Fluorophos® ALP Test System

Model FLM200

User's Guide

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FLM5 Rev16 060110

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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, dial extension 2191)
- +US 781-320-9000 (elsewhere)
- 781-320-0811 (fax)

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Safe Use

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

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

This static symbol is intended to alert the user to the presence of a specific component 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.

NOTE Additional symbol definitions are available in Appendix F.

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 ampere ratings marked on the product's 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's 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 Advanced Instruments 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 in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, 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:

- Reorient 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 the Advanced Instruments Customer Service Department by using one of the following numbers.

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

PART DESCRIPTION	PART NO.
Pipette Tips 25-75 µL (100)	FLA880
Fluorophos® Cuvettes (250)	FLA020
Printer Paper (5 rolls)	FLA835
25-µL Pipette	FLA865
75-µL Pipette	FLA885
Plunger Wire for 75-µL Pipette	FLA887
Cuvette Holder Cleaners (50 cleaners)	FLA840
Heating Block Thermometer	FLA810
20-Sample Heating Block	FLA802
Service Manual	FLM5SM
User's Guide	FLM5
Dual Excitation Filter (ACP, ALP) Assembly	FL2333
Replacement Air Filter (box of 6)	3D2340

PART DESCRIPTION	PART NO.
2 mL Pipette	FLA888
Pipette Tips 2-mL (25)	ACM205

Reagents, Calibrators & Controls

To order calibrators and standards, contact your local Advanced Instruments Distributor or the Advanced Instruments Customer Service Department by using one of the following numbers.

- 800-225-4034 (U.S. territories and Canada)
- +US 781-320-9000 (international)
- 781-320-3669 (fax)

ALP Pasteurization Assay

PART DESCRIPTION	PART NO.
Cheese Extraction Buffer	FLA005
ALP Test Kit	FLA124
225-Test ALP Reagent/Cuvettes Kit	FLA225
ALP Calibrator Set	FLA250
ALP PhosphaCheck® Control	FLA260
ALP Daily Instrument Control	FLA280

Notes:

Foreword

Principles of Fluorescence

The technology of fluorescence has been used in analytical assays for over 75 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 called 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 moleules 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 major 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.

Fluorometric Assays

The FLM200 Fluorometer is designed to perform two fluorometric assays, the Alkaline Phosphotase (ALP) assay and the Acid Phosphotase (ACP) assay. The Fluorophos® ALP Assay is a rapid test for verifying proper pasteurization of dairy products by measuring the Alkaline Phosphotase (ALP) activity. This User's Guide provides information specific to performing the Fluorophos® ALP assay.

The ThermaZyme® ACP assay and Fluorophos® ACP Juice Pasteurization assay utilize materials, reagents, substrate buffers, and Fluorometer setup that are different from the Fluorophos® ALP assay. Information specific to these tests is not included in this User's Guide. To obtain more information about ThermaZyme® ACP assay or Fluorophos® ACP Juice Pasteurization assay, please contact Advanced Instruments, Inc.

1 Installation & Setup

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 on your instrument.*

Step 1: Find a location for the instrument

When choosing a location for the 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 near the left side of the instrument where proper ventilation is needed. Your instrument's cuvette heating block also requires a small amount of space (6.5 × 8.5 × 3.5 inches, 16.5 × 21.5 × 9.0 cm) Note that the heating block will perform optimally if placed in a draft-free location.

- **AC voltage selector.** Your FLM200 is equipped with a voltage selector located on the rear of the instrument within the fuse holder. The setting should match the table, below. If not, seek qualified assistance to make the required change. A change may require fuse replacement (see Chapter 4, *Fuse replacement*).

Measured VAC	Position
100-110	100V
110-120	120V
200-235	230V
235-240	240V

- **Electric outlet availability.** Your instrument and cuvette heating block will need to operate within five feet of a properly grounded, three-pronged electrical outlet capable of continuously supplying 1 ampere at 200-240V to 1.25 amperes at 100-120V. If the instrument and heating block are not grounded properly, their 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 the fluorometer, take the following steps.

a. Carefully unpack the 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's 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.

Figure 1-A: Model FLM200 Fluorophos® Test System and Supplies

Quantity	Part No.	Description
1	FLM200	The Fluorophos® Test System
1	———	Power Cord (as specified)
1	FL0408	Paper Roll Holder
1	FL0425	Printer Maintenance Kit with Instructions
2 rolls	———	Printer Paper (re-order package of 5 rolls as FLA835)
1	FL2999	Black Body Calibration Tool
1	FLA020	Fluorophos® Cuvettes
1 pack	———	Cuvette Holder Cleaners (re-order pkg of 50 as FLA840)
1	FLA802	Heating Block (optional)
1	FLA810	Heating Block Thermometer (optional)
1	FLM5-6	Warranty Card

Table 1-A: Model FLM200 Packing List

Figure 1-B: FLMA02 ALP Starter Test Kit

Quantity	Part No.	Description
1	FLA865	25-µL Sample Pipette
1	FLA885	75-µL Sample Pipette
1	FLA888	2000-µL Sample Pipette
1	FLA224	Fluorophos ALP 225-Test Reagent Set
1	FLA250	Fluorophos ALP Calibrator Set
1	FLA260	Fluorophos ALP Pasteurization Controls
1	FLA280	Fluorophos ALP Daily Instrument Control
1 pack	ACM205	2-ml Pipette Tips (25)
1 pack	FLA880	25 - 75-µL Pipette Tips (100)
1	FLM5	Fluorophos ALP Test System User's Guide

Table 1-B: FLMA02 ALP Starter Test Kit Packing List

e. Fill out the postage-paid 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 it to 781-320-8181.

Step 3: Obtain additional items

Items needed, but not supplied by the manufacturer, include:

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

Step 4: Set up heating block

a. Plug the heating block cord into a grounded outlet.

b. Switch the heating block power switch to the ON position. The power light on the front panel will illuminate.

c. Insert the heating block thermometer into the heating block thermometer well.

d. Allow the heating block to warm up for at least 30 minutes to set temperature and stabilize. The ready light on the front panel will illuminate.

e. Use the heating block thermometer to check the temperature of the heating block.

f. If the temperature is not 38 ± 1°C, use the provided temperature adjustment tool and the setscrew accessible through the front panel to adjust the temperature. To do this, you will need to use very small increments and allow the temperature to stabilize after each adjustment.

g. When stable at 38 ± 1°C, the heating block is properly set up and ready to use. **Turn off the heating block when not in use.**

Step 5: Check excitation filter

Your instrument is shipped with a dual excitation filter assembly, which houses two filters for ALP and ACP testing, respectively. Determine which test(s) you will be running and check your filter to ensure that the correct filter is in place. If testing dairy products, make sure that the ALP filter is in place.

Facing the front of the instrument, the excitation filter assembly is located behind the cuvette holder, and is accessible through the cuvette door (see Figure 3). The two ends of the dual filter assembly are marked ACP and ALP, respectively. When the filter is installed, the visible end of the filter will indicate which filter is currently in place. If the current filter is not the one you need to use, simply remove the filter holder, flip it around and replace it in such a way that the correct filter name is showing.

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 6: Power-up and run diagnostic test

a. Plug the fluorometer power cord into a properly grounded outlet.

b. Switch the fluorometer power switch into the ON position. The display will report software and hardware identification and assistance information, as well as the date and time.

c. The display will then begin a 15-minute countdown, during which the cuvette chamber will stabilize at the required operating temperature.

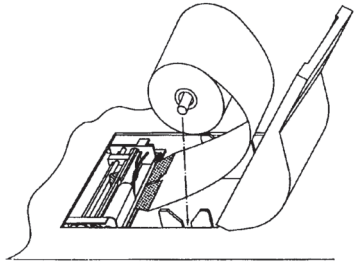

Figure 2: Loading Printer Paper

d. When the countdown ends, the instrument will run some brief diagnostic tests to ensure the instrument is functioning properly.

e. After the diagnostic tests are successfully completed, the fluorometer will display "**[CALIB] Calibration/Product [TEST]**," and will be ready for use.

Step 7: Load printer paper

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

a. Unroll 6-8 inches (15-20 cm) of paper from the paper roll.

b. Fold both corners of the end of the paper to form a triangular point, approximately 2-3 inches or 5-8 cm long. Crease the paper folds so that the doubled part will not be much thicker than two thicknesses of paper.

c. Insert the roller into the paper roll.

d. Open the printer door on the top right side of the instrument case.

e. Holding the roller in place with one hand, use the other hand to feed the triangular pointed end of the paper from the bottom of the roll into the opening between the back of the printer frame and the printer circuit board.

f. Push the paper in and/or use the "FEED" button on the keypad to feed the paper in far enough for the end to protrude from the top of the printer.

g. Center the paper roll and roller between the V-shaped roller guides behind the printer, and press down firmly to seat the roller. When both ends of the roller are in proper position, the paper roll should be level. If the paper roll is not level, carefully reseat the roller between the roller guides.

h. Press FEED or pull enough paper through the printer to take up any slack, and feed the end out through the slot at the back of the window in the printer door.

NOTE:  The paper should only be driven or pulled forward through the printer; attempting to pull the paper backward through the printer may cause printer damage.

i. Close the printer door and take up any paper slack by pulling the end of the paper.

If the printer paper becomes jammed under the print head, a protective circuit will shut off the printer without deactivating the display or the rest of the instrument.

To reactivate the printer, shut off the instrument power. Then clear the paper jam and turn the power back on. The instrument will complete the warm-up cycle and the printer will report "Fluorometer Ready" if the paper jam has been successfully cleared.

Step 8: Set date and time

At this time, you should set the date and time functions on the instrument. To set these items, use the instrument's setup mode as follows:

a. When the fluorometer display scrolls "**[CALIB] Calibration/ Product [TEST]**", press the SETUP keypad to enter the setup mode. The display will read "**1:A/D Test**".

b. Use the < or > keypad to cycle through setup menu options until you see "**2: Set Date/Time**".

c. At "**Set Date/Time**", press START to display the current date and time.

d. Press CLEAR to clear the entire setting and display the present format ("**mm/dd/yy**" or "**dd/mm/yy**"), and use the < keypad to toggle to the date format you require.

e. Use the > keypad to toggle to "**24hr**" format, "**am**", or "**pm**", as required.

f. Enter the current date and time (including zeros) via the numeric keypad, one digit at a time from left to right. For example, September 9, 1999 would be entered 090999. Entering a two-digit year will not affect instrument operation. Your instrument will automatically default to the correct four-digit date, and all printouts and readouts will use the correct four-digit year.

g. Press ENTER to store the new setting (or STOP to restore the original setting). If an invalid setting is attempted, the instrument will reject the setting and beep when ENTER is pressed.

Notes:

2 Instrument Operation

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. You should read ***both*** *this chapter and the test chapter before running any tests on the fluorometer.*

Hazardous material cautions

- **WARNING**: The specimens used in this product may be hazardous to your health and to the health of others. Follow national and local recognized safety standards and/or protocols for handling and disposing of such specimens.
- If a hazardous material is spilled on or inside the equipment, the user is responsible for taking the appropriate decontamination steps, as outlined by the user's national safety council or equivalent agency for hazardous materials, and those 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 below before proceeding further.

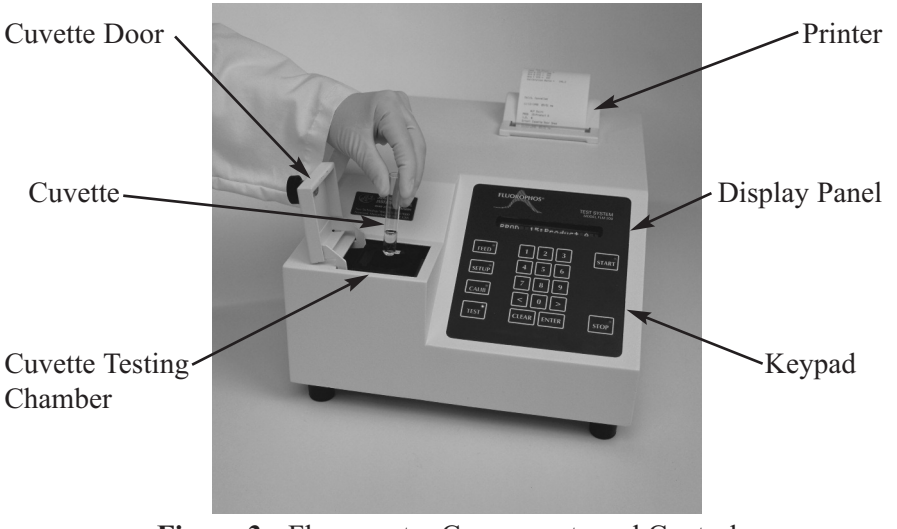

Figure 3: Fluorometer Components and Controls

Power panel

Contains the power plug, fuses and rocker-type power switch. The upper half of the switch is the power on position. The fluorometer is intended to be left on with the display reading "**2: A/D Test**" during normal operating hours.

Serial port

The Advanced® Fluorometer 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, including test results, all error messages, and most display data from the diagnostic menu.

Printer

The printer is located beneath the slotted door in the top right side of the fluorometer. Lift the front of the printer door and check that adequate thermal paper is properly loaded.

Display panel
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.

Keypad
The keypad contains spill-proof pressure pads for operator input to the instrument.

Cuvette door
The cuvette door, located to the left of the keypad, protects the cuvette testing chamber.

Function and components of the heating block

Operation of the heating block will be quicker and easier if you become familiar with the locations and functions of the components, systems and controls described below before proceeding further.

Power switch
The rocker-style power switch is located on the power entry assembly on the back of the instrument.

Power light
The power light on the front of the heating block will illuminate when power is being received by the block.

Ready light
The ready light on the front of the heating block will illuminate when the set temperature is reached.

Temperature adjustment screw
The temperature adjustment screw is used in conjunction with the thermometer to set the heating block to a desired temperature.

Thermometer and thermometer well
The thermometer is placed in the thermometer well to monitor the heating block temperature setting.

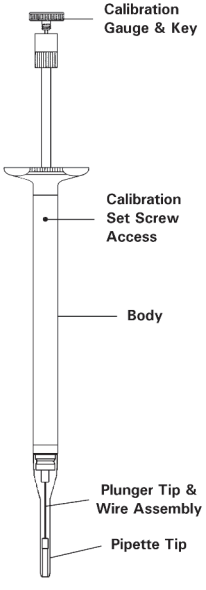

Figure 4: Fluorometer Pipette

Using the 25-µL or 75-µL pipette

The Advanced® positive displacement pipettes are factory-set for precise fixed-volume delivery and shipped ready for use. Calibration adjustment keys and detailed instructions are provided with each pipette. Operate the pipette as follows:

a. Depress the plunger fully.

b. Immerse the pipette tip about 1/4 inch 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 inch 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 once more and, while depressed, remove the pipette from the cuvette.

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. Power up the fluorometer and heating block. Both are designed to operate at 38°C and must be turned on and allowed to warm to operating temperature before use. Allow the heating block to warm for 30 minutes.

b. Instrument will run start-up diagnostics and count down 15 minutes while the cuvette chamber in the instrument warms to 38°C. If the instrument cannot meet the required operating conditions for any reason, an error message will be displayed.

c. When the fluorometer and heating block 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 switchpads presents the name of the channel on which the last test or calibration was accomplished. At this point, either press ENTER to select that test or use the arrow switchpads to step through the menu of available channels 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.
- Whenever significant service procedures are performed (e.g., lamp or filter change).
- When adjustments are made to bring A-D Mode into specification.

The fluorometer will not allow tests to be performed using an un-calibrated channel; "**Calibration Required**" will be displayed when any new channel is defined. However, 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.

Briefly, 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(s) instructions for further calibration instructions.

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 be able to run several different tests. Each test has its own specific test procedure. Please see the chapter detailing the performance of specific test(s) and 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 switchpad at "**[CALIB] Calibration/Product [TEST]**". The display will change to "**1:A/D Test**".

b. To select another function on the SETUP menu, repeatedly press > or < 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 available, but are used in troubleshooting, and will be discussed in the next chapter.

Date/Time

At the date and time display, press CLEAR to clear the entire setting and display "**mm/dd/yy hh:mm am**" (the required format) or repeatedly press < to clear the setting one digit at a time from the right. Enter the desired numbers via the numeric keypad, one digit at a time from the left. Use > to toggle to "**am**" or "**pm**", as required. Press ENTER to store the new setting (or STOP to restore the original setting). The instrument will beep and reject any invalid date or time.

Print Avg F/min

At "**[Y]< avg f/min *[N]**", press < or > to print or suppress the printing of the average increase in fluorescence which is displayed just before the test results. The current setting is indicated by the symbol, "*****". Press ENTER to store the new setting or STOP to restore the original setting.

Sub Blank Testing

This option is designed for factory use and may not be used or reset in the field.

Dis/Enable Beeper

At "**[<] Dis/Enable [*]**", press < or > to disable or enable the keypad beeper. The current setting is indicated by the symbol, "*****". Neither selection will affect the invalid-selection beeper but when the keypad beeper is enabled, an invalid selection will invoke two beeps, when disabled, only one. Press ENTER to store the new setting or STOP to restore the original setting.

Dis/Enable Xon/Xoff

At "**[<] Dis/Enable [*]**", press < or > to enable or disable serial-port Xon/Xoff handshaking. The current setting is indicated by the symbol, "*****". Press ENTER to store the new setting or STOP to restore the original setting.

Set Serial Rate

At "**Serial Rate = xxxx**", the serial-port baud rate may be reset by means of the < or > switchpad to either 300, 1200 or 9600 baud. Press ENTER to store the new setting or STOP to restore the original setting.

Zero Range Adjust

The fluorometer zero ranges are individually set at the factory and should not be reset.

Serial Number

Fluorometer serial numbers are set at the factory for instrument identification and may not be reset in the field.

Product/Test

This option is designed for factory use and may not be used or reset in the field.

Assistance

Provides the manufacturer's name, address, telephone, fax and internet information and the instrument model, software version and serial number.

Notes:

3 Fluorophos® ALP Milk Pasteurization Assay

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

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 micro-organisms 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 information

Numerous fluorometric analytical methods have been developed for analytes of interest to the dairy industry, and Shipe has reviewed some of them[1].

In 1935, Kay and Graham developed an alkaline phosphatase (ALP) assay which could be used to detect the completeness of

(1) Shipe, W.F. Fluorometric Methods: Applications and Limitations in Challenges to Contemporary Dairy Analytical Techniques. Royal Society of Chemistry, London, 1984.

pasteurization in dairy products[2]. 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 Scharer[3] and Cornell[4] methods still use phenylphosphate as in the original Kay and Graham procedure; the Aschaffenburg and Mullen procedure uses p-nitrophenyl phosphate[5]; and the Rutgers assay uses phenolphthalein monophosphate[6].

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. First, the American Public Health Association (APHA)[7] and the Association of

(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, 16th Edition, 1992. American Public Health Association, Washington, DC.

Official Analytical Chemists (AOAC)[8] have approved methods using butanol extraction followed by spectrophotometric measurement. Second, both AOAC and the International Dairy Federation (IDF) have approved methods in which the indophenol is read after protein precipitation with barium and zinc salts[8, 9].

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 dialysate[8].

The IDF has approved a p-nitrophenyl phosphate assay in which the p-nitrophenol is read after protein precipitation following a two-hour incubation [10]. These quantitative methods have served the dairy industry well and have aided in monitoring milk quality throughout the world for over fifty years.

In late 1988, Advanced Instruments, Inc., presented preliminary data on a new approach to ALP testing in dairy products[11]. 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.

(8) Official Methods of Analysis, 16th Edition, 1996. Association of Official Analytical Chemists, Arlington, VA.

(9) Detection of Phosphatase Activity, IDF Standard 63:1971. International Dairy Federation, Brussels, Belgium.

(10) Detection of Phosphatase Activity, IDF Standard 82A:1987. 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.

The Fluorophos® method has been collaboratively studied[12] and received IMS/FDA, AOAC, SMEDP and IDF approval[13] and ISO approval[14].

The Fluorophos® method was correlated to Salmonella and Listeria inactivation by Eckner in 1992[15]. The application of the Fluorophos® method to the determination of both high and very low levels of ALP activity in milk, cream and cheese was reported by Lechner and Regensburger in 1993[16], and the fluorometric method for cheese was approved by ISO in 2003[17]. Introduction of this new approach to ALP testing will provide a rapid, modern, and cost effective improvement in quality assurance testing in the dairy industry.

The Fluorophos® ALP reaction

The Advanced Instruments fluorometric assay for Alkaline Phosphatase (ALP) in fluid dairy products is based on a specially designed enzyme substrate called Fluorophos®. The high sensitivity of fluorometry permits direct and continuous monitoring of the action of ALP on the substrate. Fluorophos® is an aromatic ortho-phosphoric monoester compound which is non-fluorescent in solution. When acted upon by ALP, Fluorophos® loses its phosphate radical and becomes a highly fluorescent molecule called Fluoroyellow®. The reaction is kinetic and can be measured with a fluorometer.

In this procedure, an aliquot of dairy product is added to 2.0 mL of substrate. The rate of product formation is monitored for three minutes in a compact fluorometer thermostated at 38°C. Enzyme activity is calculated

(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) Milk and Milk-Based Drinks, Determination of Alkaline Phosphatase Activity, Fluorometric Method, IDF Standard 155:1992, International Dairy Federation, Brussels, Belgium

(14) ISO/DIS 11816.1 - Milk and Milk Based Drinks - Determination of Alkaline Phosphatase Activity, Fluorimetric Method

(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 Milk and Milk Products - Determination of Alkaline Phosphatase Activity - Part 2; Fluorometric Method for Cheese.

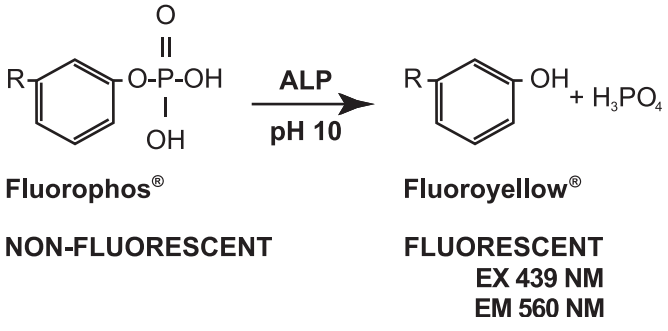

Figure 5: The Fluorophos® Reaction

in mU/L and displayed automatically at the end of the reading period. Reaction rates are linear for up to thirty minutes and to 2.0% raw milk[18].

The assay is complete in three minutes and utilizes a single stable substrate reagent. Detection limits down to 0.006% raw milk (approximately equivalent to 0.06 µg phenol in the APHA phenylphosphate "rapid" method) are possible[18].

Materials required

Contents of the Fluorophos® ALP Test Kit (FLA225):

- Fluorophos® ALP Substrate (2 bottles)
- Substrate Buffer (2 bottles)
- Glass Fluorometer Cuvettes

Materials required but not included in the Fluorophos® ALP Test Kit:

- Fluorophos® Test System with firmware version 2.0 or higher and ALP excitation filter assembly in place
- Fluorophos® ALP Test Kit (FLA124) smaller bottle size (120 mL)
- Fluorophos® ALP Calibrator Set (FLA250)
- PhosphaCheck® ALP Pasteurization Control Set (FLA260)

(18) Rocco, R.M., Fluorometric Analysis of Alkaline Phosphatase in Fluid Dairy Products, J. Food Protection 53:588-591(1990).

- Fluorophos® ALP Daily Instrument Control Kit (FLA280)
- Fluorophos® ALP Cheese Extraction Buffer (FLA005) -- only if testing cheese
- Vortex mixer
- Deionized water
- 2-mL pipette and disposable tips
- 25-µL pipette and disposable tips
- 75-µL pipette and disposable tips

Reagent storage/stability

Protect stock, working reagents and calibrators from light exposure.

- Fluorophos® ALP Test Kit
  - — Unopened, store at 2º to 8ºC (36º to 46ºF) for up to 2 years
  - — Reconstituted, store at 2º to 8ºC (36º to 46ºF) for up to 60 days
  - — In the Heating Block, store at 2º to 8ºC (36º to 46ºF) for up to 6 hours
- Fluorophos® ALP Calibrator Set
  - — Unopened, store at 2º to 8ºC (36º to 46ºF) for up to 18 months
  - — Opened, store at 2º to 8ºC (36º to 46ºF) for up to 60 days
  - — In the Heating Block, store at 2º to 8ºC (36º to 46ºF) for up to 6 hours
- PhosphaCheck® ALP Pasteurization Control Set
  - — Unopened, store at 2º to 8ºC (36º to 46ºF) for up to 18 months
  - — Opened, store at 2º to 8ºC (36º to 46ºF) for up to 72 hours
- Fluorophos® ALP Daily Instrument Control
  - — Unopened, store at 2º to 8ºC (36º to 46ºF) for up to 18 months
  - — Opened, store at 2º to 8ºC (36º to 46ºF) for up to 60 days
  - — In the Heating Block, store at 2º to 8ºC (36º to 46ºF) for up to 6 hours

- Fluorophos® ALP Cheese Extraction Buffer
  — Unopened/Opened, store at 2º to 8ºC (36º to 46ºF) for up to 3 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 Material Safety Data Sheets for complete hazard information.

NOTE: Do not freeze any of the above 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

1. Mix gently by inversion prior to each use to assure optimal results.
2. Label two (2) cuvettes for each of the three (3) calibrators.
3. Dispense 2.0 mL of the appropriate calibrator into each clean, labeled cuvette.
4. Place the cuvette in the 38 ± 1ºC 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.

1. Using a 75-µL pipette, add a 75-µL sample of the prepared product to each of the pre-warmed calibrator cuvettes.

2. Cover with parafilm and mix gently by inversion or vortex mixer.
3. Return each cuvette to the heating block. Calibration must be completed within ten (10) minutes after the addition of the sample.
4. Press **CALIB**.
5. Select "**ALP Dairy**" by pressing < or >, then press **ENTER**.
6. Select the product type to be calibrated by pressing < or >, or by using the numbered keys. When the desired product is displayed, select it by pressing **ENTER**.
   - If the channel has been previously calibrated, a printout of that calibration will be provided.

"PROD 1 Whole Milk"	**"PROD 12 Heavy Cream"**
"PROD 2 Low Fat 1%"	**"PROD 13 Dry Milk"**
"PROD 3 Low Fat 2%"	**"PROD 14 Sour Cream"**
"PROD 4 Skim Milk"	**"PROD 15 Product A"**
"PROD 5 Choc Milk"	**"PROD 16 Product B"**
"PROD 6 Cheese"	**"PROD 17 Product C"**
"PROD 7 Butter"	**"PROD 18 Product D"**
"PROD 8 Buttermilk"	**"PROD 19 Product E"**
"PROD 9 UHT Milk"	**"PROD 20 Product F"**
"PROD 10 Cream"	
"PROD 11 Light Cream"	

7. The fluorometer will display "**Insert A STD: [START]**".
8. Gently mix one (1) of the cuvettes containing Calibrator A and the sample.
9. Open the cuvette chamber door and place Calibrator A in the chamber. Shut the door and press **START**.
10. After thirty (30) seconds, the fluorometer will make a final reading (~200 FLU) and display "**Insert A STD: [START]**". Repeat with a second sample of Calibrator A.

11. Follow the prompts for Calibrators B and C. If the calibration was successful, then the fluorometer display will read "**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 when **STOP** is pressed, 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 example above) 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:

1. All colored (flavored) products will require separate calibration curves.
2. 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.
3. 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.
4. 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 ± 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.

1. Allow one bottle of Fluorophos Substrate and one bottle of ALP Substrate Buffer to come to room temperature.
2. To reconstitute, carefully decant the entire contents of the Substrate Buffer into the Fluorophos Substrate bottle. Mix by gentle inversion and allow to sit at room temperature for at least 30 minutes, or until fully dissolved.

Daily instrument checks

1. Dispense 2.0 mL of the Daily Instrument Control into a clean, labeled cuvette.
2. Place the cuvette in the 38 ± 1ºC heating block and allow to warm for fifteen (15) minutes.
3. On the fluorometer, press **SETUP**. Select menu item **A/D TEST** by pressing < or >.
4. With nothing in the cuvette chamber, press **START**. The display should read 302 ± 4.
   - If the value is outside of the specified range, clean the excitation and emission filters and repeat the A/D Test.
5. Insert the pre-warmed Daily Instrument Control cuvette into the cuvette chamber and close the door.
6. Once the display has stabilized, it should read 602 ± 15. Record this value.
   - If the value is outside of the specified range, the value can be corrected to 602 by slowly adjusting the potentiometer screw (R15 resistor) clockwise or counterclockwise, as necessary.
   - If the instrument is adjusted to 602 or has experienced excessive drift, it should be recalibrated with the Calibrator Set (P/N FLA250).
7. Dispense 2.0 mL of the reconstituted substrate into a clean, labeled cuvette.

8. Place the cuvette in the 38 ± 1ºC heating block and allow to warm for fifteen (15) minutes.
9. After 15 minutes, insert the pre-warmed reconstituted substrate cuvette into the cuvette chamber and close the door.
10. Once the display has stabilized, it should read < 1200. Record this 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. This can be avoided by keeping the reconstituted substrate at 2-8ºC, protected from light, and pouring off the amount that is going to be used into a clean vessel for pipetting.

Sample preparation

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

1. **Whole milk, skim milk, low fat milk, chocolate milk, half and half, and heavy cream:** Mix the sample thoroughly before testing.
2. **Buttermilk, sour cream, and creamed cottage cheese:** Homogenize in a mechanical blender before testing.
3. **Concentrated and dry-milk products:** Reconstitute the product with laboratory grade water as necessary and test according to the procedure for the original product.
4. **Butter:** To each labeled 16 × 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).

5. **Cheese:** To each labeled 16 × 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, etc., 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 type of problem usually have greater than 10% fat and/or are very highly-colored products such as chocolate and blueberry ice cream mixes, for example.

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

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

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

If the specified 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-µL sample. You can use the specially designed 25-µL 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

Once 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.

1. Dispense 2 mL of the reconstituted Fluorophos® ALP Substrate into each of enough fluorometer cuvettes for no more than one day's tests. Preheat for at least 15 minutes in the heating block to bring the reagent to a temperature of 38°C. Reconstituted Fluorophos® ALP Substrate is stable for 6 hours in the heating block.
2. Pipette 75 µL 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.
3. Mix well with the vortex mixer.
4. Place the cuvette in the fluorometer cuvette holder and close the cuvette door.
5. At "**[CALIB] Calibration/Product [TEST]**", press TEST.

6. If necessary, use the < or > switchpad to select "**ALP Dairy**" and press ENTER.
7. As necessary, use the < or > switchpad to change to an appropriate channel for the product to be tested and press ENTER again.
8. Using the fluorometer keypad, type an identification number for the sample being tested and press ENTER.
9. Make sure the cuvette door is shut and press START to begin the test. The display will count down 60 seconds while the substrate and sample are being stabilized at 38°C.
10. After 60 seconds the fluorometer will begin to measure and display the fluorescence of the sample in fluorescence units (FLU).
11. At the end of three minutes, the fluorometer will display the average increase in fluorescence and the ALP activity in mU/L.
12. Remove the cuvette from the cuvette holder.
13. If desired, test another sample in the same manner.
14. 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 confirmatory tests: Differentiating bovine (residual) ALP from microbial and/or reactivated ALP.

Notes:

4 Instrument Maintenance

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.

Cleanup of spills

If a liquid is spilled into the fluorometer or heating block, the power cord should be unplugged 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 either the fluorometer or the electrical parts of the heating block in water under any circumstance.

Cleanup of heating block spills:

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. Use a test-tube brush to clean the heating block wells. Take care not to scratch the anodized finish.

Cleanup of cuvette holder spills:

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, etc., 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: it may have picked up sharp bits of broken glass from the cuvette holder. Repeat with new cuvette holder cleaners as necessary until the cuvette chamber is empty. Once all liquid has been removed from the instrument, remove filters and tip or invert fluorometer as necessary to remove any broken glass remaining in the cuvette holder.

e. 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.

f. With the power switch in the off position, connect the power cord to the instrument and the power outlet.

g. Place the power switch in the on position and allow the instrument to warm up.

h. Run controls to verify instrument performance. If problems are evident when running control values, obtain Hot-Line® Service as described in Chapter 5.

Reagent storage

See information supplied with each reagent for storage instructions.

Air filter replacement

The air filter should be replaced monthly or whenever it becomes clogged with dust so as to affect instrument performance. To change air filters, simply slide old filter out through back panel and replace with new filter cartridge.

Optical filter replacement

The fluorometer has been equipped with ALP and ACP excitation filters and a dual filter holder assembly. The assembly may be used by customers who run ALP tests, ACP tests, or both.

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

To replace filter assembly:

1. Shut off the fluorometer power switch and unplug the power cord from the power outlet.
2. 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.
3. Facing the front of the instrument, the excitation filter holder is located behind 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.
4. Slide in the new filter holder. Note that the ends of the dual filter assembly are marked ACP and ALP respectively. When the filter is in place, the label on the end of the filter will indicate which filter is currently in place. If the current filter is not ALP, simply remove the

filter holder, flip it around and replace it in such a way that the ALP filter name is showing.

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.

Excitation and emission filters should be replaced 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 un-usable 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.

1. Switch the power switch to the off position and disconnect the power cord.
2. Use a small flat-bladed screwdriver or similar tool to pry open the fuse holder door. Remove the fuse holder inside.

3. Double-check the values marked on the fuses. Use 5 × 20 mm, 250V, time delay (Type T) fuses. For 100-120V operation, use 2-Amp fuses; for 200-240V operation use 1-Amp fuses.
4. Re-install the fuse holder into the back of the instrument and close the fuse holder door.
5. Re-connect the power cord and switch the power switch to the on position. The instrument should start up as normal.

Lamp replacement

The instrument contains a Halogen lamp with a rated effective lifetime of 10,000 hours.

The lamp is not a user-accessible area and should be changed by a qualified service technician, per the instructions found in the service manual for this instrument. Users are cautioned that opening this instrument exposes them to Hazardous Live Voltages in excess of 600 volts AC and DC.

Instruments operated on an 8-hour day may need lamp replacement after approximately 1200 days of operation. If the instrument is left on continuously, this number falls to 416 days.

Notes:

5 Troubleshooting & 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.

Service & maintenance cautions

- Do not perform any service or maintenance yourself, except as detailed in the User's Guide.

- Unplug the power cord prior to opening or removing covers, or else you may be exposed to electric shock, excessive temperatures, or mechanical hazards. Voltages in excess of 600 VAC and VDC are present inside this product.
- 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. Replace the fuse only 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 user's guide for instructions on routine adjustments, instrument care and troubleshooting. If this information does not solve the problem, call the appropriate number below.

- 800-225-4034 (toll-free within the USA and Canada; after normal business hours, dial extension 2191)
- +US 781-320-9000 (elsewhere)
- 781-320-0811 (fax)

When contacting our service personnel, please have the model and serial numbers from the label on the back of the instrument, and the symptoms of the problem ready. You should use a telephone as close to the 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, the service technician may assist you in making minor repairs over the phone, providing you with recommended parts (or part numbers), or may have you ship the instrument for factory repair.

If you need to return an instrument for repair or replacement:

- Notify our service department to obtain an RMA.
- Be sure to contact Advanced Instruments before shipping to avoid any delays.
- 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.
- 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.

- Do not tip, store, or ship the fluorometer without first removing any liquid-containing cuvette from the cuvette chamber. 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 the 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 back panel of the instrument. Switch 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 holder door. Remove the fuse holder inside and 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 the instrument is designed in such a way that any incomplete task will be associated with an error message, many self-explanatory, that will help you discover the source of the 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 switchpad at "**[CALIB] Calibration/Product [TEST]**". The display will change to "**1: A/D Test**".

b. To select another test on the SETUP menu, repeatedly press > or < until the desired item is displayed.

c. To activate a displayed test, press START.

d. Press STOP to exit the SETUP 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.

Serial Loop Test

This menu item is no longer supported.

Display/Print Test

Display/print test is a simple check of the display and printer. Press START to run the test. On entry, a series of characters should scroll across the display (and a single line of the same characters should print out on the printer). 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 printer, as well as the display. Press STOP to end the test.

Keypad Test

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

Photomultiplier tube voltage adjustment

The voltage supplied to the photomultiplier tube (PMT) can be adjusted to compensate for long-term changes in its output.

For instruments with Serial Suffix D or later:

1. Pipette 2 mL of Calibrator C into a cuvette and place the cuvette into the cuvette heater to warm at 38º C for 15 minutes.
2. Place Calibrator C into the cuvette holder, close the door and press SETUP.
3. Advance the menu to "**10: A/D Test**" by pressing < or >, or by using the number keypad. Press START.
4. Change the fluorescence level to 602 ± 2 FLU for ALP by slowly adjusting the potentiometer on the left side of the instrument enclosure (see figure 6).
5. Wait approximately five minutes and recheck the reading. Re-adjust to the above limits if the reading has shifted.
6. Exit the Setup menu.

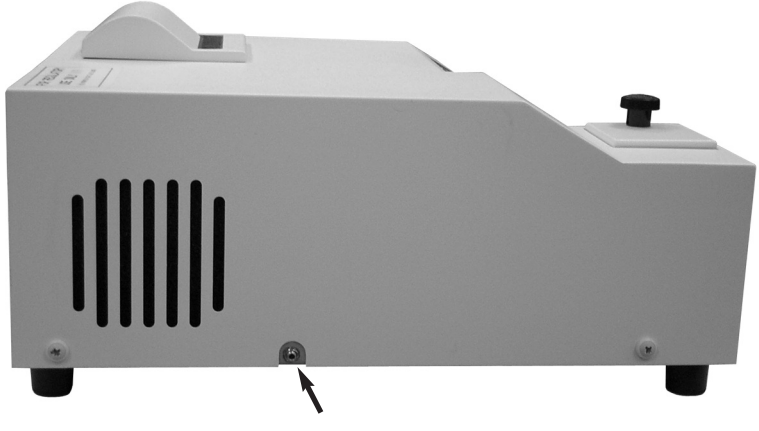

Figure 6: PMT Voltage Potentiometer (Serial Suffix D and Later)

NOTE Prior to calibrating or testing products, check the calibration ratio of the A, B, and C calibrators (with no milk added) on an empty channel. The calibration ratio should be 151 ± 7.

If the fluorescence cannot be set to the required value, the PMT gain will have to be changed. Contact Advanced Instruments for Hot-line® Service.

For instruments with Serial Suffix prior to D:

Instruments prior to Serial suffix D are not equipped with an external accessible potentiometer. Adjusting the PMT voltage requires removal of the enclosure and exposure to potentially hazardous voltages. Contact Advanced Instruments for Hot-line® Service.

Notes:

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:

Calibration Ratio = [(CAL C-A) + 2 (CAL B-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:

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:

Lin. CAL A (0 µmoles/L FY) = 191-191 = 0

Lin. CAL B (17.24 x 10^{-3} µmoles/L FY) = 317-191 = 126

Lin. CAL C (34.48 x 10^{-3} µmoles/L FY) = 448-191 = 257

In every case, the value of Calibrator C must be twice (± 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® FLM200 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 print-

out of the calibrator values and the average increase in fluorescence of the sample, as follows:

1. **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.

2. **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).

3. **Calculation equation:**

   a. Calculate the µmoles FY/min/0.075 mL sample using the corrected fluorescence reading of the "B" calibrator (Calibration Ratio used for automatic fluorometer calculations) which contains 3.448×10^{-5} µmoles of FY/2 mL:

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

   b. Determine the µmoles of FY formed by one liter of sample:

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

c. Convert U/L into mU/L:

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

d. Simplified calculation:

Avg F of corrected “B” Calibrator = (Avg B STD - Avg A STD) = (344-198) = 146

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

Using the example above (1.) and the equations in (3.) above, the following results were calculated:

Fluorescence of corrected"B" Calibrator = 146 Fluorescence Units

Avg F for Test Sample = 114.5 Fluorescence Units/min

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

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.

mU/L (10) = mU/kg

Percent raw milk and ALP enzyme activity

U.S. Federal and IDF Standards require that cows milk intended for human consumption contain less than 1.0 µg phenol/mL/15 min of ALP activity. This value of 1.0 µg phenol/mL/15 min is equivalent to 500 mU/L of ALP enzyme activity using the Fluorophos® test method.

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 µg phenol/mL/15 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 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 µg phenol/mL/15 min activity the original mixed-herd milk sample must have contained 1,000 µg phenol/mL/15 min activity. When 1,000 µg phenol/mL/15 min activity is diluted to 0.1% (v/v) (× 10,000 dilution), the expected value will be 1.0 µg phenol/mL/15 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 µg/mL/15 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.

% Raw Milk (v/v) in Whole Milk[1]	ALP ACTIVITY[3]		
	Fluorophos mU/L	AOAC 979.13 µg phenol/mL/15 min	IDF 82:1987 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
0.100	500.0	<1.00[4]	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

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 acti-vity in mixed-herd raw milk before pasteurization will vary due to species and seasonal variations.
4. Cutoff for acceptable milk is µ1 mg phenol/mL/15 min. Pasteurized Milk Ordinance (PMO) Grade A, 1985 Revision, U.S. Department of Health and Human Services, FDA, Washington, D.C.
5. Cutoff according to Codex Alimentarius Complete mission, World Health Organization, July, 1989. Joint FAO/WHO Standards Programme.

Differentiating microbial from bovine milk ALP

1. **Principle:** Alkaline Phosphatase (ALP EC 3.1.3.1) synthesized by microorganisms is heat stable at 145ºF or 63°C, whereas bovine milk ALP is inactivated at this temperature.
2. **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± 0.5°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 5 minutes in a cold water bath.

d. Re-test for ALP activity. The flow diagram in figure 7 will assist in interpretation of the results.

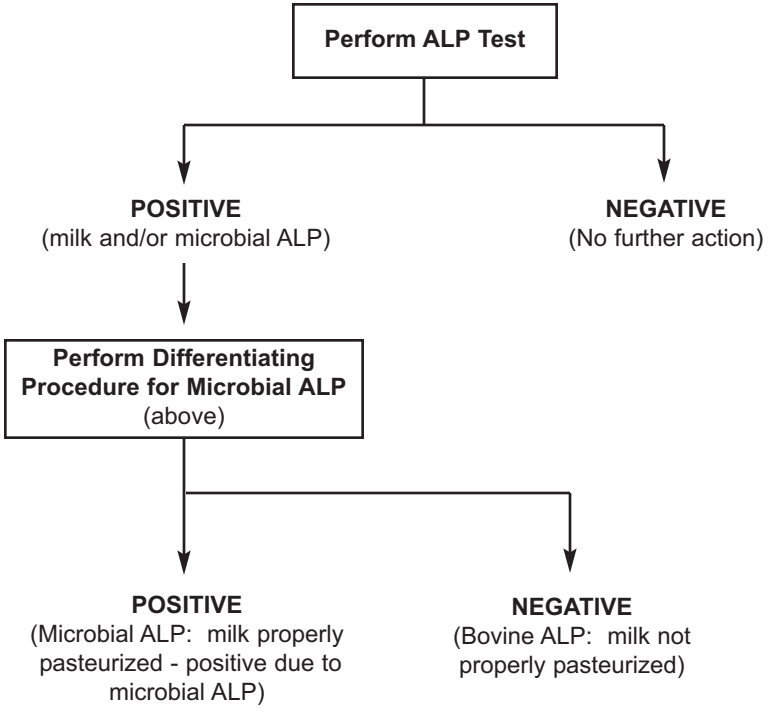

Figure 7: Microbial ALP Test Flow Diagram

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 $Mg(C_2H_3O_2)_2 \cdot 4H_2O$ 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.

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 in step 2, above) 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 5 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, above.

h. Run an ALP activity test on the "Test"-sample diluted product from step 3.g, above.

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, above), 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).

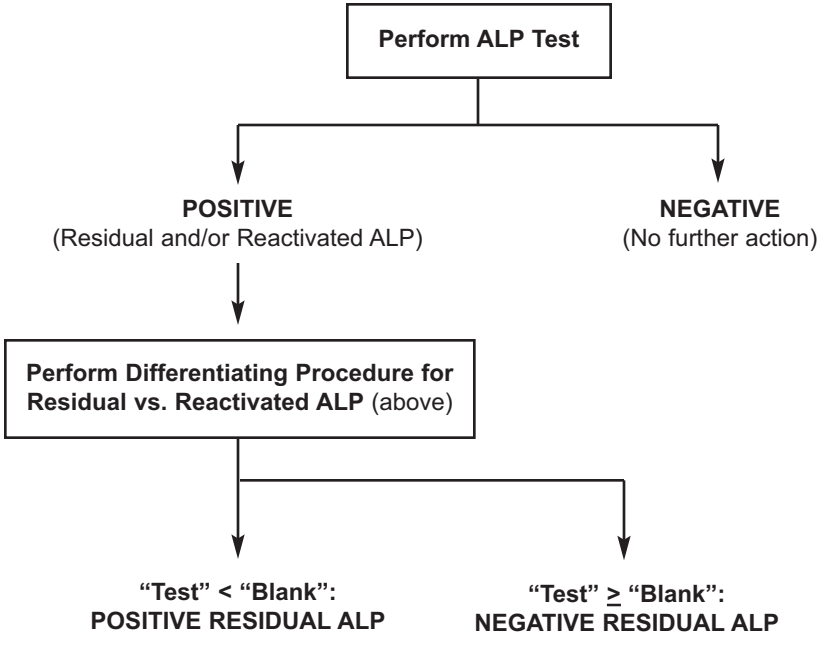

Figure 8: Residual ALP Test Flow Diagram

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.

Appendix B

Troubleshooting Table

Problem/Message	Explanation
 "Error: Cuvette Door Open"	Test procedure is cancelled. Close the cuvette door and restart the test.
"Low Cuvette Temperature" "High Cuvette Temperature"	The temperature of the fluorometer cuvette chamber (and/or the contents of the cuvette in the cuvette chamber) is not at 38 ± 0.2°C. Wait for the cuvette chamber temperature to stabilize and the fluorometer display to read, "Fluorometer Ready". Repeat the test with reagents pre-warmed as recommended in the instructions in the test kit.
"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.
"Error: Tubes Reversed?"	The substrate blank and the standard were tested in reversed order or the substrate blank result was over 3000 FLU. Prepare new samples and repeat the procedure.
"Calibration Not Complete"	The difference between the fluorescence of the duplicate readings on each calibrator must be less than ± 5 fluorescence units.

Problem/Message	Explanation
"Calibration Not Acceptable"	1. The calibrators may not have been brought to temperature (38°C), or the calibrators may be bad. Recalibrate using new calibrators. See the Calibration section in Chapter 2. 2. Check Calibrator C under PMT voltage. See the PMT Voltage Adjustment section in Chapter 5. 3. The optical lamp and/or excitation filter may require replacement. Contact Advanced Instruments for Hot-Line® Service.
"Value Less Than 10 mU/L"	Fifty-seven out of 60 readings in either minute 2 or minute 3 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.
"Error: Overranged, Repeat Test"	Display reads over 24,000 fluorescent units, very high activity. Repeat on dilution to confirm.
"Error: Unstable Reading, Repeat Test"	The average change in fluorescence during minute 2 is not within 20% of that during minute 3. Among the reasons for this are: 1. Substrate not at 38°C. 2. Not a uniform, well-mixed sample. 3. Very low result. 4. Very high result. Repeat the test.

Problem/Message	Explanation
Readout is unstable	The optical lamp and/or excitation filter may require replacement. Contact Advanced Instruments for Hot-Line® Service.
Calibrator C output drifts over time, or instrument requires frequent readjustment	The optical lamp and/or excitation filter may require replacement. Contact Advanced Instruments for Hot-Line® Service.

Notes:

Appendix C
Product Specifications

Electrical	
Voltage:	100 to 120 VAC or 200 to 240 VAC
Frequency:	50 or 60 Hz
Current:	
100-120 VAC:	1.2 Amp. for fluorometer , 1/4-Amp. for heating block
200-240 VAC:	0.7 Amp. for fluorometer , 1/8-Amp. for heating block
Fuses:	as listed on power label
Power:	
Fluorometer	150 Watts
Heating block	30 Watts
Memory backup:	Integral lithium cell; five years minimum in absence of power
Sample Volume:	See test kit instructions
Sample Capacity:	Single sample
Readout:	20-character dot matrix
Units:	FLU (fluorescence units)
Results Units:	See test kit instructions
Auto Ranges:	
Low:	0 to 999.9 FLU/minute
High:	1000-9999 FLU/minute
Zeroing:	automatic
Resolution:	± 1 FLU
Gain:	automatic
Drift:	Less than 3 FLU per hour
Cuvette Size:	12 × 75 mm round
Warm-up Time:	
Fluorometer	15 minutes for cuvette chamber
Heating Block	30 minutes

Optics:	90° optical bench with light source, fixed filter, detector
Lamp:	quartz halogen
Filters:	
Excitation	440 ALP nm, narrow pass/405 ACP nm, narrow pass
Emission:	530 nm, sharp cut-off
Storage Temperature:	32 to 158°F or 0 to 70°C
Communications:	RS-232C 9-pin DTE standard; baud selectable: 30, 1200, or 9600
Printer:	built-in, 40-column, thermal
Operating Temp. (ambient):	68 to 85°F or 20 to 29°C
Room Humidity:	5-80% (non-condensing)
Test Time:	3 minutes
Dimensions	inches centimeters
Height:	7.0 18
Width:	12.5 32
Depth:	16.5 42
Dimensions (Block)	inches centimeters
Height:	3.5 9.0
Width:	6.5 16.5
Depth:	8.5 21.5
Weight	pounds kilograms
Net:	25 11
Shipping:	35 16
Certification:	Intertek 98214

Appendix D
Regulatory Notices

- This product has been designed and manufactured in accordance with 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

CE

This symbol indicates conformity to relevant European directives.

c ETL us LISTED
Intertek
98214

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 UL 61010-1 2nd edition, "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 2nd edition, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use". The "C" in the lower left of the ETL mark demonstrates this listing.
EC Declaration of Conformity - EMC	This product meets the intent of Directive 2004/108/EC 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. • EN 61326-1:2006, Group 1, Class B, "Electrical Equipment for Measurement, Control, and Laboratory Use".
EC Declaration of: Conformity - Low Voltage	This product meets the intent of Directive 2006/95/EC, 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 2nd edition, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use". • IEC/EN 61010-2-010 2nd edition, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Particular Requirements for Laboratory Equipment for the Heating of Materials".

Regulatory approval type	Description
EC Declaration of Conformity - WEEE	This product meets the intent of Directive 2002/96/EC as amended by 2003/108/EC 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 2002/95/EC for "Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment" as an exempt medical device per Article 2, Paragraph 1 and per Annex 1, Category 9 of Directive 2002/96/EC.
CB Report CB Certificate	A CB report and certificate have been issued for this product. The standard used was IEC 61010-1, 2nd edition and 61010-2-010 2nd edition.

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. Bundesgesundheit 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 al Engenharia e Technologia - 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.**)

Appendix E
Warranty & Warranty Duties

LIMITED
WARRANTY
CONDITIONS

MODEL NUMBER: _____
SERIAL NUMBER: _____
INSTALLATION DATE: _____

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 except glass, plastic and parts warranted by their makers.

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.

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.

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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.

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):
    a. Beyond the useful life of the equipment.
    b. From unreasonable use.
    c. When Advanced Instruments is not immediately notified of said injury.
    d. From interpretation of results.

DEFINITIONS

1. "Useful life" is:
    a. 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.
    b. 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.
    c. Only during the time when the user has not altered the equipment in any way without written approval from Advanced Instruments.

d. Only during the time when the user has not loaned, leased or resold the equipment to any third party.

2. "Reasonable use" is use:

a. 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.

b. Under direct, on-the-job supervision of the supervisor or other professional in charge.

c. In which there are no known defects or uncorrected repairs.

d. Only for the purpose stated in the instructions provided with the instrument.

e. In which the equipment has been maintained according to the instructions provided.

3. "Immediate notification" is:

a. Recognition that time is of the essence when any accident, malpractice or product liability arises which involves Advanced Instruments equipment.

b. 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.

c. Allowing Advanced Instruments or its representatives, immediate, full, and thorough examination of Advanced Instruments equipment, and all records pertaining to such equipment.

Notes:

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.

Sample RS-232 Setup

As a typical example of a communications program setup, the following instructions will describe the process necessary for using your instrument in conjunction with Hyperterminal for Windows® 95 or later operating systems and a null modem cable, available from Advanced Instruments, as shown in the diagram below. You can use this procedure to be sure that your instrument and cable are operating correctly.

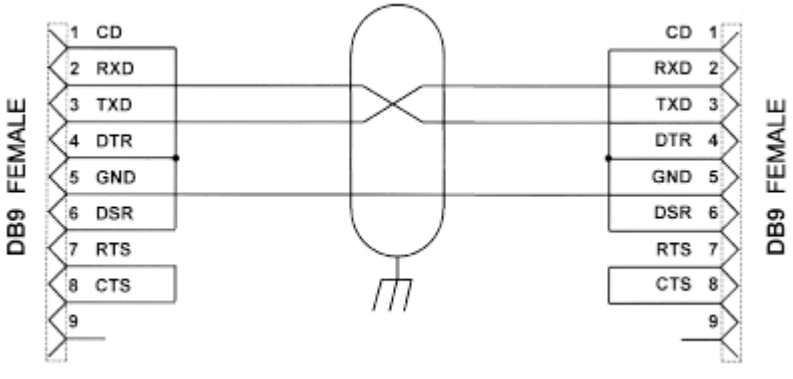
1. Create a Hyperterminal connection using the Hyperterminal program.
2. While working within that connection, choose the Properties selection from the File menu.

3. Select the Configure button.
4. Choose the following settings for the Configure menu and click on the OK button.

Bits per second	See User's Guide
Data bits	8
Parity	None
Stop bits	1
Flow control	None

5. Select the Settings menu.
6. Chose TTY for the Emulation option.
7. Select ASCII setup.
8. Set the Sending option to Echo Typed Characters Locally.
9. Set the Receiving option to Wrap Lines that Exceed Terminal Width.

You should at this point see all instrument output in the Hyperterminal window of your computer screen.



Null Modem Cable

Shield connects to metalized connector housing at both ends.

Appendix G
Symbol Definitions

	On-Off		Functional Arrow
	Feed		Printer
	Interrupt		Enter
	Test		RS232
	Start		Bar Code
	Stop		Attention
	Record Review		Caution Hot Surface
	Setup		Dangerous Voltage
	Calibration		Lifting Hazard
	Cancel; Delete		Calibrator

CONT	Content		Do Not Open Top
CONTROL	Control		Handle With Care
CONTROL –	Negative Control		Toxic
CONTROL +	Positive Control		Use Blade To Open
	Flammable		Do Not Re-Use
	Fragile	IVD	For *In Vitro* Diagnostic Use
	Irritant	CE	European Conformity
	Keep Dry		Temperature Limit
	Date Manufactured		See Instructions
STERILE	Sterile	LOT	Lot Number
	Non-Sterile		Use By; Expiration Date
SN	Serial Number	EC REP	Authorized Representative
SOLN	Solution	REF	Part Number

	Sufficient for [x] Tests		Latex-Free
	Open Here		Diluent
	Low Fluid Level		See Instructions for Temperature Guidelines
	Keep Hands Clear		Potential Puncture Hazard
	Manufacturer		Electronic Equipment - Dispose of Properly

Notes:

Appendix H

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; after normal business hours, dial extension 2191)
  - +US 781-320-9000 (elsewhere)
  - 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 I

Service Log

Model: **FLM200**

Serial Number: _____

Software Revision: _____

Date	Problem/Symptom	Action

Date	Problem/Symptom	Action

Index

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P

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