happening with your instrument. Press the < and > keys to choose the destination for the data: the printer, the serial port, or both, and press the **ENTER** key to send the data. The test result is not included in this event record.

Appendix A

Additional Technical Information

Calibration equations

Calibration ratio refers to a number used by the fluorometer for calculating unknown test values and is derived as follows:

$$\text{Calibration Ratio} = [(\text{CAL C}-\text{A}) + 2 (\text{CAL B}-\text{A})]/4$$

Assume 191 is the mean of the Calibrator A fluorescence readings, 317 the mean of Calibrator B, and 448 the mean of Calibrator C. For these averages:

$$\text{Calibration Ratio} = [(257) + 2 (126)]/4 = 127.2$$

The fluorometer also uses the calibration ratio for linearity checks during calibration and will only accept calibration readings which are linear and within specified ranges. The linearity of the calibrators may be manually checked as follows:

$$\text{Lin. CAL A (0 } \mu\text{moles/L FY)} = 191-191 = 0$$

$$\text{Lin. CAL B (17.24 } \times 10^{-3} \text{ } \mu\text{moles/L FY)} = 317-191 = 126$$

$$\text{Lin. CAL C (34.48 } \times 10^{-3} \text{ } \mu\text{moles/L FY)} = 448-191 = 257$$

In every case, the value of Calibrator C must be twice ($\pm 10\%$) the value of Calibrator B. If not, repeat calibration with fresh calibrators or a new lot of calibrators.

Manual calculations for fluid dairy products

The Advanced® FLM300 Fluorometer printout reports the ALP activity of the test sample (mU/L) as well as the average fluorescence increase (Avg F/min). Alternatively, the ALP activity may be calculated, from the printout of the calibrator values and the average increase in fluorescence of the sample, as follows:

a. Calibration: as the calibration process is accomplished, printouts *similar* to the following should result:

A STD = 198

A STD = 198

B STD = 345

B STD = 344

C STD = 475

C STD = 475

Avg A STD = 198

Avg B STD = 344

Avg C STD = 475

Calibration Ratio = 142.2

Note: The values above are examples and for instructional purposes only - Do not use for the calculation of unknown values.

b. Enzyme units: one unit of ALP is that amount of enzyme which catalyzes the transformation of one micromole of substrate per minute per liter of sample. Because of the low levels of ALP in pasteurized fluid dairy products, results are reported in milliunits per liter (mU/L).

c. Calculation equation:

1. Calculate the $\mu\text{moles FY}/\text{min}/0.075 \text{ mL}$ sample using the corrected fluorescence reading of the "B" calibrator (Calibration Ratio used for automatic fluorometer calculations) which contains $3.448 \times 10^{-5} \mu\text{moles of FY}/2 \text{ mL}$:

$$([\text{Avg F}/\text{min}/0.075 \text{ mL sample}]/[\text{Avg F of corrected "B" Calibrator}]) (3.448 \times 10^{-5}) = \mu\text{moles FY}/\text{min}/0.075 \text{ mL}$$

2. Determine the $\mu\text{moles of FY}$ formed by one liter of sample:

$$([\text{Avg F}/\text{min}/0.075 \text{ mL sample}]/[\text{Avg F of corrected "B" Calibrator}]) (3.448 \times 10^{-5}) (13333) = \text{U/L}$$

3. Convert U/L into mU/L:

$$([\text{Avg Avg F}/\text{min}/0.075 \text{ mL sample}]/[\text{Avg F of corrected "B" Calibrator}]) (3.448 \times 10^{-5}) (13333) (1000) = \text{mU/L}$$

4. Simplified calculation:

$$\text{Avg F of corrected "B" Calibrator} = (\text{Avg B STD} - \text{Avg A STD}) = (344 - 198) = 146$$

$$([\text{Avg F}/\text{min}/0.075 \text{ mL sample}]/[\text{Avg F of corrected "B" Calibrator}]) (459.7) = \text{mU/L}$$

Using the example above (a.) and the equations in (c.) above, the following results were calculated:

Fluorescence of corrected "B" Calibrator = 146 Fluorescence Units

Avg F for Test Sample = 114.5 Fluorescence Units/min

$$(114.5/146) (459.7) = 360.5 \text{ mU/L}$$

Calculation for butter and cheese

The extraction of 0.5 g of butter or cheese into 5.0 mL of extraction buffer results in a ten-fold dilution and requires the mU/L printout on the fluorometer tape to be multiplied by 10.

$$\text{mU/L} (10) = \text{mU/kg}$$

Percent raw milk and ALP enzyme activity

U.S. Federal Standards require that cows' milk intended for human consumption contain less than $0.7 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ of ALP activity. This value of $0.7 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ is equivalent to 350 mU/L of ALP enzyme activity using the Fluorophos® test method. The European Commission Regulations (EC 1664/2006) require ALP activity in heat treated milk to be less than 350 mU/L, where alkaline phosphatase activity must be determined using the Fluorophos method (ISO 11816-1).

It is important to remember that the cut-off values established by all regulatory agencies are based on residual levels of ALP "activity" not on weighed-in values for the enzyme or on a percent of raw milk remaining in the finished product. Experience over the years has shown, however, that a 0.1% (v/v) dilution of mixed-herd raw milk will give approximately 1 to $2 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ or 500 mU/L of ALP activity.

The reason that the values obtained with a 0.1% (v/v) raw milk dilution are approximate is that the original enzyme activity of the mixed-herd raw milk sample will not always contain the same amount of enzyme from sample to sample.

The total amount of enzyme in the mixed-herd sample will vary from season to season, and, possibly, from breed to breed. For example, in order to obtain a dilution of raw milk at 0.1% (v/v) which contains exactly $1 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ activity the original mixed-herd milk sample must have contained $1,000 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ activity. When $1,000 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ activity is diluted to 0.1% (v/v) (x10,000 dilution), the expected value will be $1.0 \mu\text{g phenol}/\text{mL}/15 \text{ min}$ activity. A slight variation in biological sampling of a mixed-herd population could reduce the expected value for a 0.1% (v/v) dilution of this original mixed-herd raw milk to $0.9 \mu\text{g}/\text{mL}/15 \text{ min}$.

The table on the next page shows the results in which a sample of mixed-herd raw milk was added to heat-treated milk from 0.003 to 0.4% and the samples tested by various methods, including Fluorophos.

Appendix A Additional Technical Information

% Raw Milk (v/v) in Whole Milk ¹	ALP ACTIVITY ³		
	Fluorophos mU/L	AOAC 979.13 µg phenol/mL/15 min	µg PNP/mL/2 hrs
0.003	15.6	0.03	0.312
0.006	31.2	0.06	0.625
0.012	62.5	0.12	1.25
0.025	125.0	0.25	2.50
0.050	250.0	0.50	5.00
0.070	350.00 ⁶	0.70 ⁴	7.0
0.100	500.0	<1.00	10.00 ⁵
0.200	1000.0	2.00	20.00
0.400	2000.0	4.00	40.00
100.00 ²	500,000.0	1000.00	10,000.00

Notes from the table above:

1. Whole milk (3.2% fat) was heated to 95°C for two minutes and found to contain less than 10 mU/L of ALP activity by the Fluorophos® ALP method. Fresh mixed-herd raw milk was then added at the above v/v concentrations.
2. Extrapolated from dilutions made as in note 1.
3. Above values are based on the assumption that the mixed-herd raw milk which is used to make the above dilutions contains exactly 5000,000 mU/L or 1000 µg phenol/mL/15 min ALP activity. This is not always true in that the total ALP activity in mixed-herd raw milk before pasteurization will vary due to species and seasonal variations.
4. Cutoff for acceptable milk is less than 0.7 µg phenol/mL/15 min. Pasteurized Milk Ordinance (PMO) Grade A, 2015 Revision, U.S. Department of Health and Human Services, FDA, Washington, D.C.
5. Cutoff according to Codex Alimentarius Commission Code of Hygienic Practice for Milk and Milk Products, CAC/RCP 57-2004.
6. Cutoff according to European Commission Regulation (EC) No 1664/2006.

Differentiating microbial from bovine milk ALP

Principle: Alkaline Phosphatase (ALP EC 3.1.3.1) synthesized by micro-organisms is heat stable at 145°F or 63°C, whereas bovine milk ALP is inactivated at this temperature.

Procedure:

- a. Pipette 1.0 mL of the milk being tested into the bottom of a labeled 16 x 150 mm glass screw-cap culture tube, without allowing any of the milk to touch or adhere to the sides of the tube.
- b. Place the screw cap on the tube, but do not seal completely, to allow for gas expansion. Place the tube in a $63 \pm 0.5^\circ\text{C}$ water bath for 30 minutes (or 66°C if the fat content is over 10%). During this time, gently mix the tube once every 10 minutes.
- c. Remove the tube from the water bath and allow to cool for at least five minutes in a cold water bath.
- d. Re-test for ALP activity. The flow diagram in Fig. A.1 will assist in interpretation of the results.

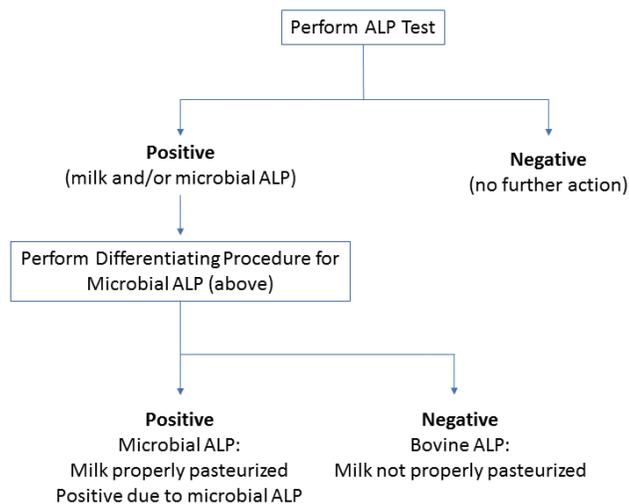


Fig. A.1: Microbial ALP Test Flow Diagram

REF: Standard Methods for the Examination of Dairy Products, 17th Ed. (Ed. H. Wehr, J. Frank) 2004, p349, American Public Health Association, Washington, D.C.

Differentiating residual from reactivated alkaline phosphatase (ALP) in fluid milk products

1. Definitions:

- a. Residual ALP: ALP enzyme remaining in the product due to raw milk contamination and/or incomplete pasteurization.
- b. Reactivated ALP: ALP enzyme that was inactivated due to pasteurization (usually UHT treatment), which recovers its activity due to storage conditions.

2. Reagent: Magnesium Acetate (40.1 mg of Mg^{++}/mL): Dissolve 35.4 g of $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$ in about 50 mL of DI water. Warm to dissolve completely, then bring to 100 mL with additional DI water. This solution is stable for 6 months at 2-8°C.

Appendix A Additional Technical Information

3. Procedure:

- a. Place 10 mL of the test product in a suitable glass container and heat in a boiling water bath for 30 minutes after the test product temperature reaches 95°C. Cool rapidly.
- b. Place 5.0 mL of the (unheated) test product in each of two 16 x 100 mm screw-cap test tubes.
- c. Add 0.1 mL of DI water to one of the test tubes to constitute a "Blank" sample.
- d. Add 0.1 mL of the Magnesium Acetate reagent (prepared previously) to the second test tube to constitute a "Test" sample.
- e. Cap both test tubes, mix well and incubate for 1 hour at 34°C. Remove the test tubes from the heated bath and cool in an ice bath for five minutes.
- f. Run an ALP activity test on the "Blank" sample.
- g. Add 1.0 mL of the "Test" sample to 5.0 mL of the heated and cooled test product from step 3.a.
- h. Run an ALP activity test on the "Test" sample diluted product from step 3.g.

4. Interpretation of Test Results:

- a. If the ALP activity of the "Test" sample diluted product (x 6) is equal to, or greater than, the ALP activity of the "Blank" sample, the original product is considered negative for residual ALP activity (as defined in item 1.a), indicating that the assayed activity was reactivated ALP in origin (as defined in item 1.b).
- b. If the ALP activity of the "Test" sample diluted product (x 6) is less than the ALP activity of the "Blank" sample, the original product is considered positive for residual ALP activity (as defined in item 1.a).

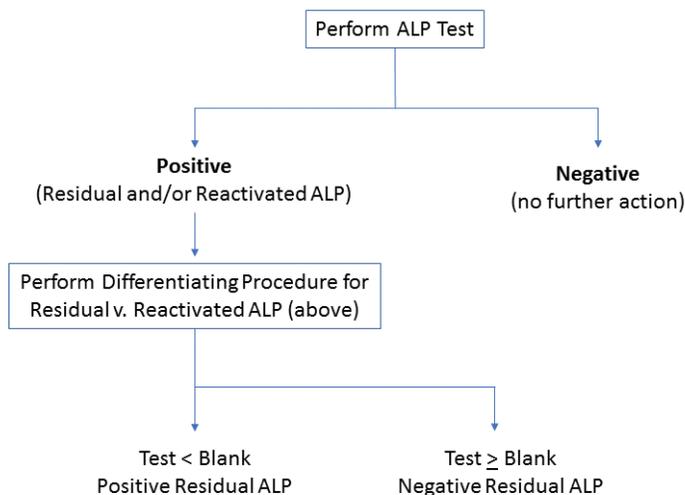


Fig. A.2: Residual ALP Test Flow Diagram

REF: Standard Methods for the Examination of Dairy Products, 17th Ed. (Ed. H. Wehr, J. Frank) 2004, p349-350, American Public Health Association, Washington, D.C.

Troubleshooting Table

Problem / Message	Explanation
Cuvette Door Open	Test procedure is cancelled. Close the cuvette door and restart the test.
Cuvette Temp. High Cuvette Temp. Low	The temperature of the fluorometer cuvette chamber (and/or the contents of the cuvette in the cuvette chamber) is not at $38 \pm 1^\circ\text{C}$. Wait for the cuvette chamber temperature to stabilize.
Incubator Temp. Low Incubator Temp. High	The temperature of the fluorometer heater block is not at $38 \pm 1^\circ\text{C}$. This error will not prevent calibration or testing.
LED Temp. Low LED Temp. High	The temperature of the sample illumination LED is not at its set point. Wait for the LED temperature to stabilize.
Calibration Required	The product channel chosen has not yet been calibrated. Perform calibration.
Unacceptable Calib Ratio	The difference in fluorescence reading between the calibrators is greater than allowed. Repeat calibration with new calibrators.
Standards Reversed? Please Repeat	The calibrators were tested in reverse order. Prepare new calibrators and repeat.
Calibration Not Complete	The difference between the fluorescence of the duplicate readings on each calibrator must be less than 6 fluorescence units.
Value < 10 mU/L or Value < 10 mU/kg	If either minute two or minute three are below or equal to zero fluorescence (indicating a very low result). Confirm this very low result by pressing TEST and START a second time and/or third time. Check to make sure sample has been added to the substrate. Repeat the entire test with new substrate and sample.
Overranged, Repeat Test	Sample reads over 24,000 fluorescent units, very high activity. Repeat on dilution to confirm.
Underranged, Repeat Test	Sample reads under 100 fluorescent units, very low activity.
Unstable Reading, Repeat Test	The average change in fluorescence during minute two is not within an acceptable range of that during minute three. Among the reasons for this are: (1) Substrate not at 38°C . (2) Not a uniform, well-mixed sample. Repeat the test.
Readout is unstable	The sample illumination LED and/or optical filters may require replacement. Contact Advanced Instruments for Hot-Line™ Service.
Calibrator C output drifts over time, or instrument requires frequent readjustment	The sample illumination LED and/or optical filters may require replacement. Contact Advanced Instruments for Hot-Line™ Service.
Low readings in A/D Test	With a Daily Instrument Control cuvette in the sample chamber, a reading near 300 FLU may indicate a problem with the sample illumination LED. If the low readings persist with the sample door open, the shutter which protects the PMT from ambient light may have failed to open. In either case, contact Advanced Instruments for Hot-Line™ Service.
System Temperature High	The instrument temperature is above 29°C . Move the instrument to a cooler location.
Temperature Too High	Temperature setting entered while adjusting incubator or cuvette temperatures is too high. Enter a lower setting.
Battery Low	Internal battery requires replacement. Contact Advanced Instruments for Hot-Line™ Service.
Powerup Diag Failed	One of the internal diagnostics failed during powerup. Power down the instrument, wait 30 seconds, and power up again. If this continues, contact Advanced Instruments for Hot-Line™ Service.
Error Reading Barcode	The number of barcode characters is greater than 13. Barcode must be 13 characters or less.

Appendix C

Product Specifications

Item	Specification
Electrical	
Power requirement	100 to 240 VAC (50/60 Hz)
Fuses (2)	250V time delay (Type T): 1 Amp
Power consumption	180W
Memory backup	Integral lithium cell; 10-year life (typical); not user-replaceable
Sample Volume	See test kit instructions
Sample Capacity	Single sample
Readout	20-character vacuum fluorescent display
Units	FLU (fluorescence unit)
Results units	See test kit instructions
Zeroing	Automatic
Resolution	1 FLU
Drift	Less than 3 FLU per hour
Cuvette size	12 x 75 mm round
Warm-up time	
Cuvette chamber	15 minutes
Heating block	30 minutes
Test time	3 minutes
Optics	90° optical bench with light source, fixed filters, detector
Light source	Light emitting diode
Filters	
Excitation	440 nm bandpass
Emission	550 nm longpass
Storage temperature	32 to 158°F / 0 to 70°C
Operating temperature	68 to 85°F / 20 to 29°C
Room humidity	5-80% relative humidity (non-condensing)
Sound level	71.5 dB(A) maximum at operator's position
Communications	Integrated thermal printer DTE EIA-232/V.24(RS-232) serial port (baud selectable: 1200, 2400, 4800, 9600 or 19200) optional barcode reader

Item	Specification	
Dimensions	inches	centimeters
Height	6.5	17
Width	12.5	32
Depth	16.5	42
Weight	pounds	kilograms
Net	18	8
Shipping	34	15
Certification	   <p>Refer to Regulatory Notices (Appendix D) for applicable standards.</p>	
IEC Protection Class	I	
Over-Voltage Category	II	
Pollution Degree	2	
Moisture Protection	IPX0 (ordinary)	

Appendix D

Regulatory Notices

This product has been designed and manufactured to meet the intent of U.S., Canadian, and European regulatory requirements as outlined below. Modifications made to this product that are not expressly approved in writing by the manufacturer will void the user's authority to operate this product, previously issued factory approvals, and the user's rights under the warranty.

The distributor or dealer may have applied additional local, national, or international approvals to this product. Consult the distributor or dealer for more information and documentation.

Connections to this product must be made with shielded cables. Use of non-shielded cables may violate RFI/EMI limits.

Symbol conventions



This symbol indicates conformity to relevant European directives.



This symbol indicates the product was tested to conform to relevant Canadian and U.S. safety standards by Intertek Testing Services NA, Inc. The ETL mark is approved in the United States as a Nationally Recognized Testing Lab (NRTL) by OSHA, and in Canada by the Standards Council of Canada.

Regulatory approval type	Description
U.S. Safety	This product has been listed by ETL testing laboratories as being in compliance with the requirements of UL61010-1 & 61010-2-010, "Electrical Equipment for Laboratory Use". The "US" in the lower right of the ETL mark demonstrates this listing.
Canadian Safety	This product has been listed by ETL testing laboratories as being in compliance with the requirements of CAN/CSA C22.2 No.61010-1 & 61010-2-010, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use". The "C" in the lower left of the ETL mark demonstrates this listing.
CE Declaration of Conformity: EMC	This product meets the intent of Directive 2014/30/EU for Electromagnetic Compatibility. Compliance was demonstrated using the following standards, as listed in the Official Journal of the European Communities: Consult the Declaration of Conformance certificate shipped with the product for the latest update. IEC 61326-1: Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1 General Requirements.
CE Declaration of Conformity: Low Voltage	This product meets the intent of Directive 2014/35/EU, the Low Voltage Directive. Compliance was demonstrated using the following standards, as listed in the Official Journal of the European Communities. Consult the Declaration of Conformance certificate shipped with the product (if required) for the latest update. IEC/EN 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use". IEC/EN 61010-2-010, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Particular Requirements for Laboratory Equipment for the Heating of Materials".
EC Declaration of Conformity: WEEE	This product meets the intent of Directive 2012/19/EU for Waste Electrical and Electronic Equipment (WEEE). Consult the Declaration of Conformance certificate shipped with the product (if required) for the latest update.
FCC: Part 15, Subpart B, Class B	This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.
Canadian ICES-003	This Class B digital apparatus complies with Canadian ICES-003. Cet appareil numérique de la classe B est conforme à la norme NMB-003 du Canada.
EC Declaration of Conformity: RoHS	This product meets the intent of Directive 2011/65/EU for "Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment."
CB Report CB Certificate	A CB report and certificate have been issued for this product. The standard used was IEC 61010-1 and 61010-2-010. A copy of the certificate is available upon request.

Fluorophos® Test System Approvals, as of June 2004

(Covering: Milk from cows, sheep and goats; Cheeses and Creams)

Regulatory Approvals

1. AOAC First Action - 1991
2. AOAC International 1995 15th Ed. Sec. 979.13 (AOAC International Final Action) 1995
3. International Dairy Federation (IDF) - 1992
4. International Standards Organization (ISO) - ISO/DIS 11816 - 2:2001
5. Interstate Milk Shippers (IMS) - 1993
6. FDA 8th Edition / 1995 Cheese
7. NCIMS - 2001 Method extension granted for cream products
8. Official Journal of the EU, named Reference Method, ISO 11816-1,6 May 2007 (alternative methods must be validated against the Fluorometric method)
9. Official Journal of the French Republic - 2004

Independent Laboratory Reviews

1. NIZO (Holland)
2. Bundesgesundheits Adm (Germany)
3. Weihenstephan (Germany)
4. SMR (Sweden)
5. Milk Marketing Board (England and Wales)
6. Food Standards Agency (UK)
7. Hannah Research Institute (Scotland)
8. University of Milan (Italy)
9. Instituto Nacional de Engenharia e Tecnologia - Lisbon (Portugal)
10. Universidad Autonoma de Bellaterra - Barcelona (Spain)
11. Nestle (Switzerland)
12. Black - Gilbert Chandler Institute (Australia)
13. University of Tennessee (U.S.A.)
14. University of Wisconsin (U.S.A.)

Warranty & Warranty Duties

By accepting and operating this instrument, the user and Advanced Instruments agree to the following responsibilities which constitute contractual warranties and conditions between the seller and the user for the maximum benefit and usefulness of the instrument.

LIMITED WARRANTY CONDITIONS

Advanced Instruments warrants that for 12 months from date of shipment, each new product sold or manufactured is free from defects in material and workmanship under normal installation, use and service.

Advanced Instruments agrees that such defects will be remedied or new parts furnished in exchange for any defective part delivered by the owner, transportation charges prepaid, to the factory for our examination. If, in our judgment, the part contains any such defect it will be replaced, charging only for transportation.

This warranty does not extend to any instrument subjected to negligence, accident, abuse, misuse or other improper operation, or which has been tampered with, altered or repaired by anyone other than Advanced Instruments or its authorized service representatives. Nor does this warranty extend to any consequential damages caused by defects or improper use, or secondary damage resulting from prior problems.

The above provisions do not extend the original warranty period of any instrument or part thereof which has been replaced or repaired thereunder.

Remedies made under the terms of this warranty in no case include any obligation or responsibility for transportation charges or arrangements.

All results, calibrations, effectiveness, medical diagnoses and interpretations are the responsibility of the user.

MODEL NUMBER: _____

SERIAL NUMBER: _____

INSTALLATION DATE: _____

THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, REPRESENTATIONS AND CONDITIONS OF ANY KIND, EXPRESSED OR IMPLIED IN FACT OR BY LAW. IN NO EVENT SHALL ADVANCED INSTRUMENTS BE LIABLE UNDER THIS WARRANTY OR OTHERWISE IN ANY MATTER FOR ANY REMOTE OR CONSEQUENTIAL DAMAGES RESULTING FROM THE BREACH OF THIS WARRANTY OR CONDITION IMPLIED BY LAW OR OTHERWISE, OR FOR ANY DAMAGE TO THE INSTRUMENT OR ANY PART THEREOF CAUSED BY IMPROPER INSTALLATION, UNAUTHORIZED REPAIR OR ALTERATION, NEGLIGENCE, ABUSE OR MISUSE INCLUDING USE OF IMPROPER GLASSWARE OR REAGENTS, ACCIDENT, OR ACT OF GOD.

Attention is called to the user's responsibility regarding reasonable use, useful life of the instrument and immediate notification of accident outlined in the warranty duties section of the user's guide and/or service manual.

No person is authorized to assume for us any other liability in connection with the sale or service of this equipment.

Advanced Instruments reserves the right, at any time without notice, to make changes in price, color, material, specifications and models without incurring liability to modify products previously produced, and to add or discontinue models and accessories.

This warranty gives you specific legal rights, and you may also have other rights which vary from state to state.

AI1YW REV 0

Appendix E Warranty & Warranty Duties

Advanced Instruments warrants that it:

1. Has produced equipment equal to or exceeding that of any competitive product in the same price range in standards of design, material and workmanship.
2. Knows of no defects in design or materials which may cause bodily injury.
3. Will endeavor to advise the User of changes or improvements in the instrument as they are developed, so that the User may take steps to improve the safety and performance of his equipment throughout its useful life.
4. Will replace or repair equipment according to the guarantee on the attached warranty.
5. Will cooperate closely in common defense of any accident involving this equipment, or third-party suit against the User or operating personnel, if advised immediately by the User of the occurrence of any accident.

The User warrants that:

1. The instrument will be used reasonably.
2. The instrument will be regularly maintained according to this manual, including a log of all service, tests and repairs performed on the equipment, and records will be kept of all requests for repair made to Advanced Instruments where such repairs were beyond the ability of local service personnel.
3. The instrument will not be altered without written approval from Advanced Instruments.
4. Advanced Instruments will be notified immediately if any injury occurs in any association with the instrument and will be allowed prompt and thorough examination of the instrument in question.
5. Advanced Instruments will be held harmless in cases of injury arising (see definitions below):
 - Beyond the useful life of the equipment.
 - From unreasonable use.
 - When Advanced Instruments is not immediately notified of said injury.
 - From interpretation of results.

Definitions**Useful life**

- The same as the depreciation life in the Internal Revenue Service guidelines, whether or not the user actually depreciates the instrument, but not to exceed 10 years from date of delivery to the user.
- Only during the time the equipment has been maintained on a regular basis as prescribed by Advanced Instruments. If the User is in an area which has no local service, Advanced Instruments may require a local service person (understood to mean the person actually performing the “hands-on” service of the equipment) to attend and pass a reasonable maintenance and repair course.
- Only during the time when the User has not altered the equipment in any way without written approval from Advanced Instruments.
- Only during the time when the User has not loaned, leased or resold the equipment to any third party.

Reasonable use

- According to the instructions supplied by Advanced Instruments (assuming English-reading personnel or supervision). If neither the supervisor nor the operator reads English, the User agrees to obtain accurate translations of the instrument labels, instructions, user's guides and/or manuals provided.

- Under direct, on-the-job supervision of the supervisor or other professional in charge.
- In which there are no known defects or uncorrected repairs.
- Only for the purpose stated in the instructions provided with the instrument.
- In which the equipment has been maintained according to the instructions provided.

Immediate notification

- Recognition that time is of the essence when any accident, malpractice or product liability arises which involves Advanced Instruments equipment.
- Notification to Advanced Instruments immediately (the same day, if possible) in the event of injury to any person in circumstances involving Advanced Instruments equipment in which Advanced Instruments might be named as a defendant in any form of litigation.
- Allowing Advanced Instruments or its representatives, immediate, full, and thorough examination of Advanced Instruments equipment, and all records pertaining to such equipment.

Appendix F

Supplemental RS-232 Information

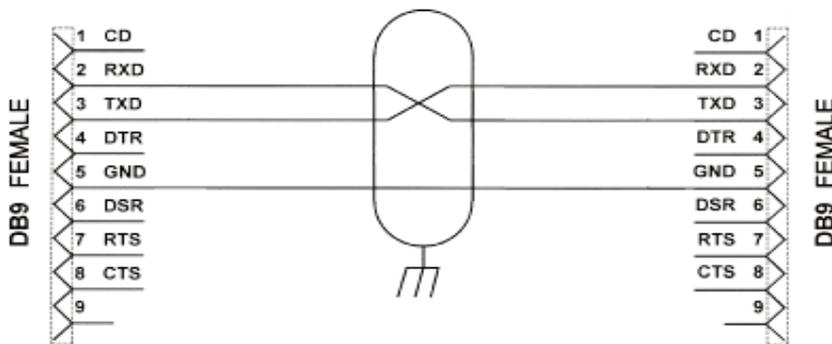
The DB-9 RS-232 port on your instrument conforms to the DTE EIA-232 standard and can reliably communicate over shielded cable up to 10 meters in length, depending on the baud rate you use. Almost every item of information displayed by your instrument is also transmitted over the RS-232 port, including test results, all error messages, and most display data from the diagnostic menu.

Data is transmitted asynchronously as 1 start bit, 8 data bits and 1 stop bit, with no parity. Each message transmitted from the communication port is terminated by the sequence, Carriage Return (0D Hex), Line Feed (0A Hex). Note that your instrument is only capable of outputting information. At this time, there is no protocol for talking to the instrument.

This product's serial port output is based on "ASCII" code protocols.

No special codes are used to identify errors, test results, or normal operational messages. Connection to any data collection or LIMS system will require the User or a third party to write custom interface software in order to recognize and parse the text strings of interest. Advanced Instruments does not maintain a central list of text strings used in the various versions of software created, nor have we adopted any standard protocol with respect to the format of these messages. To determine the format and type(s) of text strings contained in this instrument, user should use a terminal program to capture and save the output for evaluation by the interface programmer.

At this time Advanced Instruments has not investigated any third-party software for use or recommendation for the purpose of importing data into PC applications, such as Microsoft® Excel or a customer's LIMS system.



NOTE: Shield connects to metalized connector housing at both ends.

Fig. F.1: Null Modem Cable

Symbol Definitions

The below list of symbols is for reference only. Not all symbols shown may be used on this instrument or in the accompanying documentation.

	On/off		Functional arrow		Content
	Feed		Printer		Control
	Interrupt		Enter		Negative control
	Test		RS232		Positive control
	Start		Bar code		Flammable
	Stop		Caution hot surface		Fragile
	Record review		Lifting hazard		Irritant
	Setup		Calibrator		Keep dry
	Calibration		Date manufactured		Sterile
	Cancel; Delete				

Appendix G Symbol Definitions

	Non-sterile		European conformity		Low fluid level
	Serial number		Temperature limit		Keep hands clear
	Solution		See instructions		Manufacturer
	Do not open top		Lot number		Latex-free
	Handle with care		Use by; Expiration date		Diluent
	Use blade to open		Authorized representative		See instructions for temperature guidelines
	Do not re-use		Part number		Potential puncture hazard
	For In Vitro diagnostic use		Sufficient for [x] tests		Electronic equipment-dispose of properly
			Open here		

Product Disposal and Recycling

International concern about environmental pollution resulting from improper disposal of products and materials at the end of their useful life has resulted in an increase in legislation to control the methods and procedures used to handle waste electrical and electronic equipment. While the regulatory status in some regions of the world has progressed to the point where formal legislation is already in effect, many other regions are in the process of creating similar legislation or adopting that already in existence in other areas. The result in the years ahead will be more stringent control over disposal of products and recycling of their components once they are withdrawn from use.

Since regulations governing the disposal of your instrument and accessories may vary depending upon your geographic location, the following guidelines are provided to assist you in identifying the options available to you once the decision has been made to replace or dispose of this product:

Contact the supplier who sold you the product. Whether this was Advanced Instruments itself, or one of its authorized dealers, this supplier should be knowledgeable about the national and local regulations

governing disposal and recycling of products in your area. In some cases, this supplier may be legally obligated to accept the product from you and arrange for proper disposal or recycling with no further involvement on your part. Alternately, the supplier can provide you with specific instructions for actions that you can take to dispose of the product properly.

Contact your local government agency responsible for waste collection and disposal. They can identify procedures and restrictions in effect to ensure proper disposal, and available locations where products can be sent.

Contact Advanced Instruments Hot-Line™ Service:

800-225-4034 (toll-free within the USA and Canada,
8:00am–4:30pm EST; after normal business hours,
follow voicemail prompts)

+1 781-320-9000 (elsewhere)

+1 781-320-0811 (fax)

Service personnel will provide you with contact information for local disposal, or instructions for returning the product directly to Advanced Instruments.

Appendix J

Index

A

- A/D test 28
- Alkaline phosphatase *see* *ALP*
- ALP 18
- ALP pasteurization assay 18
 - Calibration 15
 - Calibration curves 21
 - High turbidity procedure 23
 - Reagents, reconstituting 21
 - Reagents, storage 19
 - Sample preparation 22
 - Test 23
- Assistance 29

B

- Barcode test 28
- Beeper test 29

C

- Calculations
 - Butter and cheese 31
 - Fluid Dairy Products 30
- Calibration 15
 - ALP Pasteurization 20
 - Equations 30
 - Frequency 15
- Chamber temperature adjustment 11
- Cuvette door 14

D

- Daily Instrument Checks 21

- Daily Instrument Control 21
- Daily Instrument Control adjustment 21
- Data capture 17
- Date/Time 12
- Date, setting 12
- Diagnostic checks 28
- Dis/Enable Beeper 17
- Display panel 14
- Display/Print test 29

E

- Event Record 29
- Excitation filter 11

F

- Fuse replacement 26

H

- Heating block 11, 17
- Heating block adjustment 11
- High-turbidity test 22, 23

I

- Installation and setup 9

K

- Keypad keys 14
- Keypad test 29

M

- Maintenance 27

O

Obtaining service 27
Operating settings 16
Optical filters
 Care 26
 Replacement 26
Output Avg F/min 17

P

Percent raw milk 31
Pipette 14
 Maintenance 26
Power panel 13
Power requirements 9
Print Avg F/min 17
Print calibration ratios 17
Printer 11, 13
 Loading paper 11
Product names 17
Product specifications 36

R

Reagents
 Handling 20
 Preparing 16
 Reconstituting 21
 Storage 19
Regulatory Notices 38
RS-232 information 44

S

Serial number 17
Serial port 13
Service 27

Service log 48
Set serial rate 17
Space requirements 9
Specifications 36
Spills 25
Symbol definitions 5, 45

T

Time, setting 12
Troubleshooting checks 28
Troubleshooting table 35

U

Unpacking 9

V

Ventilation requirements 9

W

Warranty duties 41

Copyright

This user's guide is copyrighted by Advanced Instruments with all rights reserved. Under copyright laws, this guide may not be reproduced in any form, in whole or part, without the prior written consent of Advanced Instruments.

Advanced Instruments has reviewed this guide thoroughly. All material contained within is believed reliable, but the accuracy and completeness are not guaranteed or warranted, and are not intended to be representations or warranties concerning the product described.

Windows CE® and Microsoft Excel® are registered trademarks of Microsoft Corporation in the United States and other countries. Intel® is a registered trademark of Intel Corporation in the United States and other countries. Halamid® is a registered trade mark of VEIP Disinfectants. All other trademarks are the property of Advanced Instruments.

Hot-Line™ Technical Service

If you have any questions or problems regarding the proper operation of your instrument, please contact our Hot-Line™ Technical Service department by calling one of the following numbers.

800-225-4034 (toll-free within the USA and Canada,
8:00am–4:30pm EST; after normal business hours,
dial 781-320-9000)

+1 781-320-9000 (elsewhere)

+1 781-320-0811 (fax)



Two Technology Way | Norwood, MA 02062

For more information | 800-225-4034 | +1-781-320-9000

Online | aicompanies.com | info@aicompanies.com



The management system governing the manufacturing of this product is ISO 9001 registered.

Hot-Line™ Technical Service Advanced Instruments and worldwide distributor network provides 24/7 comprehensive customer service and technical support.

This user's guide is copyrighted by Advanced Instruments with all rights reserved. Under copyright laws, this guide may not be reproduced in any form, in whole or part, without the prior written consent of Advanced Instruments.

©2017 Advanced Instruments. Fluorophos® is a registered trademark of Advanced Instruments. All other trademarks are the property of their respective companies.