

UV-Vis

Orion AquaMate 7000 Vis
Orion AquaMate 8000 UV-Vis
User Guide

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Thermo
S C I E N T I F I C

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WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

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Contents

Preface

Congratulations on your purchase of a Thermo Scientific spectrophotometer! Our spectrophotometers integrate advanced hardware features with the power and flexibility of a wide range of accessories.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

Note Notes contain helpful supplementary information.

IMPORTANT Follow instructions labeled “Important” to avoid damaging the system hardware or losing data.

CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

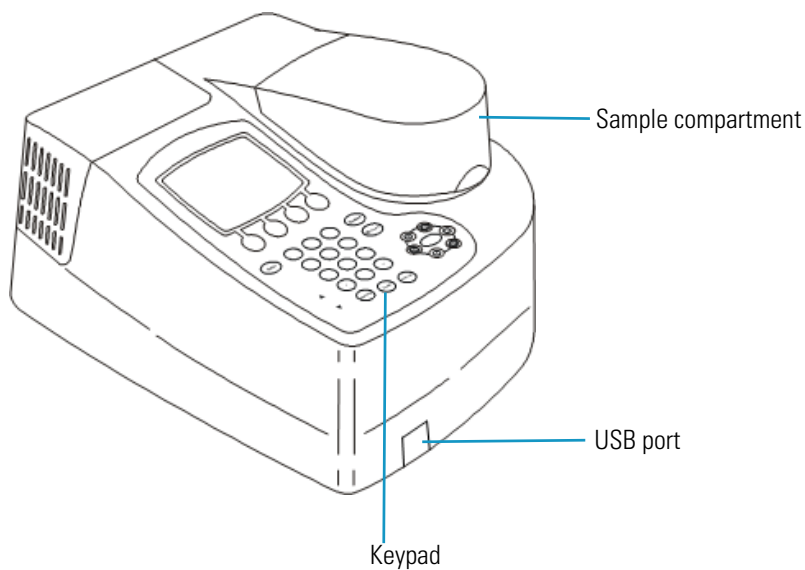
Spectrophotometer Basics

This chapter describes:

- Spectrophotometer Components
- Cell Holders
- Selecting and Positioning Cuvettes
- Z-dimensions

Spectrophotometer Components

Here are some major components visible on the outside of a typical instrument:

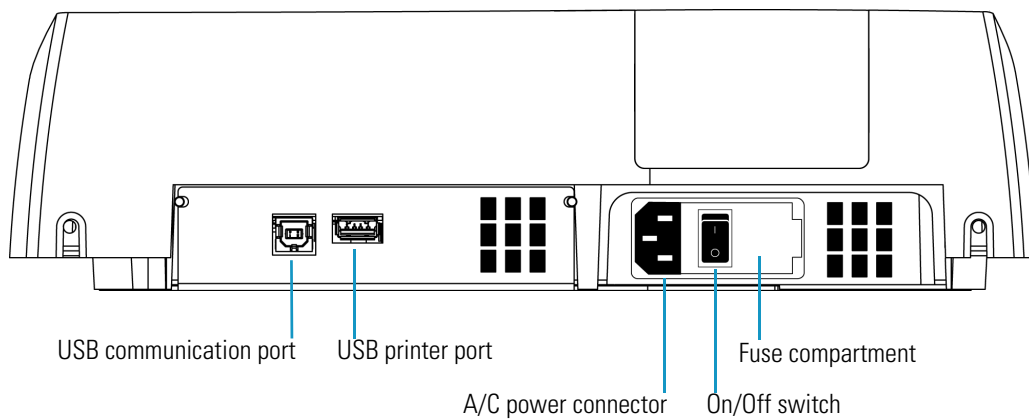


Connectors

The connectors are on the back of the instrument:

1 Spectrophotometer Basics





Spectrophotometer Components







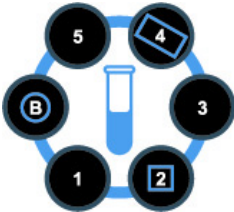






WARNING Avoid shock hazard. Always turn off the instrument and unplug it from the wall outlet or power strip before you unplug the power cord from the instrument connector.

About the Keypad



Key or button	Function
 Function keys	<ul style="list-style-type: none"> • Called “Function” keys. • Performs a specific function as displayed above each key. • Functions will change depending on the software screen. • Some function keys may not be active.
	<ul style="list-style-type: none"> • Clears the value being entered. • Returns to the previous screen.
	<ul style="list-style-type: none"> • Deletes the last character entered.
	<ul style="list-style-type: none"> • Accepts highlighted, entered, or selected values. • Advances to the next parameter or screen.

Key or button	Function
	<ul style="list-style-type: none"> Prints the method or results to the selected printer. If “PC” is selected for the printer, sends the method or results to the USB port.
	<ul style="list-style-type: none"> Displays a menu of software applications.
	<ul style="list-style-type: none"> Displays the Utility screen.
 	<ul style="list-style-type: none"> Controls the location of the cursor. Highlights the value or option for the selection.
	<ul style="list-style-type: none"> Enters numbers, a decimal point and a minus sign for values.
	<ul style="list-style-type: none"> Cell position keys. Selects the cell holder position to be measured. B = blank and 1-5 = sample positions.
	<ul style="list-style-type: none">    = positions when used in Auto 3 mode.

Cell Holders

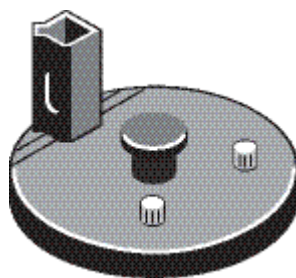
The Orion AquaMate 8000 UV-Vis includes a special cell holder with positions for a vial, a square cuvette, and rectangular long path cuvettes up to 50mm in length. 6-position and single cell holders are also included for traditional spectrophotometry applications, allowing you to take advantage of the full capabilities of the instrument.

The Orion AquaMate 7000 Vis includes only the three position water analysis cell holder. Other cell holders are available for purchase as optional accessories.

6-Position Cell Holder



Single Cell Holder



Note If the Cell Positioner method option is set to Auto 6, whenever you press **Run Test** to start a measurement, the instrument attempts to initialize the cell positioner. If a single cell holder is installed, the message “Error, Single Cell Holder found. Use Single Cell Holder?” appears. Press **Accept Change** to continue the measurement with a single cell, or install the 6-cell changer and press **Cancel Change**.

See the parts list for a more detailed list of available accessories.

Selecting and Positioning Cuvettes

The compatible wavelength range for different types of cells depends on the material used.

Cell Type	Wavelength
Optical Glass	360 nm to > 1100 nm
Borosilicate Glass	330 nm to > 1100 nm
Disposable:	
Quartz	190 nm to > 1100 nm
Polystyrene	> 340 nm
Methacrylate	> 300 nm
Acrylic	> 280 nm
UV-transparent	> 220 nm

Note See the manufacturer’s specifications and work within the recommended range.

Note The pathlength of test tubes is not as well defined as that of square cuvettes.

Other Guidelines

Position cuvettes and test tubes so that the clear sides face the light beam, one clear side facing the front of the instrument and the other facing the back.

Note Always place test tubes in the instrument in exactly the same orientation in the light beam. An alignment mark on the test tube helps you orient the test tubes consistently and correctly.

When using small aperture (small volume) cells:

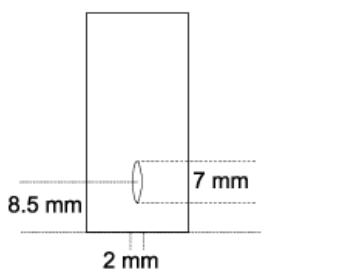
- Always use cells with black masking
- Use the same cell (or cuvette) for your blank and your samples

Z-dimensions

The figure below illustrates the position of the light beam in the instrument.

Beam size specifications are shown below.

- Distance from bottom of cuvette to center of beam (Z-dimension): 8.5 mm
- Beam dimensions: 2 mm (wide) by 7 mm (tall)



Setting Up the Instrument

Setting up the instrument involves:

- [Entering Parameter Values](#)
- [Setting Utility Parameters](#)

Entering Parameter Values

The following sections describe the use of the keypad to interact with menus and controls. These sections provide instructions for:

- [Numeric Entry](#)
- [Menu Selection](#)
- [On/Off Toggle](#)
- [Alphanumeric Entry](#)

Numeric Entry

With the parameter (e.g., Wavelength) highlighted, start typing the numeric value. An Entry window with the value range appears. Type the complete entry and press **Enter**.

Advanced A-%T-C	17:02 28Feb07		
	BLANK		
Test Name	Copper		
Measurement Mode	Conc/Std		
Wavelength	546nm		
Ref. Wavelength Correction	On		
Ref. Wavelength	320nm		
Delay Time (min:sec)	0:00		
Conc of Standard	Undefined		
Factor	Undefined		
Units	C		
Sample Positioner	Auto 6		
Number of Samples	1		
<table border="1"> <tr> <td>Entry: 4</td> </tr> <tr> <td>Enter Wavelength (190 - 1100)</td> </tr> </table>		Entry: 4	Enter Wavelength (190 - 1100)
Entry: 4			
Enter Wavelength (190 - 1100)			

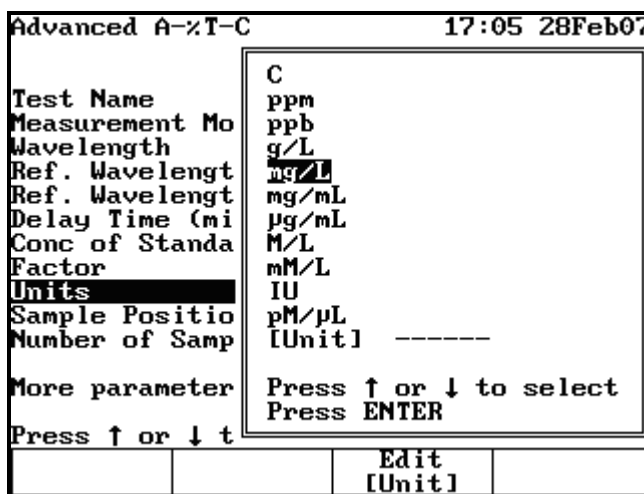
2 Setting Up the Instrument

Entering Parameter Values

Alternatively, you can press **Enter** to display the Entry window with the value range and then type the complete entry and press **Enter**.

Menu Selection

With the parameter (Units, or Sample Positioner) highlighted, press **Enter** to display the selection list. Highlight the appropriate item and press **Enter**.

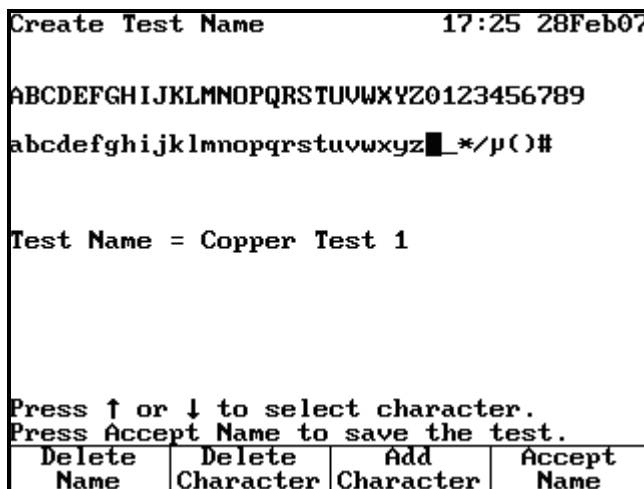


On/Off Toggle

With the parameter (e.g., AutoPrint) highlighted, press **Enter** to toggle to the opposite value.

Alphanumeric Entry

With the parameter (e.g., Test Name) highlighted, press **Enter**. The Name Entry screen appears. Highlight the desired character and press **Add Character**. When you are finished, press **Accept Name**.



Setting Utility Parameters

The Utility menu lets you set certain non-test hardware parameters, such as the date and time, standby setting, screen contrast settings and printer setup. You can also access a directory of all stored tests and the calculator function.

You cannot set utility parameters or change the utility when the instrument is carrying out a measurement.

- Press **Utility** on the keypad.

Utility	4:09pm 5Aug09
Software Revision:	4.000d
Instrument Serial Number:	2R01234567
Printer	PC
Date/Time Setup	4:09pm 5Aug09
Standby	Off
Baseline Expiration (hr:min)	Off
Beeper	On
Language	English
% of lamp life used	0.87%
Screen Contrast	
Printout Contrast	
Stored Tests Directory	
USB Drive Files	
Calculator	
Press ↑ or ↓ to select parameter.	

Setting the Date and Time

Highlight **Date/Time Setup** and press **Enter**.

Utility	3:39pm 16Mar07		
Set Date	16Mar07		
Time Format	am/pm		
Set Time	3:39pm		
Press ↑ or ↓ to select item to change.			
Set Day	Set Month	Set Year	

You can modify the date, time format and time.

❖ **To set the date**

1. Highlight **Set Date** and press **Enter**.
2. Press **Set Date**, type the date and press **Enter**.
3. Press **Set Month**, highlight the correct month and press **Enter**.
4. Press **Set Year**, type the year and then **Enter**.
5. Press **Esc** to save the settings.

❖ **To select the time format**

You can set the instrument to display the time in either am/pm or 24-hour format. To change the format, highlight **Time Format** and press **Enter** until the desired format (AM/PM or 24 hour) appears.

❖ **To set the time**

1. Highlight **Set Time** and press **Enter**.
2. To set the hour, press **Set Hour**, type in the hour and press **Enter**.
3. To set the minutes, press **Set Minute**, type in the minute and press **Enter**.
4. To select between AM and PM (if in AM/PM time format), press **Set AM/PM** until the appropriate setting appears.

Note Your changes are saved automatically (even during power down) by battery backup.

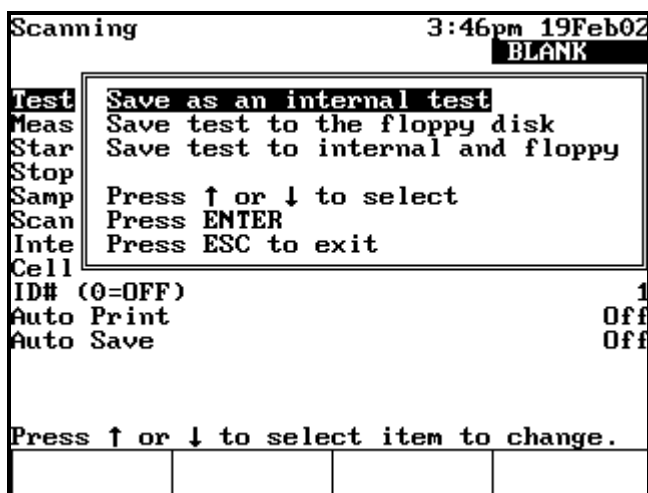
Standby Mode

To prolong lamp life, your spectrophotometer has been pre-set at the factory to automatically go into standby mode after 15 minutes of inactivity.

Since the tungsten lamp of the Orion AquaMate Vis spectrophotometer requires a 30-minute warm-up, the spectrophotometer has been pre-set at the factory to Standby Mode OFF.

❖ **To change Standby Mode time**

1. Highlight **Standby** and press **Enter**.



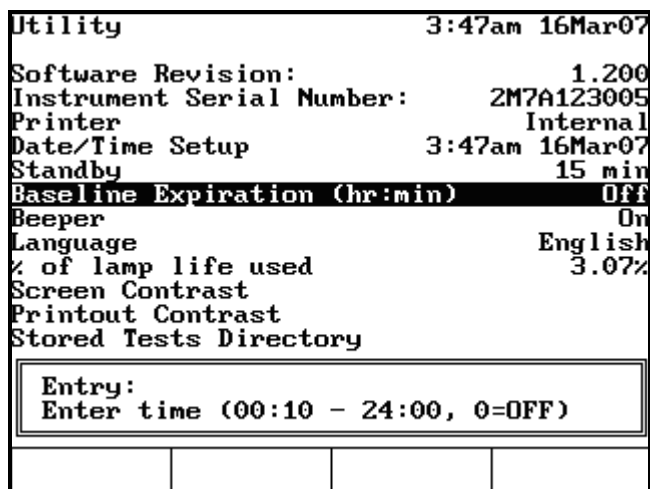
2. Highlight the length of time you want the instrument to wait before entering standby mode and press **Enter**.

Setting Baseline Expiration Time

If you will be performing scans on your samples, you can set a time limit for which a collected baseline will be valid. This is particularly useful when measurements are made in a production setting across multiple shifts or when the nature of the blank sample changes dramatically with time.

❖ To set the baseline expiration time

1. Highlight **Baseline Expiration (hr:min)** and press **Enter**.



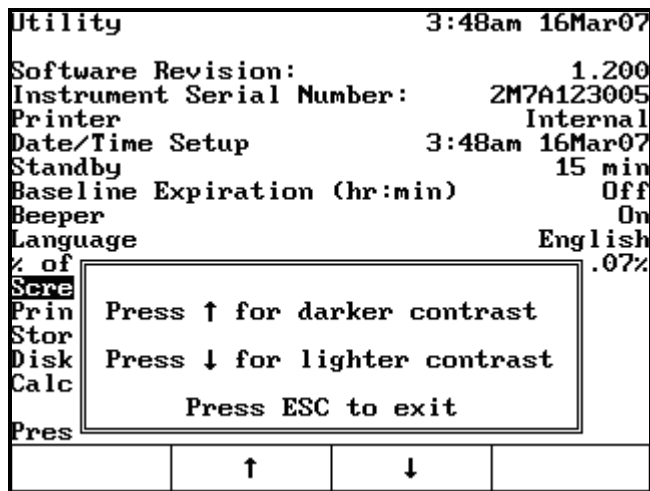
2. Enter the desired time in the **Entry baseline expiration time** field and press **Enter**.

Setting the Screen Contrast

To make it easier to read the display, you can adjust the screen contrast on the instrument.

❖ **To set the screen contrast**

1. Highlight **Screen Contrast** and press **Enter**.



2. Adjust the contrast by following the instructions on the screen.
3. Press **Esc**.

Accessories

This chapter briefly describes the sampling and system accessories that are available for your spectrophotometer. Complete descriptions and operating instructions are included with the accessories.

You can install or remove these accessories without switching off the instrument.

Cell Holders and Cell Holder Accessories

The AquaMate is shipped with a custom cell holder that accommodates a vial up to 25 mm in diameter, a rectangular cell up to 50 mm long, or a 10 mm pathlength square cuvette. An additional single cell platform and 6-cell carousel for 10 mm square cells is included with the Orion AquaMate 8000 UV-Vis.

Cell Holder Configurations

The following table shows the available cell holders and their accessories. You can install or remove accessories without switching off the instrument.

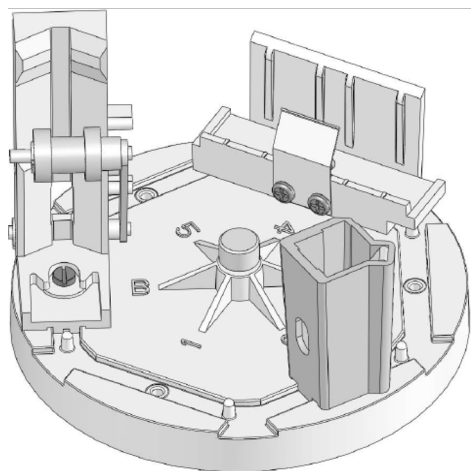
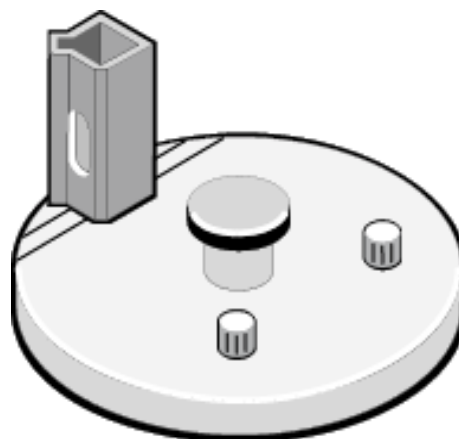
3 Accessories

Cell Holders and Cell Holder Accessories

Cell Changer System

Single Cell System

Standard Cell Holders



Cell Holder Initialization

When you press **Run Test** to start a measurement, the instrument displays the message “Calibrating and Checking Turret, Please Wait” while it attempts to initialize the cell changer at the Blank position. With the Water Analysis carousel, the spectrophotometer will rotate the carousel to the default position, then rotate the selected cell holder back into the measurement position. This ensures that the cell holder is properly positioned in the beam.

If a single cell holder has been installed, the message “Error, Single Cell Holder found. Use Single Cell Holder?” appears. Press **Accept Change** to continue, or install the cell changer and press **Cancel Change**.

If the 6-Position Cell Holder has been installed, it will initialize it to the “B” position. After it has initialized the cell changer, the instrument will display the data collection screen for the test.

Note If, while in this screen, you remove the cell changer and press Run Sample, the instrument will display “Fatal Error: 8 Press Esc to return to main menu.”

Pressing **Esc** returns you to the parameter menu screen of the test. Press **Run Test** and the instrument will now display the message, “Calibrating and Checking Turret, Please Wait” while it attempts to initialize the cell positioner at the Blank position.

If the cell changer is still not in the sample compartment, the instrument will display “Error, Single Cell Holder Found. Use Single Cell Holder?”

Either press **Cancel Change** and reinstall the cell changer or press **Accept Change** to continue with using the single cell holder.

To prevent the Fatal Error whenever removing the cell changer, always return to either the main menu or the test parameter menu of the test before removing the cell changer.

Changing Cell Holders

To:

- use long pathlength cells (cylindrical or rectangular)
- use test tubes
- measure solid samples in the filter holder
- regulate sample temperature via an external liquid recirculator

you must install the appropriate cell holders. The 6-Position Cell Holder installed in the spectrophotometer can easily be removed to install other accessory cell holders. See [“Removing the 6-Position Cell Holder and the Single Cell Holder”](#) on page 17.

Installing the 6-Position Cell Holder and the Single Cell Holder

❖ To install the 6-Position Cell Holder and the Single Cell Holder

1. Open the sample compartment door and let it rest on its hinge.
2. With one hand, carefully lower the cell holder straight down into the sample compartment.

3 Accessories

Cell Holders and Cell Holder Accessories

Figure 1. 6-Position Cell Holder

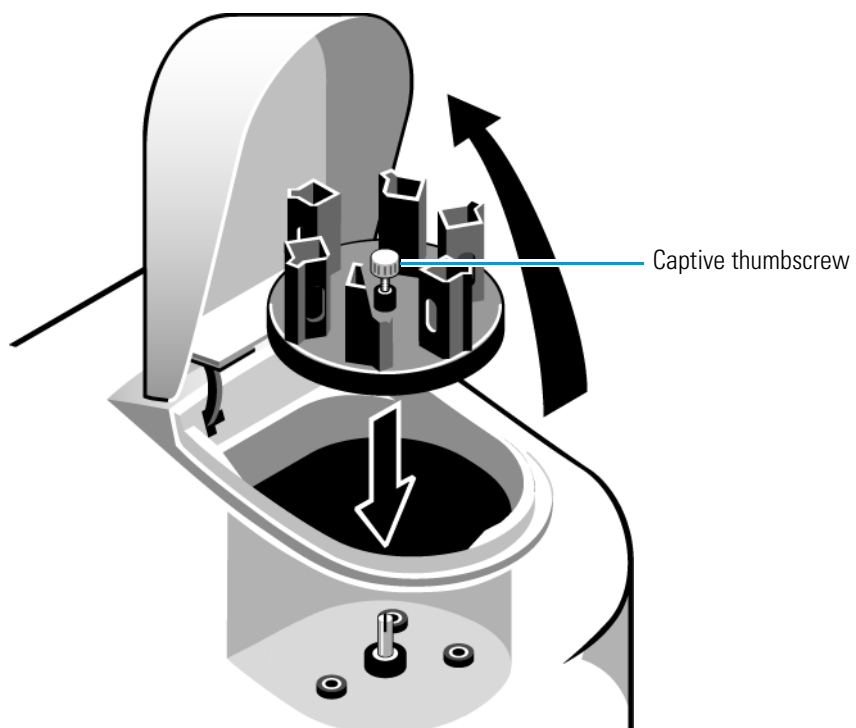
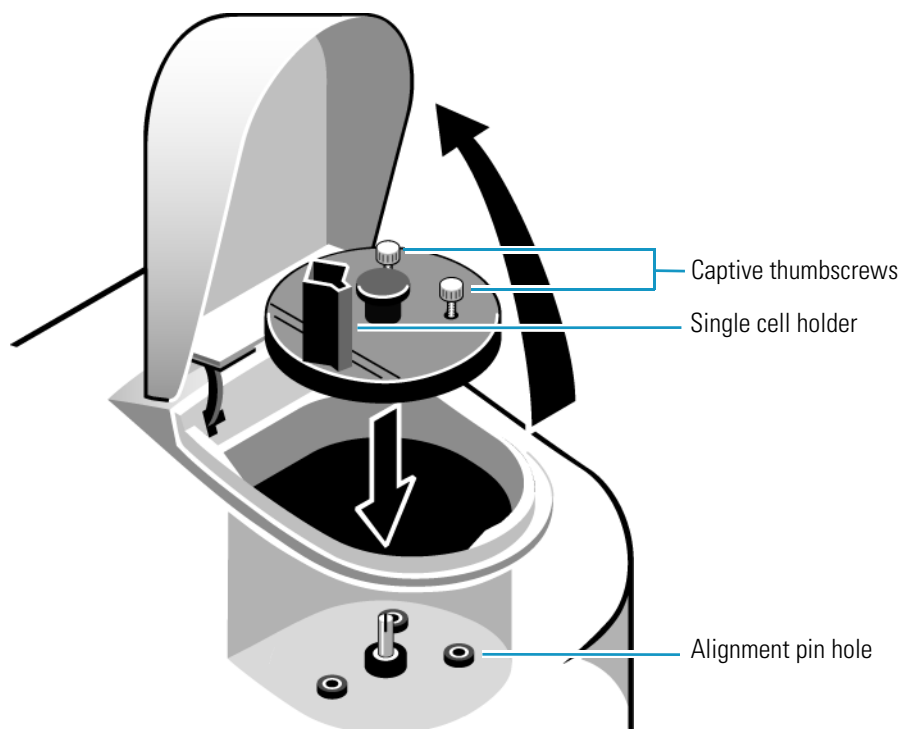


Figure 2. Single Cell Holder



3. With your other hand, tighten the captive thumbscrew(s).
4. Close the sample compartment door.

Note If the cell holder is not aligned correctly, you will not be able to tighten the thumbscrews.

Removing the 6-Position Cell Holder and the Single Cell Holder

❖ To remove the 6-Position Cell Holder and the Single Cell Holder

1. Open the sample compartment door and let it rest on its hinge.
2. With one hand, loosen the captive thumbscrew.
3. With your other hand, pull straight up on the cell holder and lift it out of the sample compartment
4. Close the sample compartment door.

Installing Accessory Cell Holders

Make sure that you have the correct cell holder baseplate installed.

Note To use 100 mm long pathlength cells, you must install the Single Cell Holder baseplate.

See “[Cell Holder Configurations](#)” on [page 13](#) for the different cell holder accessories that can be created for your instrument.

Each of the Cell Holders needs to be installed on either a single-cell baseplate or a multi-cell baseplate by removing the cell holder(s) secured to the baseplate. Each cell holder has a captive screw in the bottom of the holder that secures the holder to the baseplate. Use a flat blade screwdriver to loosen the captive screw from the baseplate and lift the cell holder from the baseplate. Then insert the new cell holder into the appropriate position and secure it to the baseplate by tightening the captive screw.

Note Remove the baseplate from the instrument before removing or installing cell holders.

See “[Installing the 6-Position Cell Holder and the Single Cell Holder](#)” on [page 15](#) for instructions on installing the complete accessory assembly in your instrument.

External Printers

Your spectrophotometer is able to print to external desktop printers supporting HP PCL 5.0 format and later.

Note PCL format does not support HP “Windows” printers.

To print to an HP PCL printer, connect the USB cable to the printer and to the USB port on the back of the instrument (see “[Connectors](#)” on [page 1](#)).

Note The instrument is compatible with most HP PCL printers; brands other than HP will also be supported if they use the PCL5 communications protocol. If the printer is not purchased from Thermo Scientific, it is your responsibility to determine whether the printer is compatible with the instrument. Contact technical support or your sales representative for more information.

Sample Positioner Setting

The spectrophotometer lets you use many different cell holders and accessories to take measurements. When you select your test parameters, you select the type of measurement required and indicate the number of samples. You can choose from the following measurement options:

Auto 6

Measure one blank and up to five samples without changing cuvettes in the cell changer. The instrument automatically measures the blank and advances the cell changer to measure the remaining samples.

Auto 3

Measure one blank and two samples without changing cuvettes in the cell changer. The instrument automatically measures the blank and advances the cell changer to measure the samples in positions 2 and 4.

Manual 3

This setting is used only for the Water Analysis test. Use the buttons on the keypad to position the desired sample holder in the beam for measurement.

Single Cell Holder

Place the blank in the cell holder, measure it, place a sample in the cell holder, and then measure your sample. This process is completely manual. The cell position buttons do not function when you select Single Cell Holder.

Note You can cause the 6-Cell Changer to function as a single cell holder and measure only one cell by selecting this option. However, we recommend that the single cell holder be used for accessories requiring a high degree of positioning repeatability, such as the nanoCell accessory, small aperture microcells, the coupling module of fiber optics probes, and flowcells used with a sipper system.

Manual 6

Measure a blank and up to five samples without changing cuvettes in the cell changer, using the cell position buttons to advance the cell changer to the appropriate position for the next measurement. Place the blank in the blank position and your samples in the other cell positions. Regardless of where the cell holder is positioned, when you press **Measure Blank** the cell holder automatically goes to the blank position and measures the blank. However, you can use the cell position buttons to select a different position for the measurement.

Note When you have the Cell Changer installed, the instrument always considers the material in the B position as a blank. This means that even after measuring your blank the first time, you can place samples only in positions 1 through 5.

Cell Correction

Every test setup screen provides access to Cell Correction.

Note Cell Correction is not active from the Main (Basic ATC) screen.

Note Cell Correction is active only when the 6-Position Cell Holder is set to either Auto 6 or Auto 3. The feature is not active when the cell holder is set to 1-Cell or Manual 6, nor when the Single Cell Holder is installed.

Before running Cell Correction:

- Clean the inside and outside of all the cells to be matched.
- Fill the cells with distilled water (or other blank solution), and place them in the sample compartment (see “[Selecting and Positioning Cuvettes](#)” on [page 5](#)). Place the blank cuvette in Cell “B” of the cell changer.

Cell Correction

❖ To run Cell Correction

1. Load the test type or stored test.

Advanced A-ZT-C		15:26 21Feb07	
		BLANK	
Test Name			
Measurement Mode	Absorbance		
Wavelength	546.0nm		
Ref. Wavelength Correction	On		
Ref. Wavelength	545.0nm		
Delay Time (min:sec)	0:00		
Sample Positioner	Auto 6		
Number of Samples	1		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save	Stored	Run
	Test	Tests	Test

2. If Cell Correction is not visible, highlight **More parameters** and press **Enter**.

3. Highlight **Cell Correction** and press **Enter**.

Advanced A-T-C		15:28 21Feb07	
BLANK			
Cell Correction			On
Setup Correction			
ID# (0=OFF)			1
Low/High Limits	-9999/9999		
Statistics			Off
More parameters...			
*Cell Correction Not Calculated			
Press ↑ or ↓ to select item to change.			
	Save	Stored	
	Test	Tests	

Cell Correction is now activated, as indicated by the word **On**.

Note When Cell Correction is activated, additional parameter lines are added to the screen above the Cell Correction line. If the Cell Correction line is no longer visible, highlight **More Parameters** and press **Enter**.

4. Highlight **Setup Correction** and press **Enter**.
5. Highlight **Correction Mode** and press **Enter** to set the mode to either:

Scan – Cell Correction is run on a blank and one sample cell for the range of wavelengths you specify in Scanning mode.

Discrete nms – Cell Correction is run on a blank and up to five sample cells for up to 31 user-specified, discrete wavelengths.

Cell Correction		4:35am 21Jan01	
BLANK			
Correction Mode			Discrete nms
Date Corrected	-----		
Sample Positioner	Auto		6
Number of Matched Cuvettes			1
Press ↑ or ↓ to select item to change.			
Set nms			Run Corr.

6. If you selected Scan mode in the preceding step, specify the **Start Wavelength** and the **Stop Wavelength** values.

7. Press **Run Corr.** to start Cell Correction.

If you selected Discrete nms mode, first specify the wavelengths using the following procedures, and then Cell Correction.

Cell Correction measures the other cells against the blank and records, stores and dates the measurements. From these measurements Cell Correction establishes the required correction factors, which then are automatically applied during all subsequent tests (if Cell Correction is activated).

Specifying Wavelengths for Discrete nms Mode

❖ To specify wavelengths for Discrete nms mode

1. Highlight **Sample Positioner** and press **Enter** to set this parameter to either Auto 3 (when using three large cell holders) or Auto 6 (when using six small cell holders).
2. Highlight **Number of Matched Cuvettes** and press **Enter**.
3. Specify the number of cells you are matching and press **Enter**.

Cell Correction	3:12pm 24Jan01
	BLANK
Correction Mode	Discrete nms
Date Corrected	----
Sample Positioner	Auto 6
Number of Matched Cuvettes	1
Entry:	
Enter Number of Samples (1 - 5)	

4. Press **Set nms** to select the wavelengths for which Cell Correction will be run.
A list of wavelengths appears.

5 Cell Correction

Cell Correction

Cell Correction		15:33 26Jun02
		BLANK
	nm	
1	600	
2	540	
3	450	
4	-----	
Entry: 405		
Enter Wavelength (190 - 1100)		

Note Match cells at all analytical wavelengths. Matching at one does not guarantee matching at others.

5. Highlight the position where you want to enter the first wavelength.
6. Press **Add nm**.
7. Enter the value for the wavelength and press **Enter**.
8. After entering all the wavelengths, press **Run/Corr.** to start Cell Correction.

The application measures the other cells against the blank and records, stores and dates the measurements. From these measurements the application establishes the required correction factors, which are applied during all subsequent tests (if Cell Correction is activated).

Managing Stored Tests

The instrument uses test method files containing the values for all parameters needed to run a test, including those for cell changer alignment and installed accessories. After setting the parameters, you can assign a unique test name and save the test. You can then restore the test and run it without having to set the parameters again.

When you power-down the instrument, the current test is maintained by battery backup. When you turn the instrument on again, the cell holder alignment and values for all parameters are the same as when the instrument was last used. If you load a saved test, the values for all parameters stored with it replace the current values of the test parameters.

Software Password

This password lets you “lock” test setups (test parameters) so they may not be overwritten or deleted. The password also lets you remove the security so you may edit the test parameters. See the Lock/Unlock section for more information on locking a test.

Note This password cannot be changed.

Password: 4363797

Note Tests stored on a USB memory device cannot be locked or unlocked.

Naming a Test

When saving a test, specify its file name using up to eight alphanumeric characters.

❖ To name a test

1. After setting the test parameters, highlight **Test Name** and press **Enter**.

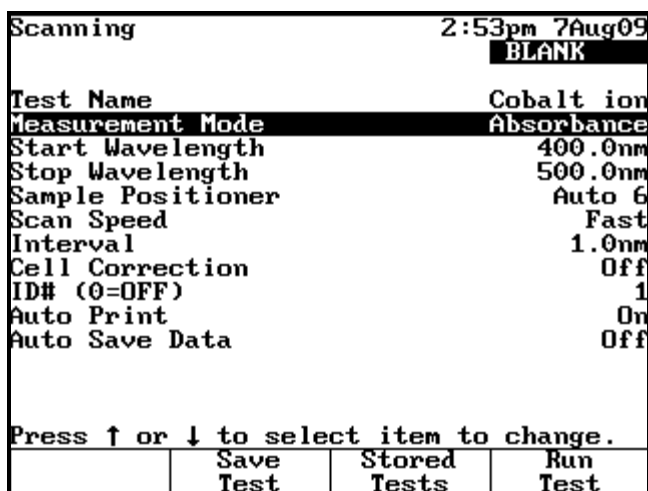
Create Test Name		12:21 21Feb07	
ABCDEFGHIJKLMNOPQRSTUVWXYZ0123456789			
abcdefghijklmnopqrstuvwxyz_*/p()#			
Test Name =			
Press ↑ or ↓ to select character.			
Press Accept Name to save the test.			
		Add Character	Accept Name

This screen lets you:

- Delete the test name
 - Delete a character in the name
 - Add a character to the name
 - Accept the name
2. Highlight the first character for the test name and press **Add Character**.
 3. Continue selecting and adding characters until the name is complete.
 4. Press **Accept Name**.

Saving a Test

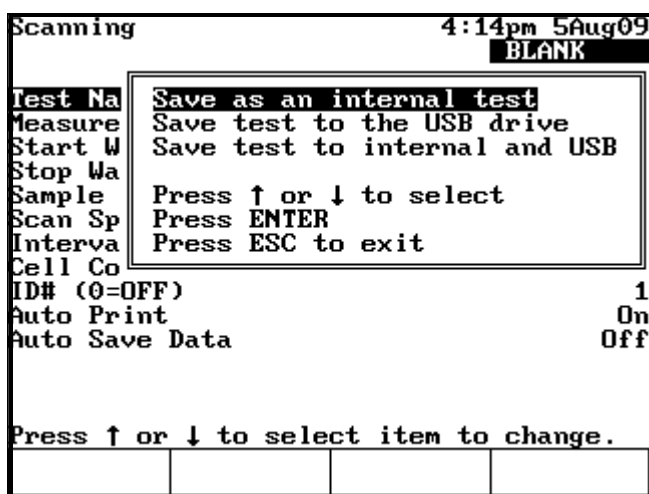
After a method has been configured, there are two options for saving a test. The test method can be saved to an external memory device using the front USB port or to the internal memory of the spectrophotometer.



❖ To save a test in the instrument library

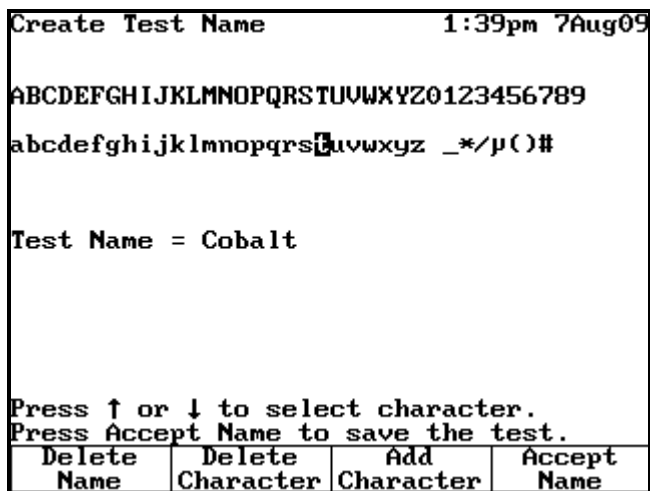
1. Press **Save Test**.

A prompt appears:



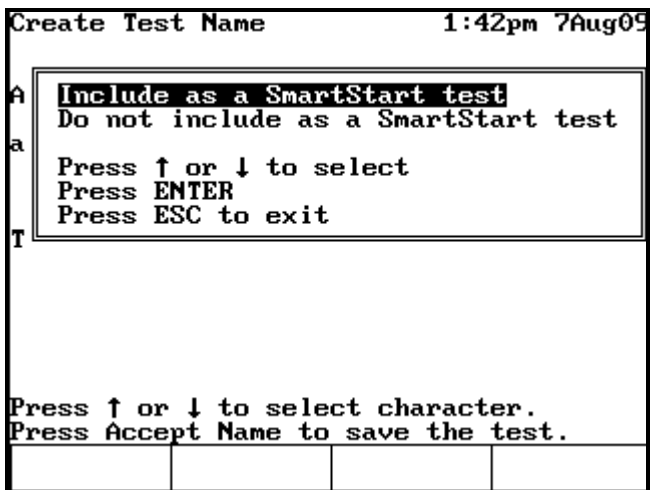
2. Select the appropriate location and press **Enter**.
3. Create or enter a name for the test.

Use the procedure in “Naming a Test” on page 25.



4. Press **Accept Name**.
5. Select whether the test is included as a SmartStart test.

See “SmartStart” on page 31.



6. Highlight the appropriate SmartStart option and press **Enter** to save the test.

Loading Test Files

You can load saved test files from the internal memory from the Utility screen.

❖ **To load test files**

1. To access all test files, press **Utility**.
2. Highlight **Stored Tests Directory** and press **Enter**.

Utility Test Directory		12:25 21Feb07
Nucleic Acid Tests		
DNA (260/280)		Read-only
DNA (260/230)		Read-only
DNA with Scan (260/280)		Read-only
DNA with Scan (260/230)		Read-only
dsDNA		Read-only
ssDNA, RNA		Read-only
Oligos (entered factor)		Read-only
Oligos (calc factor)		Read-only
Direct UV (260)		Read-only
Protein Tests		
Bradford-Standard		Read-only
Bradford-Micro		Read-only
Lowry-Standard		Read-only
More tests...		
Press ↑ or ↓ to select test.		
Select Test		Load Test

Note To view the stored tests of a particular test type, press **Test**, select a test type, and press **Stored Tests**.

Lock/Unlock

To lock or unlock a test, highlight it and press **Lock/Unlock**.

Enter the password and press **Enter**.

Utility Test Directory		9:31 22Feb07
Protein Tests		
Lowry-Micro		Read-only
BCA-Standard		Read-only
BCA-Micro		Read-only
Biuret		Read-only
Direct UV (280)		Read-only
Direct UV (205)		Read-only
Warburg-Christian		Read-only
Cell Growth		
Cell Growth		Read-only
Scanning		
Assay51		22Feb07
More tests...		
Press ↑ or ↓ to select test.		
Select Test	Lock Unlock	Delete Test
		Load Test

Note To lock or unlock access to the file, you must enter the software password of this manual.

Deleting a Test

To delete a test, highlight it and press **Delete Test**.

6 Managing Stored Tests

Deleting a Test

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SmartStart

The SmartStart™ feature lets you customize the spectrophotometer by placing the most frequently used test methods on the first menu. Right after the instrument initializes, a simple menu containing only the SmartStart tests appears.

If you select one test as a SmartStart test, the instrument, when powered on, automatically loads this test and prepares for immediate measurement.

If you select more than one test as SmartStart tests, the instrument, when powered on, automatically displays a menu containing only those tests.

Note You can always access the default main menu by pressing **Test**.

❖ To set up a single test SmartStart

1. Press **Utility** to display the Utility screen.

```

Utility                               11:04 28Feb07
Software Revision:                     0.014b
Instrument Serial Number:
Printer                                 Off
Date/Time Setup                         11:04 28Feb07
Standby                                 15 min
Baseline Expiration (hr:min)           Off
Beeper                                  On
% of lamp life used                     1.21%
Screen Contrast
Printout Contrast
Stored Tests Directory
Calculator

Press ↑ or ↓ to select parameter.

```

2. Highlight **Stored Tests Directory** and press **Enter**.
3. Highlight the appropriate test and press **Select Test**.

An arrow sign “>” indicates the test has been selected for the SmartStart menu.

Press **Esc** to return to the Utility screen, or power down the instrument.

❖ **To unselect a test**

1. Press **Utility**.
2. Highlight **Stored Tests Directory** and press **Enter**.
3. Highlight the test to be removed and press **Unselect Test**.

The Main Menu will be displayed upon power up.

❖ **To set up a multiple test SmartStart**

1. Follow steps 1 through 3 in the procedure above for setting up a single test SmartStart.
2. Select the desired tests.

An arrow sign “>” indicates the tests selected for the SmartStart menu.

Press **Esc** to return to the Utility screen, or power down the instrument.

Note To remove tests from the SmartStart menu, see the procedure above for unselecting a test.

Concentration Units

This chapter covers:

- [Specifying Concentration Units](#)
- [Creating Custom Units](#)

Specifying Concentration Units

Concentration and kinetics tests include a parameter for units, which labels the results. The spectrophotometer includes a set of basic concentration units.

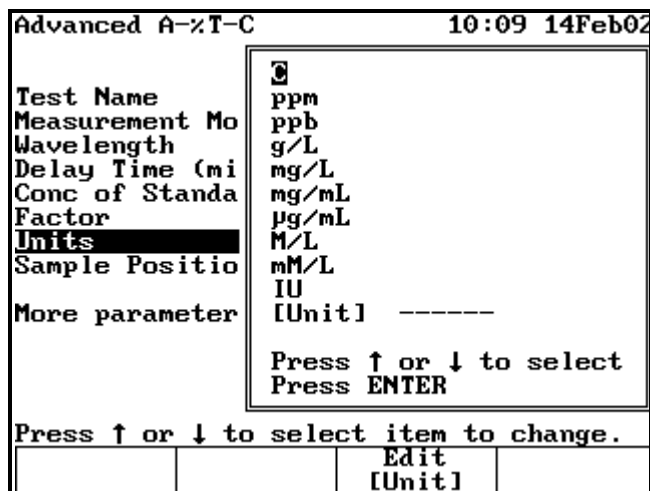
All programs in the spectrophotometer use the same list of basic units:

- C (concentration)
- $\mu\text{g/L}$
- ppm
- M/L
- ppb
- mM/L
- g/L
- IU
- mg/L
- pM/ μL
- mg/mL
- ng/ μL

Custom units can also be created; for more information, see [“Creating Custom Units”](#) on [page 34](#).

❖ **To select the units**

1. Highlight **Units** and press **Enter**.



2. Highlight the unit you want to select and press **Enter**.

Creating Custom Units

In addition to the basic concentration units, you can create a custom concentration unit and add it to the list. This custom concentration unit can be changed when desired.

Note Only one custom concentration unit is available in the list at one time.

❖ **To create custom units**

1. With the Units Selection window displayed, press **Edit [Unit]**.

Use this screen to:

- Delete the name of a unit
 - Delete a character in the name of a unit
 - Add a character to the name of a unit
 - Accept the name of a unit
2. Follow steps 2 through 4 in “[Naming a Test](#)” on [page 25](#).

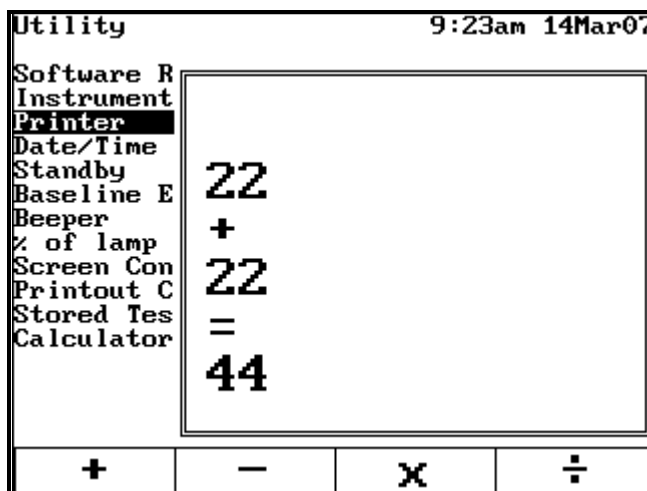
The new custom unit appears in the list of basic units.

Calculator Function

❖ To use the Calculator function

1. From the Utility menu, highlight **Calculator** and press **Enter**.
2. Use the numeric keypad to enter the desired value.
3. Press the desired function (+, -, x or ÷).
4. Enter the second desired value and press **Enter**.

Note You can only add, subtract, multiply, or divide two lines of numbers at a time.



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Water Analysis Application

The Water Analysis Application supports commercial water analysis kits and methods where the manufacturer specifies a factor or equation that yields the concentration of the analyte as a result. Thermo Scientific Orion AquaMate spectrophotometers come with a set of pre-programmed methods loaded onto a USB memory device. Up to ~90 methods can be loaded into the AquaMate's internal library and accessed at any time. For more information, see “[Managing Stored Tests](#)” on [page 25](#). The methods can also be made available from the Smart Start menu. For more information see “[SmartStart](#)” on [page 31](#).

Loading a Test Method from the USB Stick to the AquaMate

1. Access the USB memory stick using a computer.
2. Copy the desired test method(s) from the Orion, Merck and/or CHEMetrics folders to the Thermo folder.
3. Remove the USB memory stick from the computer and insert it into the USB port on the front of the AquaMate spectrophotometer.
4. When the AquaMate spectrophotometer is first powered on, it will display the initial measurement screen.
5. Press **test** to access the test types.
6. Press the ▲ ▼ keys to highlight Water Analysis and press **enter**.
7. Press the **Stored Tests** function key.
8. Press the ▲ ▼ keys to highlight Load test from USB drive and press **enter**.
9. Press the ▲ ▼ keys to highlight the test method to be loaded and press the **Load Test** function key.
10. Press the **Save Test** function key.
11. Press the ▲ ▼ keys to highlight Save as an internal test and press **enter**.
12. Press the **Accept Name** function key.
13. Press the ▲ ▼ keys to highlight Include as a SmartStart test or Do not include as a SmartStart test and press **enter**.

13. Press the **Run Test** function key to begin using the test method.

Refer to the specific reagent chemistry section for detailed instructions.

Running a Test Method from the USB Stick

1. Make sure the test method to be run is in the Thermo folder on the USB stick and the USB stick is connected to the USB port on the front of the AquaMate spectrophotometer.
2. From the initial measurement screen, press **test** to access the test types.
3. Press the ▲ ▼ keys to highlight Water Analysis and press **enter**.
4. Press the **Stored Tests** function key.
5. Press the ▲ ▼ keys to highlight Load test from USB drive and press **enter**.
6. Press the ▲ ▼ keys to highlight the appropriate test method and press the **Load Test** function key.
7. Press the **Run Test** function key.

Running a Test Method from the AquaMate Instrument Library

1. From the initial measurement screen, press **test** to access the test types.
2. Press the ▲ ▼ keys to highlight Water Analysis and press **enter**.
3. Press the **Stored Tests** function key.
4. Press the ▲ ▼ keys to highlight Load internal test and press **enter**.
5. Press the ▲ ▼ keys to highlight the appropriate test method and press the **Load Test** function key.
6. Press the **Run Test** function key.

Running a Test Method from the AquaMate SmartStart Menu

1. From the initial measurement screen, press **test** to access the test types.
2. Press the **SmartStart** function key.
3. Press the ▲ ▼ keys to highlight the appropriate test method and press the **Load Test** function key.
4. Press the **Run Test** function key.

Running a Water Test

When a method has been loaded, the following screen will appear.

```

Water Analysis                10:10am 26Sep12
                             BLANK
Test Name                    AL_C
Measurement Mode             Absorbance
Wavelength                   651nm
1pt Adjust                   Off
Reverse Color                 Off
Sample Positioner           Manual 3
ID# (0=OFF)                  1
Statistics                    Off
Auto Save Data              Off
More parameters...

Press ↑ or ↓ to select item to change.
  
```

	Save Test	Stored Tests	Run Test
--	-----------	--------------	----------

Press the **Run Test** function key. The following screen appears.

```

Water Analysis                8:03am 5Jul12
Test Name: AL_C              BLANK
ID#   Abs   Result
     546.0nm  mg/L
-----
Measure
Blank
  
```

Measure Blank			
---------------	--	--	--

Open the sample compartment. If the desired sample holder is not in the beam, press the button on the carousel control of the keypad corresponding to the correct cell holder shape (circle for vials or test-tubes, square for 10 mm square cuvettes, rectangle for long path rectangular cuvettes). If necessary, the instrument will rotate the carousel to place the correct cell holder in the measurement position.

Insert a cuvette, tube or vial containing the blank or zero solution into the sample holder.

Note Ideally, the same cuvette or vial, or one that has been matched to the sample vial, should be used.

See [Using a Reverse Color Method](#) for use of reagent blanks in reverse color methods.

10 Water Analysis Application

Running a Water Test

Close the lid and press the function key under the Measure Blank option. The instrument will set the measurement wavelength and perform a blank measurement. Open the lid and remove the blank.

Note When running a reverse color test, a reagent blank will also be required in addition to the normal blank. Insert the reagent blank if necessary and press the function key under the Rgnt Blank option. Open the lid and remove the reagent blank.

Place the cuvette, vial or tube containing your sample in the sample holder, close the lid and press the function key under the Measure Sample option. The screen will display the result as shown below.

Water Analysis		3:14pm 20Oct12	
Test Name: Al_C		BLANK	
ID#	Abs 546nm	Result C	
1	0.960	81.22	
2	0.352	29.78	
# Samples		2	
Average		55.50	
Std Dev		36.37	
Page 1, Samples 1 - 2			
Measure Blank	Save Data		Measure Sample

Note If you set Statistics to OFF in the test parameters screen, no Average or Std Dev information will be displayed.

Programming a New Method or Altering the Factor(s) in a Method

Press the Test button to go to the main menu. Use the up/down arrow keys to select Water Analysis if necessary, then press the Enter button to get to the Water Analysis test menu, below:

Water Analysis		10:10am 26Sep12	
BLANK			
Test Name	AL_C		
Measurement Mode	Absorbance		
Wavelength	651nm		
1pt Adjust	Off		
Reverse Color	Off		
Sample Positioner	Manual 3		
ID# (0=OFF)	1		
Statistics	Off		
Auto Save Data	Off		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save	Stored	Run
	Test	Tests	Test

Press the Enter button to alter the value for the highlighted parameter. Change the highlighted parameter by pressing the up/down buttons. The measurement wavelength can also be edited by simply starting to press number keys when the parameter is highlighted. If a reverse color test is being programmed, then the Reverse Color option must be enabled by setting this field to **On**.

Note The Manual 3 is the only available Sample Positioner option in the Water Analysis test.

Select More Parameters to enter the second screen of the Water Analysis test programming, below:

Water Analysis		8:01am 5Jul12	
BLANK			
Result= A + BX + CX2 + DX3			
A	-0.30800000		
B(or Factor)	1.15700000		
C	-0.74800000		
D	0.23700000		
Result Units	mg/L		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save	Stored	Run
	Test	Tests	Test

10 Water Analysis Application

Using the One Point Adjustment Calibration Feature

The AquaMate can implement factors or curves up to third order polynomials. As shown in the screen, the format for entering the equation is:

$$\text{Result} = A + Bx + Cx^2 + Dx^3$$

where “x” is the absorbance at the programmed wavelength.

For methods with a simple multiplicative factor, enter this factor as value B. e.g. If the factor given with the kit is 12.68, set B=12.68, and your equation will be

$$\text{Result} = 12.68 * \text{Abs}_\lambda$$

where ABS_λ is the measured absorbance at the wavelength specified in the first parameters screen.

A large majority of commercial water kits use factor sets that require only B, or only A and B. A limited number may require factors C and D.

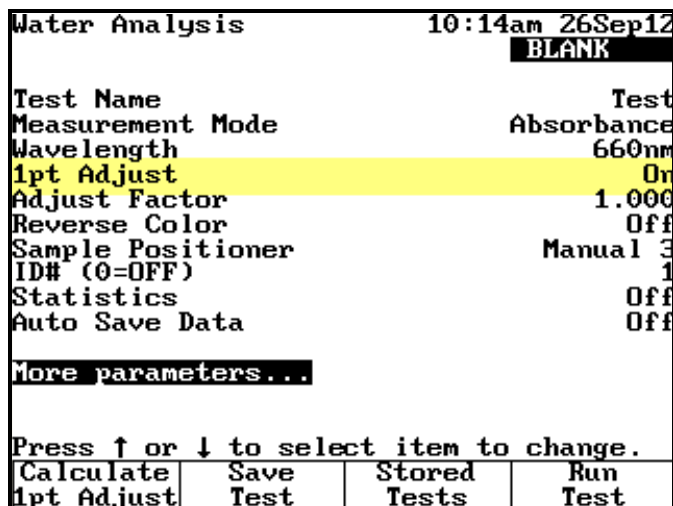
Note You can choose a result unit from a list of pre-programmed options or customize your own unit. Simply select Result Units and press enter. The list will be displayed. For more information, see “[Concentration Units](#)” on [page 33](#).

Using the One Point Adjustment Calibration Feature

Use the one point adjustment feature prior to running a preprogrammed test method to ensure accurate measurements. This procedure is recommended each time a new batch of reagents are used to account for variations in batch-to-batch reagent composition and other factors that affect the accuracy of a method with a fixed calibration curve.

When the one point adjustment feature is off, no adjustment is applied when running the test and test results are calculated exactly according to the preprogrammed equation.

1. Load the test method in the Water Analysis test menu.
2. If the 1 pt Adjust parameter is set to Off, press the ▲▼ keys to highlight 1pt Adjust and press Enter to set the parameter to On.

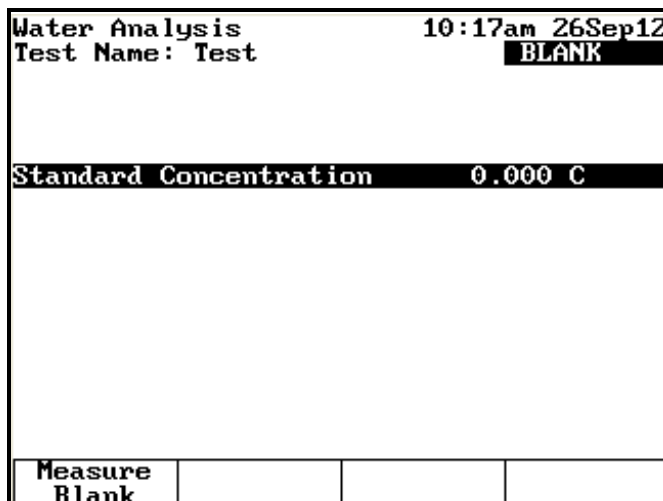


3. Prepare a blank, reagent blank (reverse color methods only) and calibration standard with a known concentration within the method range and near the expected sample concentration.

Refer to the specific reagent chemistry section for detailed instructions.

4. Press the **Calculate 1pt Adjust** function key.

The screen below will appear with the Standard Concentration: value highlighted.



5. Press **enter** to edit this field, then use the numeric keypad to enter the concentration of the standard and press **enter**.
6. Wipe the exterior of the blank vial and place the blank vial into the holder in the sample chamber.
7. Close the sample chamber door.
8. Press the **Measure Blank** function key.

10 Water Analysis Application

Using the One Point Adjustment Calibration Feature

9. Open the sample chamber door and remove the vial from the sample chamber.
10. For reverse color methods only:
 - a. Wipe the exterior of the reagent blank vial and place the reagent blank vial into the holder in the sample chamber. Close the sample chamber door.
 - b. Press the **Measure Rgnt Blank** function key.
 - c. Open the sample chamber door and remove the vial from the sample chamber.
11. Wipe the exterior of the standard vial and place the standard vial into the holder in the sample chamber.
12. Close the sample chamber door.
13. Press the **Measure Standard** function key.
14. Open the sample chamber door and remove the vial from the sample chamber.

The display will show the standard concentration that was entered in step 5, the measured concentration without a correction and the calculated adjustment correction factor.

Water Analysis		10:23am 26Sep12	
Test Name: Test		BLANK	
Standard Concentration		2.000 mg/L	
Measured Concentration		1.848 mg/L	
Adjust Correction		1.082	
Measure Blank	Accept		Measure Standard

Typically a correction factor of 0.7 to 1.3 (within 30%) is acceptable.

15. Press the **Accept** function key if the calculated adjustment correction factor is acceptable.

The screen returns to the Water Analysis test menu and displays the adjustment factor among the parameters.

Water Analysis		10:24am 26Sep12	
BLANK			
Test Name	Test		
Measurement Mode	Absorbance		
Wavelength	660nm		
1pt Adjust	On		
Adjust Factor	1.082		
Reverse Color	Off		
Sample Positioner	Manual 3		
ID# (0=OFF)	1		
Statistics	Off		
Auto Save Data	Off		
More parameters...			
Press ↑ or ↓ to select item to change.			
Calculate	Save	Stored	Run
1pt Adjust	Test	Tests	Test

16. To save the adjustment correction factor to the test method, press the **Save Test** function key and overwrite the existing test method or save as a new test method.
17. Press the **Run Test** function key.

When running a test with an adjustment correction factor in use, the display will show (1pt Adj) next to the test name.

Water Analysis		10:25am 26Sep12	
Test Name: Test(1pt Adj)		BLANK	
ID#	Abs 660nm	Result mg/L	
1	1.348	2.918	
2	1.348	2.918	
3	0.925	2.002	
Page 1, Samples 1 - 3			
Measure Blank	Save Data		Measure Sample

Using a Reverse Color Method

Reverse color methods are recognizable as those which have a negative factor (i.e., the slope of the calibration curve is negative). For these methods, the chemistry used to make the measurement causes a dark color (high absorbance) for very low concentrations of analyte, and an increasingly lighter color (lower absorbance) as the concentration of the analyte increases. Chemical Oxygen Demand (COD) is an example of a test that is often run using a reverse color chemistry.

10 Water Analysis Application

Using a Reverse Color Method

Reverse color methods require that the instrument measure both a clear blank and a reagent blank to properly calibrate the instrument before measuring test samples.

Using a Preprogrammed Reverse Color Method

Load the method from the library. An example of a reverse color method screen is shown below.

Water Analysis		10:28am 26Sep12	
BLANK			
Test Name	COD		
Measurement Mode	Absorbance		
Wavelength	340nm		
1pt Adjust	Off		
Reverse Color	On		
Sample Positioner	Manual 3		
ID# (0=OFF)	1		
Statistics	Off		
Auto Save Data	Off		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save Test	Stored Tests	Run Test

To run the method, follow the sequence of steps below:

1. Press the **Run Test** function key. The following screen appears:

Water Analysis		10:29am 26Sep12	
Test Name: COD		BLANK	
	Abs	Result	
ID#	340nm	mg/L	
-----	-----	-----	
Measure Blank			

2. Place a cuvette, tube or vial containing a clear, colorless blank solution (such as distilled water) in the measurement position and press the **Measure Blank** function key. **The Measure Rgnt Blank** option will appear.

Water Analysis		10:29am 26Sep12
Test Name: COD		BLANK
ID#	Abs 340nm	Result mg/L
-----	-----	-----
Measure Blank		Measure Rgnt Blank

- Place a reagent blank (typically a solution prepared using the test kit and a sample known to have a zero concentration of the analyte, such as distilled water) in the measurement position and press the **Measure Rgnt Blank** function key. The **Measure Sample** option appears.

Water Analysis		10:30am 26Sep12
Test Name: COD		BLANK
ID#	Abs 340nm	Result mg/L
-----	-----	-----
Measure Blank		Measure Rgnt Blank
		Measure Sample

- Place your sample in the measurement position and press the **Measure Sample** function key. The data for your sample measurement are displayed. Continue to press the **Measure Sample** function key after placing each sample in the measurement position in the sample compartment.

Note If you leave the method by pressing the **Esc** key or the **Test** key on the instrument keypad you will need to re-measure the clear and reagent blanks in order to measure more samples using the reverse color test.

10 Water Analysis Application

Using a Reverse Color Method

Water Analysis		10:32am 26Sep12	
Test Name: COD		BLANK	
ID#	Abs 590nm	Result mg/L	
1	1.509	2.791	
2	0.996	5.690	

Page 1, Samples 1 - 2

Measure Blank	Save Data	Measure Rgmt Blank	Measure Sample
---------------	-----------	--------------------	----------------

Programming a Reverse Color Method

To program a new reverse color test, go to the main water test menu, scroll down to the Reverse Color option and press the Enter key. The value for Reverse Color will change to On.

Water Analysis		10:28am 26Sep12	
		BLANK	
Test Name	COD		
Measurement Mode	Absorbance		
Wavelength	340nm		
1pt Adjust	Off		
Reverse Color	On		
Sample Positioner	Manual 3		
ID# (0=OFF)	1		
Statistics	Off		
Auto Save Data	Off		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save Test	Stored Tests	Run Test

Scroll down to More Parameters... and press the Enter key.

Set the factor (or polynomial equation) using the values supplied with the test kit or calculated by your laboratory during development of the test.

Water Analysis		10:27am 26Sep12	
		BLANK	
Result= A + BX + CX2 + DX3			
A		0.00000000	
B(or Factor)		-5.65000000	
C		0.00000000	
D		0.00000000	
Result Units		mg/L	
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save Test	Stored Tests	Run Test

Scroll to the desired line, press the Enter key, and then type in the numeric value of the factor. Press the Enter key to confirm the value. Saving your method after creation is recommended. Press the **Save Test** function key to save your method to the library and/or a USB memory stick once you have programmed it. Press the **Run Test** function key to begin the test. See the previous section for a detailed explanation of the blanking and measurement procedure.

10 Water Analysis Application

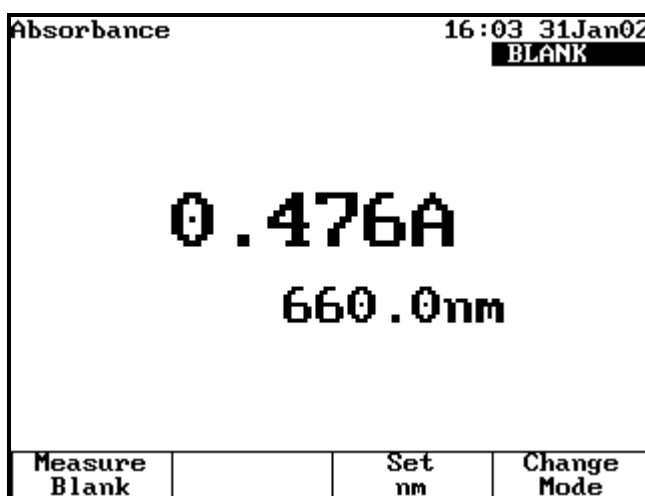
Using a Reverse Color Method

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Abs and %T Measurements—Basic A-%T-C

Basic ATC mode puts the instrument into an “instant measurement” mode. The user simply walks up to the instrument, inserts a sample and measures it. Depending on whether the mode is set to Absorbance (A), % Transmittance (%T), or Concentration, the result appears, along with the type of measurement, the date and time, the wavelength and the cell position used for the measurement.

To toggle between Absorbance, %Transmittance, and Concentration, press **Change Mode**. You can toggle modes whenever you see Change Mode.



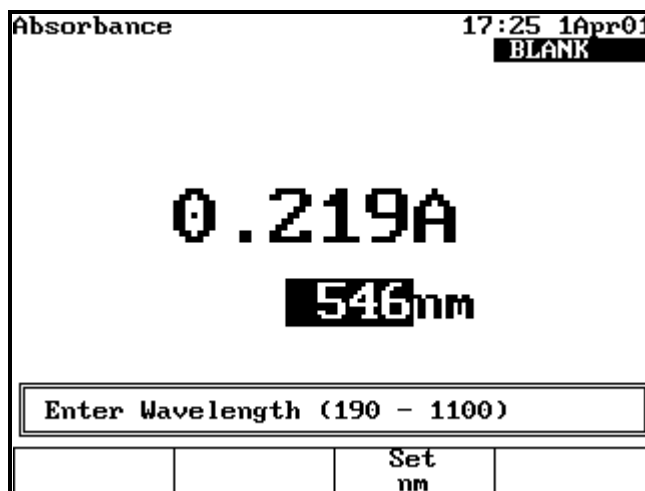
When Basic ATC is set to Absorbance or %T, these capabilities are provided:

- [Setting the Wavelength](#)
- [Measuring a Blank](#)
- [Measuring Samples](#)

Setting the Wavelength

❖ To set the wavelength

1. Press **Set nm** or any number key to set the wavelength.



2. Enter the wavelength for taking measurements and press **Set nm** again.

Measuring a Blank

❖ To measure a blank

1. Place the blank in the cell holder.
If a 6-Position Cell Changer is installed, place the blank in the B position.
2. To enter an absorbance or transmittance value for the blank, press a number key and enter the desired value in the **Entry** field.
3. Press **Measure Blank**.

Measuring Samples

If a 6-Position Cell Changer is installed, place the samples in the cell positions and press the corresponding cell position button to move the cell holder to the measuring position. The absorbance (ABS) or percent transmittance (%T) measurement appears on the display.

If a Single Cell Holder is installed, remove the blank and place the sample in the cell holder. The absorbance or %Transmittance measurement appears on the display.

Abs and %T Measurements—Advanced A-%T-C

Use the Advanced A-%T-C application for Absorbance or %Transmittance measurements that include Cell Correction or a measurement delay time, or for automating the measurement of multiple samples with a cell changer. This section covers:

- Selecting the measurement mode (Absorbance or %Transmittance)
- Cell Correction
- Recalling a Test
- Setting Up Test Parameters
- Taking Measurements

To get started, press **Test**, highlight **Advanced A-%T-C** and press **Enter**.

Advanced A-%T-C		11:42 4Feb02	
		BLANK	
Test Name -----			
Measurement Mode	Absorbance		
Wavelength	546.0nm		
Delay Time (min:sec)	0:00		
Sample Positioner	Auto 6		
Number of Samples	1		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save Test	Stored Tests	Run Test

Recalling a Test

❖ To recall a test

1. In the Advanced A %T C screen, press **Stored Tests**.
2. Highlight the test you want to recall and press **Enter**.

Use this screen for:

- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Saving a Test](#)
- Viewing the list of stored tests
- [Taking Measurements](#)

Setting Up Test Parameters

❖ To set up test parameters

1. Highlight the desired parameter.

Some parameters appear only if you select one of the concentration modes, while others appear regardless of the selected measurement mode. See “Parameters” on [page 125](#) for a complete list.

2. When the parameters are set, press **Save Test** to save the test or **Measure Sample** to measure a blank or samples.

Taking Measurements

❖ To take measurements automatically (using Auto 6 or Auto 3)

1. Press **Run Sample**.
2. Install the blank and samples.
3. Press **Measure Sample**.

❖ To take measurements manually (using Manual 6 or Single Cell Holder)

1. Press **Run Sample**.
2. When prompted, place the blank and samples into the cell holder.

If the 6-Position Cell Holder is installed, place the blank in the B position and the samples in positions 1 to 5.

3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

4. Press **Measure Sample**.

The sample measurement appears. If a 6-Position Cell Holder is installed, press the cell position buttons to reposition the cell holder and measure the rest of the samples manually.

12 Abs and %T Measurements—Advanced A-%T-C

Taking Measurements

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Basic Concentration Measurements—Basic A-%T-C

This chapter covers:

- [Basic Concentration Measurements](#)
- [Using Conc/Std to Measure Concentration](#)
- [Using Conc/Factor to Measure Concentration](#)
- [Measuring Samples](#)

Basic Concentration Measurements

Use Basic ATC mode to make basic concentration measurements. This basic concentration mode is useful for very simple comparisons that do not require a standard curve. You cannot save data in this application or factors used when a single standard is measured. For better accuracy and the ability to save and recall methods and data, use the Standard Curve application mode described in “[Concentration Measurements—Standard Curve Application](#)” on [page 91](#).

Measuring concentration using the Basic ATC mode is similar to measuring Absorbance or %T. Basic ATC mode lets you measure concentration using either a factor or one standard to convert absorbance readings to concentration units.

- When using a factor, specify the factor and concentration units.
- When using a standard, specify the concentration of the standard and measure its absorbance.

When Basic ATC is set to Conc/Std or Conc/Factor, you can perform these tasks.

- [Setting the Wavelength and Mode](#)
- [Measuring a Blank](#)
- Measuring a standard or entering a factor
- [Measuring Samples](#)

The steps for taking measurements in the two modes are similar—the only difference is whether you measure a standard or enter a factor.

Setting the Wavelength and Mode

❖ **To set the wavelength and mode**

1. Press **Set nm** or any other number key to set the wavelength.
2. Enter the wavelength for taking measurements and press **Set nm** again.
3. Press **Change Mode** until the appropriate measurement mode (Concentration with Standard or Concentration with Factor) appears.

Using Conc/Std to Measure Concentration

In this mode a standard sample of well known concentration is used to determine the concentration of samples. The concentration of unknown samples is determined ratiometrically from the measured standard. The measurement can be expressed mathematically as

$$\frac{\text{Standard Concentration}}{\text{Abs / \%T of Standard}} = \frac{\text{Sample Concentration}}{\text{Abs / \%T of Unknown Sample}}$$

where the standard concentration is precisely known, the Abs/%T of the standard and the Abs/%T of the unknown sample are measured, and the sample concentration is calculated.

❖ **To use Conc/Std to measure concentration**

1. If necessary, press **Change Mode** to switch to the Concentration with Standard mode.



If a 6-Position Cell Changer is installed, place the blank in the B position, and the standard in position 1.

2. Press **Measure Blank**.

If the Single Cell Changer is installed, remove the blank and place the standard in the cell changer.

3. Press **Units/Standard** to set the units and measure the standard.
4. Press **Enter Conc**, enter the concentration value of the standard and press **Enter**.
5. Press **Select Units**, highlight the appropriate unit in the list and press **Enter**.
6. Press **Measure Standard**.

The instrument measures the absorbance of the standard and displays the absorbance and calculated factor.

Using Conc/Factor to Measure Concentration

In this mode a concentration factor is used to determine the concentration of samples. The concentration of unknown samples is determined ratiometrically from the entered factor. The measurement can be expressed mathematically as

$$(\text{Abs} / \%T \text{ of Sample}) * \text{Factor} = \text{Concentration of Sample}$$

where the factor is entered, the Abs/%T of the sample is measured, and the sample concentration is calculated.

❖ To use Conc/Factor to measure concentration

1. If necessary, press **Change Mode** to switch to the Conc With Factor mode.
2. Press **Units/Factor** to set the factor and select the units.

Conc with Factor		15:29 6Mar01	
		BLANK	
Factor	45		
Units	C		
Press ↑ or ↓ to select item to change.			
	Enter Factor	Select Units	

3. Press **Enter Factor**.
4. Type the desired factor value.

5. Press **Enter** **Factor** to accept the factor and return to the screen displaying the factor and units.
6. Press **Select Units**.
7. Highlight the appropriate unit in the list and press **Enter**.
8. Press **Esc** to return to the Conc With Factor screen.

Measuring Samples

If the 6-Position Cell Changer is installed, place the sample you want to measure in one of the cell positions and press the corresponding cell position button to move the cell changer to the measuring position.

The measurement appears.

If the Single Cell Changer is installed, remove the blank and place the sample in the cell changer. The measurement appears.

Concentration Measurements—Advanced A-%T-C

Use the Advanced A-%T-C application for concentration measurements for:

- Selecting a measurement mode (concentration with one standard or concentration with a factor).
- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Measuring a Standard](#) or [Entering a Factor](#) (only if you select either concentration with one standard or concentration with a factor)
- [Measuring a Blank](#)
- [Measuring Samples](#)

To get started, press **Test**, highlight **Advanced A-%T-C** and press **Enter**.

Advanced A-%T-C		15:40 26Jun02	
		BLANK	
Test Name		AAA	
Measurement Mode		Conc/Std	
Wavelength		546nm	
Ref. Wavelength Correction		Off	
Delay Time (min:sec)		0:00	
Conc of Standard		100.0	
Factor		1587	
Units		C	
Sample Positioner		Auto 6	
Number of Samples		4	
More parameters...			
Press ↑ or ↓ to select item to change.			
Run	Save	Stored	Run
Standard	Test	Tests	Test

Recalling a Test

❖ **To recall a test**

1. Press **Stored Tests**.
2. Highlight the test and press **Enter** or **Load Test** to display the parameters for the selected test.

Setting Up Test Parameters

❖ **To set up test parameters**

1. Highlight the desired parameter.

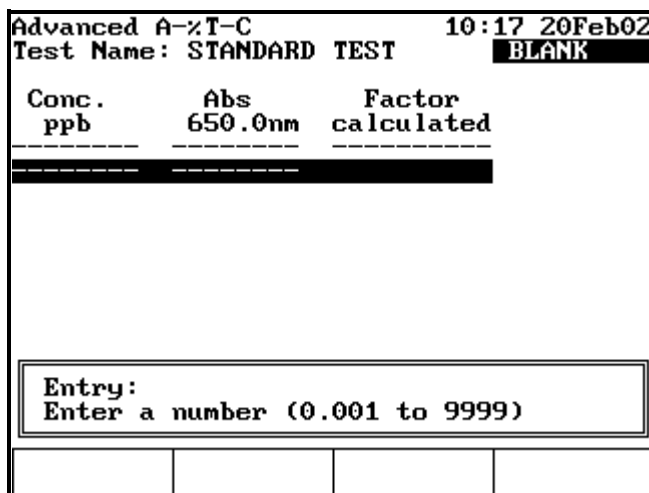
Some parameters appear only if you select a concentration mode, while others appear regardless of the selected measurement mode.

See [Parameters](#) for a complete list.
2. When the parameters are set, press **Save Test** to save the test or **Run Standard** to measure a standard (if in Conc/Std), or press **Run Test** (if in Conc/Factor).

Measuring a Standard

❖ **To measure a standard automatically (using Auto 6 or Auto 3)**

1. With Measurement Mode set to Conc/Std, press **Run Standard**.



2. Enter the concentration of the standard and press **Enter**.
3. Press **Measure Blank**.

4. Insert the blank and standard.
5. Press **Enter** to measure the blank and samples and display the absorbance and calculated factor.

❖ **To measure a standard manually (using Manual 6 or Single Cell Holder)**

1. Press **Run Standard**.
2. Enter the concentration of the standard and press **Enter**.
3. Insert the blank and standard.
4. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

5. Press **Measure Standard**.

The instrument measures the absorbance of the standard and displays the absorbance and calculated factor.

Entering a Factor

❖ **To enter a factor**

Highlight **Factor**. To change the factor, enter the correct factor.

To change the units, highlight **Units** and select the correct units.

Measuring Samples

❖ **To measure a sample automatically (using Auto 6 or Auto 3)**

1. Press **Run Test**.
2. When prompted, place the blank and samples in their cell positions and press **Enter**.
The instrument measures the blank and samples and displays the sample measurements.
3. Press **Measure Sample** to measure additional samples.

❖ **To measure samples manually (using Manual 6 or Single Cell Holder)**

1. Press **Run Test**.
2. Insert the blank and sample.
A 6-Position Cell Holder can hold five samples.

14 Concentration Measurements—Advanced A-%T-C

Measuring Samples

3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

4. Press **Measure Sample**.

If a 6-Position Cell Holder is installed, press the cell position buttons to reposition the cell holder and measure the rest of the samples manually.

Scanning

The wavelength scanning application lets you measure the absorption or percent transmission spectrum of a sample. You can use scans to determine peak wavelengths or to evaluate the quality of a material.

Use the Scanning application for:

- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Collecting a Baseline Scan](#)
- [Scanning a Sample](#)
- [Viewing and Manipulating Scan Data](#)
- [Rescaling Graphical Scan Data](#)
- [Determining Peak Height Using a 3-point Net Equation](#)
- [Calculating the Area Under a Curve](#)
- [Labeling Peaks and Valleys](#)

Note The Scanning application lets you measure only one sample at a time. Auto 6, Auto 3 and Manual 6 are not available for scanned measurements.

Note To set a baseline expiration time, press **Utility** and then highlight **Baseline Expiration**. Press **Enter** and set the desired time.

To get started, press **Test**, highlight **Scanning** and press **Enter**.

Recalling a Test

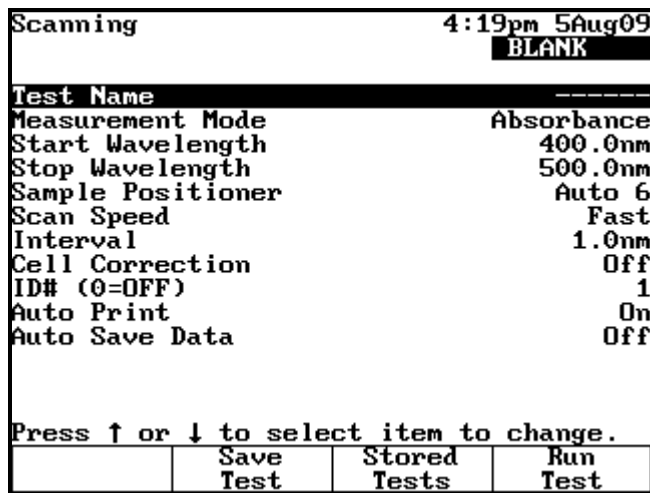
❖ To recall a test

1. Press **Stored Tests**.
2. Highlight the test you want to recall and press **Enter**.

The parameters for the selected test appear.

This screen provides these capabilities:

- [Setting Up Test Parameters](#)
- Setting up Cell Correction



The screenshot shows a terminal-style interface for the 'Scanning' menu. At the top right, it displays the time '4:19pm' and date '5Aug09'. Below this, the word 'BLANK' is highlighted in a black box. The main menu lists various test parameters with their current values:

Test Name	
Measurement Mode	Absorbance
Start Wavelength	400.0nm
Stop Wavelength	500.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	1.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off

At the bottom of the screen, there is a prompt: 'Press ↑ or ↓ to select item to change.' Below this prompt is a navigation bar with three buttons: 'Save Test', 'Stored Tests', and 'Run Test'.

Setting Up Test Parameters

❖ To set up test parameters

1. Highlight the desired parameter.
2. When the parameters are set, press **Save Test** to save the test or **Run Test** to measure a blank or a sample.

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

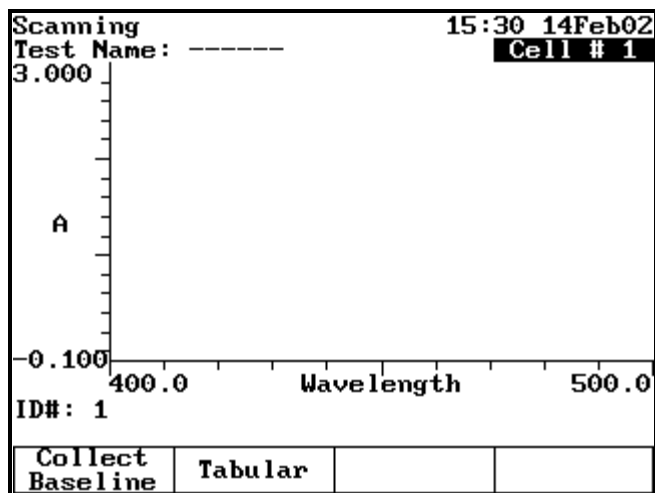
Collecting a Baseline Scan

Note If a 6-Position Cell Holder is installed, be sure to place the blank in the B position. The instrument always uses the B position to collect the baseline.

❖ To collect a baseline scan

1. Press **Run Test**.

- Place the blank in the B position.



- Press **Collect Baseline**.

While the spectrophotometer is measuring the baseline, a status message appears indicating the progress of the baseline scan. After the baseline is measured, this message disappears.

Note To switch between tabular and graphical displays, press **Graph/Tabular**.

Scanning a Sample

Note If a 6-Position Cell Holder is installed, be sure to place the sample in cell position #1. The instrument always uses cell position #1 to scan the sample.

❖ To scan a sample

- Press **Run Test**.

If a 6-Position Cell Holder is installed, place the sample in cell position #1.

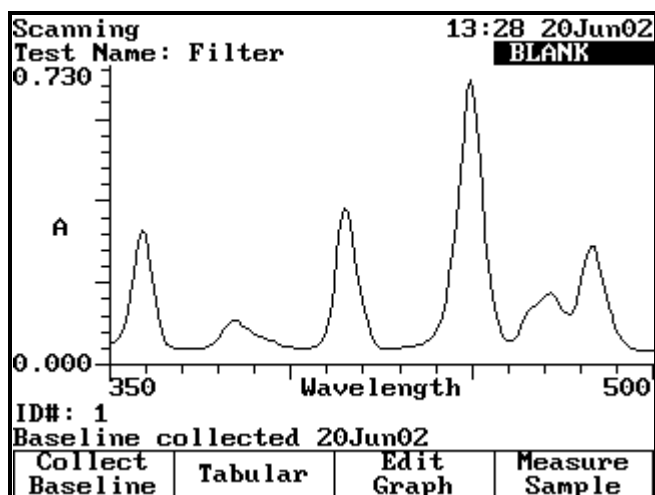
- Press **Measure Sample**.

Note To switch between tabular and graphical displays, press **Graph/Tabular**.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

15 Scanning

Viewing and Manipulating Scan Data



Viewing and Manipulating Scan Data

The Scanning application lets you view and manipulate results in graphical or tabular form.

When working with graphical scan data, press **Edit Graph** before performing other functions on the data.

The edit graph screen provides these capabilities:

- [Rescaling Graphical Scan Data](#)
- [Determining Peak Height Using a 3-point Net Equation](#)
- [Performing Calculations on the Scan Data](#)
- [Labeling Peaks and Valleys](#)

Rescaling Graphical Scan Data

You can modify the scale of your scan data plot automatically or manually. When you select Auto Scale, the instrument scales the X- and Y-axes so all the data appears on the plot. When you select Manual Scale, you select specific minimum and maximum values for the axes. When you modify the scale, the instrument recalculates and displays the new data plot.

Press **Edit Scale** to modify the scale. In the edit scale screen, you can:

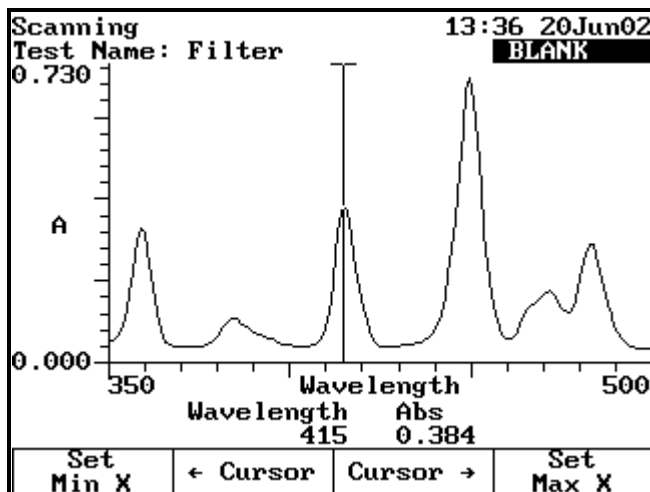
- Use **Auto Scale** to change the scale and display the new graph.
- Use **Manual Scale** to change the scale and display the new graph.
- Use the cursor to identify specific points along the X-axis.

❖ To use the Auto Scale function

With your scan data displayed on the edit scale screen, press **Auto Scale**. The instrument adjusts the minimum and maximum values for the X- and Y-axes so all the data appears on the plot.

❖ To use the Cursor

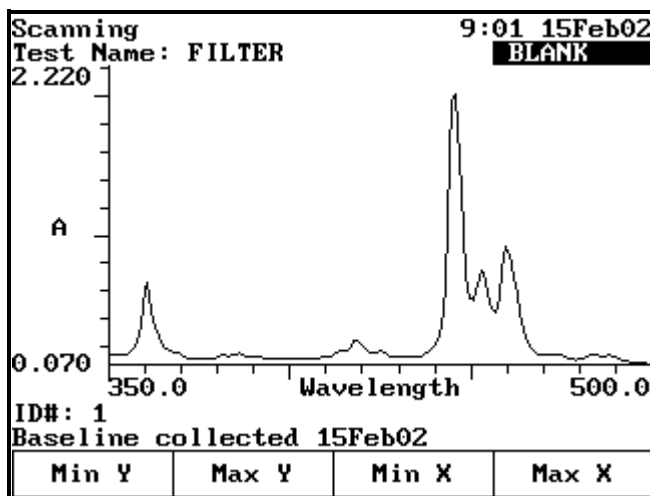
1. With your scan data displayed on the edit scale screen, press **Cursor**.



2. Use **Cursor ←** to move to the desired minimum wavelength value. Press **Set Min X** to redraw the plot using the new minimum wavelength value.
3. Repeat using **Cursor →** and **Set Max X** to set the new maximum wavelength.

❖ To use the Manual Scale function

1. Press **Manual Scale** to display the manual scale options.



2. To set the minimum or maximum value for the X- or Y-axis, press **Min Y**, **Max Y**, **Min X** or **Max X**.

15 Scanning

Viewing and Manipulating Scan Data

3. Enter the correct value and then press **Min Y**, **Max Y**, **Min X** or **Max X** to accept it.

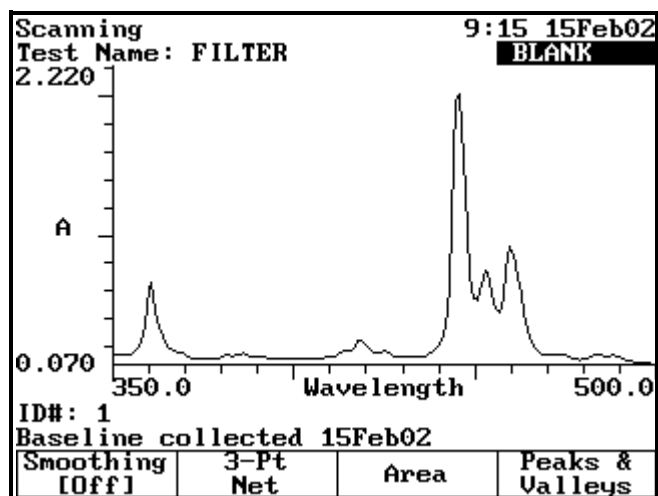
The instrument redraws the plot using the entered minimum and maximum values.

Performing Calculations on the Scan Data

You can modify your graph by performing calculations on the data. In the Edit Graph screen, press **Math**.

The Math screen provides these capabilities:

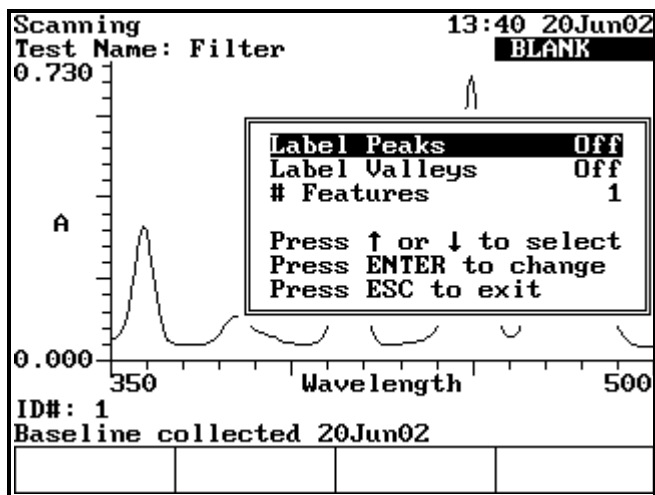
- Labeling Peaks and Valleys
- Smoothing Data
- Calculating the Area Under a Curve
- Determining Peak Height Using a 3-point Net Equation



Labeling Peaks and Valleys

❖ To label valleys and peaks

1. With your scan displayed on the edit graph screen, press **Math**.



2. Press **Peaks & Valleys** to display the Label Peaks and Valleys window.
3. Select the type of labels to display and press **Enter**.

The instrument labels the selected items on your scan data plot.

Note Up to nine peaks or valleys can be calculated and displayed.

Smoothing Data

If your scan shows sampling noise, you can smooth the data with the smoothing function.

❖ To smooth data

With your scan data displayed on the edit graph screen, press **Smoothing [On]**.

Determining Peak Height Using a 3-point Net Equation

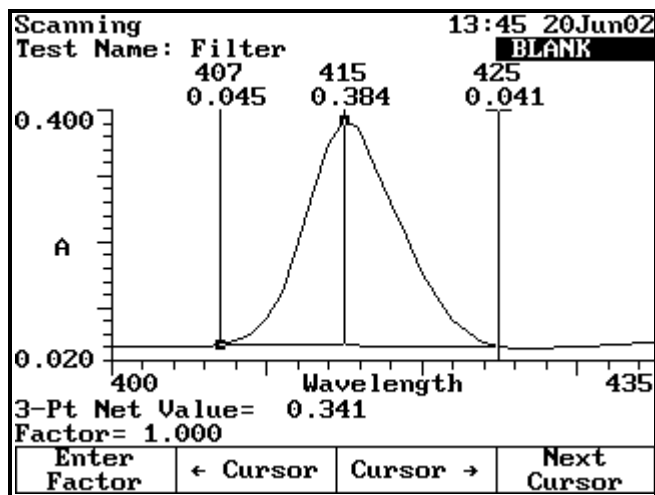
❖ To determine 3-point net measurements

1. With your scan data displayed on the edit graph screen, press **Math**.
2. Press **3-Pt Net**.

The 3-point net measurement screen shows the cursor options and three cursor lines (designated for the left, center and right wavelengths).

15 Scanning

Viewing and Manipulating Scan Data



- Use **Cursor** → and **Cursor** ← to position the left cursor line to the desired wavelength value.

The instrument calculates the 3-point net absorbance for the selected wavelengths.

- Continue selecting the other wavelengths by pressing **Next Cursor** to activate the center and right cursor lines.

Select the wavelengths with **Cursor** ← and **Cursor** →.

Repeat until all three wavelengths have been selected.

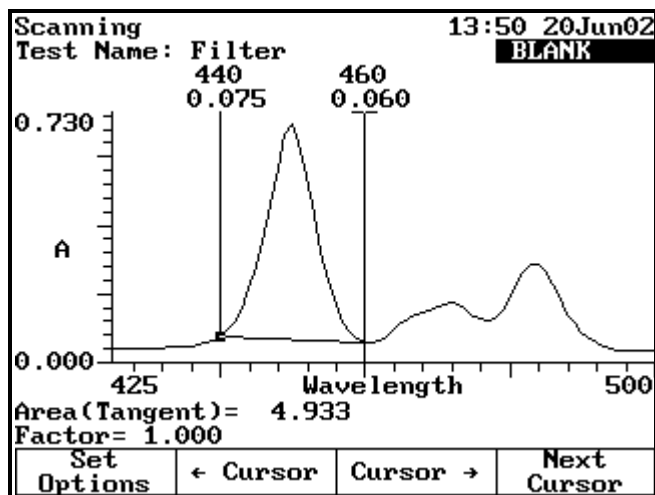
- Press **Enter Factor** to access the set factor box. Enter the desired factor and press **Enter**.

The instrument calculates the value for the 3-point net absorbance for the selected wavelengths, multiplied by the selected factor.

Calculating the Area Under a Curve

❖ To calculate the area under a curve

- With your scan data displayed on the edit graph screen, press **Math**.
- Press **Area** to display the Area Under the Curve measurement screen.



- Use **Cursor** → and **Cursor** ← to position the left cursor line to the desired wavelength value.

The instrument calculates the area under the curve for the selected wavelengths.

- Continue selecting the other wavelengths by pressing **Next Cursor** to activate the next cursor line.

Select the wavelength with **Cursor** → and **Cursor** ←.

- Press **Set Options** to access the set options window.
- Highlight **Factor**. Enter the desired factor and press **Enter**.
- Highlight **Calculation baseline**.
- Press **Enter** to toggle between Zero and Tangent.
- Press **Esc** to return to the area under a curve screen.

The instrument calculates the area under a curve for the selected wavelengths, factor and calculation method.

Viewing and Rescaling Tabular Scan Data

When working with tabular scan data, you must press **Edit Data** before performing other functions on the data.

15 Scanning

Viewing and Manipulating Scan Data

Scanning		10:05 15Feb02	
Test Name: FILTER		BLANK	
Wavelength	Abs		
→ 350.0	0.140		
351.0	0.139		
352.0	0.140		
353.0	0.139		
354.0	0.137		
355.0	0.138		
356.0	0.150		
357.0	0.180		
358.0	0.231		
359.0	0.364		
ID#: 1			
Baseline collected 15Feb02			
Use all data	Graph	Start nm	End nm

❖ To use all the scan data

With your table of scan data displayed on the edit screen, press **Use All Data**.

❖ To select specific start and end wavelengths

1. With your table of scan data displayed on the edit screen, highlight the appropriate data point in the table.
2. Press **Start nm** or **End nm**.

The instrument highlights the selected data points.

To display the plot using the highlighted data points, press **Graph**.

Multiwavelength

The Multiwavelength application lets you make multiple fixed-wavelength measurements. It is a fast alternative to scanning if the wavelengths of interest are well known.

Use Multiwavelength for:

- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Adding Wavelengths and Factors](#)
- [Deleting Wavelengths and Factors](#)
- [Taking Measurements](#)

To get started, press **Test**, highlight **Multiwavelength** and press **Enter**.

Multiwavelength		10:30 22Feb07	
		BLANK	

Test Name			
Measurement Mode	Absorbance		
Sample Positioner	Auto 6		
Number of Samples	1		
Cell Correction	Off		
ID# (0=OFF)	1		
Press ↑ or ↓ to select item to change.			
Set nms	Save Test	Stored Tests	

Recalling a Test

❖ To recall a test

1. Press **Stored Tests**.

2. Highlight the test to recall and press **Enter** to display its parameters.

This screen provides these capabilities:

- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Saving a Test](#)
- Viewing the list of stored tests
- [Taking Measurements](#)

Setting Up Test Parameters

❖ To set up test parameters

In the Multiwavelength screen, highlight the desired parameter.

See the procedures below for instructions on [adding](#) or [deleting](#) wavelengths and factors.

If you have previously selected the wavelengths to measure, press **Save Test** to save the test or **Run Test** to measure a blank or samples.

If you have not selected the wavelengths, you can add wavelengths and factors as shown below.

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

Adding Wavelengths and Factors

Note You can enter factors only when the measurement mode is set to Concentration/Factor.

❖ To add wavelengths and factors

1. Press **Set nms**.

Multiwavelength		4:20pm 5Aug09	
Test Name: -----		BLANK	
WL#	nm	Factor	
1	340.0	56.85	
2	532.0	630.8	
3	633.0	0.052	
4	-----	-----	

Page 1
Press ↑ or ↓ to view data

Edit	Add nm		Run Test
------	--------	--	----------

2. Highlight a position for entering the first wavelength and factor pair.
3. Press **Add nm**.
4. Enter the values for the wavelength and factor and press **Enter**.
5. When the values are correct, press **Add nm**.
6. Continue until you have entered all the wavelengths and factors.

Deleting Wavelengths and Factors

❖ To delete wavelengths and factors

1. Press **Set nms**.

Note If no wavelength values have been entered, the wavelength and factor columns will be empty.

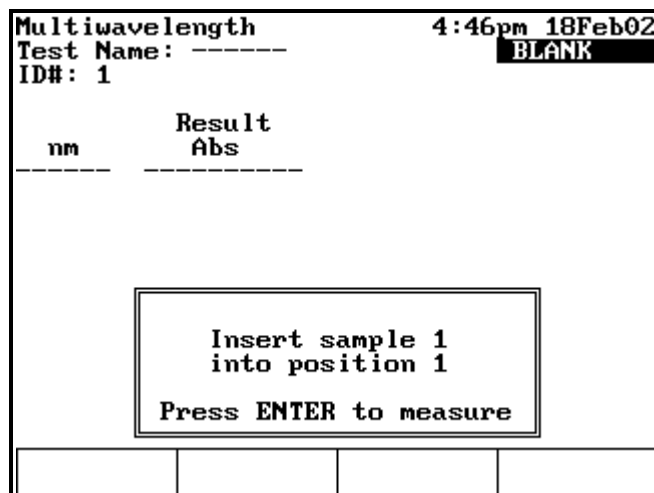
2. Highlight the first wavelength and factor pair to delete.
3. Press **Delete nm**.

Taking Measurements

You can access Multiwavelength acquisition from either the Set nms screen shown above or from the Multiwavelength setup screen.

❖ To take measurements automatically (Auto 6 or Auto 3)

1. Press **Run Test** to display the Multiwavelength measurement screen.



2. Install the blank and samples.
3. Press **Enter**.

❖ **To take measurements manually (using Manual 6 or Single Cell Holder)**

1. With the Multiwavelength screen displayed and the parameters set, press **Run Test**.
2. Install the blank and samples.

If a 6-Position Cell Holder is installed, place the blank in the B position. The holder can hold five samples.

3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

4. With the list of wavelengths (and factors) displayed, press **Measure Samples** to measure and display the absorbance at each wavelength.

If you set the measurement mode to Concentration/Factor, the calculated concentration at each wavelength also appears.

If a 6-Position Cell Holder is installed, press **Cell Position** to reposition the cell holder and measure the rest of the samples manually.

Absorbance Ratio

The Absorbance Ratio application lets you measure the absorption ratio of two different wavelengths. Reference wavelength correction is available to eliminate the effects of a sample matrix. Typically used in quality control applications, an absorbance ratio provides a convenient and quick diagnostic test for sample quality.

Use Absorbance Ratio for:

- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Measuring a Blank](#)
- [Measuring Samples](#)

To get started, press **Test**, highlight **Absorbance Ratio** and press **Enter**.

Recalling a Test

Absorbance Ratio		10:32 20Feb02	
		BLANK	
Test Name		456 [Saved]	
Wavelength 1		260.0nm	
Wavelength 2		280.0nm	
Ref. Wavelength Correction		Off	
Sample Positioner		Auto 6	
Number of Samples		5	
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save	Stored	Run
	Test	Tests	Test

❖ To recall a test

1. In the Absorbance Ratio screen, press **Stored Tests**.

2. Highlight the test to recall and press **Enter**.

This screen provides these capabilities:

- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Saving a Test](#)
- Viewing the list of stored tests
- [Measuring a Blank](#)

Setting Up Test Parameters

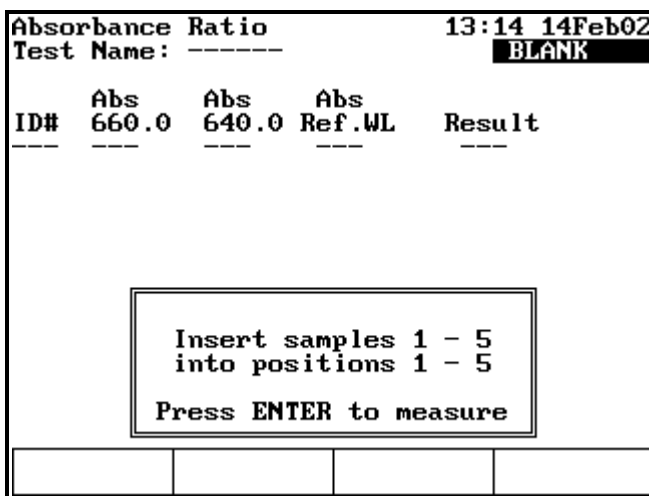
❖ To set up test parameters

1. In the Absorbance Ratio screen, highlight the desired parameter.
2. When the parameters are set, press **Save Test** to save the test or **Run Test** to measure a blank or samples.

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

Measuring Samples



❖ **To measure samples automatically (using Auto 6 or Auto 3)**

1. Install the blank and samples.
2. Press **Enter**.

❖ **To measure samples manually (using Manual 6 or Single Cell Holder)**

1. In the Absorbance Ratio screen, press **Run Test**.
2. Install the blank and sample.

A 6-Position Cell Holder can hold five samples.

3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

4. Press **Measure Sample**.

If a 6-Position Cell Holder is installed, press the cell position buttons to reposition the cell holder and measure the rest of the samples manually.

17 Absorbance Ratio

Measuring Samples

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Absorbance Difference

The Absorbance Difference application lets you measure the difference in absorption at two different wavelengths. Reference wavelength correction is available to eliminate the effects of a sample matrix. Typically used in quality control applications, an absorbance difference application provides a convenient and quick diagnostic test for sample quality.

Use Absorbance Difference for:

- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Measuring a Blank](#)
- [Measuring Samples](#)

To get started, press **Test**, highlight **Absorbance Difference** and press **Enter**.

```

Absorbance Difference      12:59pm 3Jan02
                          BLANK
Test Name                  -----
Wavelength 1              546.0nm
Wavelength 2              546.0nm
Ref. Wavelength Correction Off
Factor 1                   1.000
Factor 2                   1.000
Units                     ppm
Sample Positioner         Auto 6
Number of Samples         1

More parameters...

Press ↑ or ↓ to select item to change.
  
```

	Save Test	Stored Tests	Run Test
--	--------------	-----------------	-------------

Recalling a Test

❖ To recall a test

1. In the Absorbance Ratio screen (see “Using the Absorbance Ratio Screen” on page 84), press **Stored Tests**.
2. Highlight the test to recall and press **Enter**.

Using the Absorbance Ratio Screen

Use this screen for:

- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Saving a Test](#)
- Viewing the list of stored tests
- [Measuring a Blank](#)
- [Measuring Samples](#)

Setting Up Test Parameters

❖ To set up test parameters

1. In the Absorbance Ratio screen, highlight the parameter to set.
2. When the parameters are set, press **Save Test** to save the test or **Run Test** to measure a blank or samples.

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

Measuring Samples

Absorbance Difference				13:29 14Feb02				
Test Name: TEST 9				BLANK				
ID#	Abs 429.0	Abs 630.0	Abs Ref.WL	Result ppm				
<div style="border: 1px solid black; padding: 5px; width: fit-content; margin: auto;"> Insert samples 1 - 5 into positions 1 - 5 Press ENTER to measure </div>								
<table border="1" style="width: 100%; height: 20px;"> <tr> <td style="width: 25%;"></td> <td style="width: 25%;"></td> <td style="width: 25%;"></td> <td style="width: 25%;"></td> </tr> </table>								

❖ **To measure samples automatically (using Auto 6 or Auto 3)**

1. In the Absorbance Difference screen, press **Run Test**.
2. Install the blank and samples.
3. Press **Enter**.

❖ **To measure samples manually (using Manual 6 or Single Cell Holder)**

1. In the Absorbance Difference screen, press **Run Test**.
2. Install the blank and samples.

A 6-Position Cell Holder can hold five samples.

3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

Absorbance Difference				13:39 14Feb02				
Test Name: TEST 9				Cell # 1				
ID#	Abs 429.0	Abs 630.0	Abs Ref.WL	Result ppm				
<table border="1" style="width: 100%; height: 20px;"> <tr> <td style="width: 25%;">Measure Blank</td> <td style="width: 25%;"></td> <td style="width: 25%;"></td> <td style="width: 25%;"></td> </tr> </table>					Measure Blank			
Measure Blank								

18 Absorbance Difference

Measuring Samples

4. Press **Measure Sample**.

If a 6-Position Cell Holder is installed, press the position buttons to reposition the cell holder and measure the rest of the samples manually.

3-Point Net

The 3-Point Net application lets you determine the height of a peak based on a sloping baseline drawn between two wavelengths on either side of the peak. This type of analysis is beneficial when the precise peak height is needed for a particular assay. A factor can be multiplied by the measured peak height to give the concentration of the measured analyte in the appropriate concentration units.

Use 3-Point Net for:

- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Taking Measurements](#)

To get started, press **Test**, highlight **3-Point Net** and press **Enter**.

3-Point Net		10:11 15Feb02	
		BLANK	
Test Name		BILIRUBIN	
Wavelength 1		545.0nm	
Wavelength 2		546.0nm	
Wavelength 3		547.0nm	
Factor		1.000	
Units		C	
Sample Positioner		Auto 6	
Number of Samples		1	
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save Test	Stored Tests	Run Test

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

Recalling a Test

❖ To recall a test

1. Press **Stored Tests**.
2. Highlight the test to recall and press **Enter** to display its parameters.

This screen provides these capabilities:

- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Saving a Test](#)
- Viewing the list of stored tests
- [Taking Measurements](#)

Setting Up Test Parameters

❖ To set up test parameters

1. Highlight the desired parameter.
2. When the parameters are set, press **Save Test** to save the test or **Run Test** to measure a blank or samples.

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

Taking Measurements

❖ To take measurements automatically (Auto 6 or Auto 3)

1. With the 3-Point Net Setup screen displayed and the parameters set, press **Run Test** to display the 3-Point Net measurement screen.

3-Point Net				10:25 15Feb02
Test Name: BILIRUBIN				BLANK
ID#	Abs	Abs	Abs	Result
	429.0	515.0	630.0	C
<div style="border: 1px solid black; padding: 5px; margin: 10px auto; width: fit-content;"> Insert sample 1 into position 1 Press ENTER to measure </div>				

2. Install the blank and samples.
3. Press **Enter**.

❖ **To take measurements manually (using Manual 6 or Single Cell Holder)**

1. Press **Run Test**.

3-Point Net				9:26 4Feb02
Test Name: 3_PTNET				Cell # 1
ID#	Abs	Abs	Abs	Result
	630.0	660.0	680.0	µg/mL
<div style="border: 1px solid black; padding: 5px; margin: 10px auto; width: fit-content;"> Measure Blank </div>				

2. Install the blank and samples.

If a 6-Position Cell Holder is installed, place the blank in the B position. The holder can hold five samples.

3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its cell position.

4. Press **Measure Sample**.

If a 6-Position Cell Holder is installed, press the cell position buttons to reposition the cell holder and measure the rest of the samples manually.

Concentration Measurements—Standard Curve Application

The Standard Curve application lets you perform a quantitative analysis experiment using a multipoint calibration curve. A calibration curve is composed of standards of well-known concentration. A fit of this standard curve is used to measure the concentration of samples.

Use Standard Curve for:

- Creating a standard curve (set up the parameters and then measure the standards for the curve)
- [Cell Correction](#)
- [Measuring Samples](#)
- Viewing calibration curve data: select between graphical and tabular displays
- [Editing a Standard Curve](#): change the number of standards, select a different curve fit or delete points from the curve.
- Viewing and saving sample data measured using the standard curve

To get started, press **Test**, highlight **Standard Curve** and press **Enter**.

Standard Curve		15:28 4Feb02	
		BLANK	
Test Name	AMMONIA		
Date Standards Measured	-----		
Wavelength	660.0nm		
Ref. Wavelength Correction	Off		
Curve Fit	Linear		
Number of Standards	5		
Units	ppm		
Sample Positioner	Auto 6		
Number of Samples	1		
More parameters...			
Press ↑ or ↓ to select item to change.			
Run Standards	Save Test	Stored Tests	

Recalling a Standard Curve

❖ **To recall a standard curve**

1. Press **Stored Tests**.
2. Highlight the test to recall and press **Enter**.

Setting the Parameters for a Standard Curve

❖ **To set the parameters for a standard curve**

1. Place the standards in their cell positions.
2. Set parameters for measuring the standards.

See “[Parameters](#)” on [page 125](#) for a complete list.

- a. Enter the **Test Name**, **Wavelength**, **Reference Wavelength Correction** and **Reference Wavelength**.
- b. Select the **Curve Fit**, **Units** and **Sample Positioner**.
- c. Set **Number of Standards** and **Number of Samples**.
- d. Enter the low and high limits.
- e. Select the settings for **Statistics** and **AutoPrint**.
- f. Run **Cell Correction**.

Measuring the Standards for a Standard Curve

❖ **To measure standards automatically (using Auto 6 or Auto 3)**

1. Install the blank and standards.
2. When the parameters are correct, press **Run Standards**.

Standard Curve		15:39 4Feb02
Test Name: AMMONIA		BLANK
Std #	Conc. ppm	Abs 660.0nm
1	0.120	-----
2	0.200	-----
3	-----	-----
4	-----	-----
5	-----	-----

Entry: Enter a number (0.001 to 9999)
--

3. Set **Entry concentration** and press **Enter**.
4. Press **Measure Standards**.

The [Standards screen](#) shows the absorbance of each standard, along with the slope, intercept and correlation coefficient of the standard curve.

❖ **To measure standards manually (using Manual 6 or Single Cell Holder)**

1. Install the blank and standards.
2. When the parameters are correct, press **Run Standards**.

Standard Curve		12:50 14Feb02
Test Name: AMMONIA		BLANK
Std #	Conc. mg/L	Abs 660.0nm
1	25.00	-----
2	45.00	-----
3	55.00	-----
4	75.00	-----
5	100.0	-----

Page 1, Standards 1 - 5			
Measure Blank	Save Test	Edit Standards	

3. Set **Entry concentration**.
4. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

5. Press **Measure Standards**.

If a 6-Position Cell Holder is installed, press the cell position buttons to reposition the cell holder and measure the rest of the standards manually.

When all of the standards have been measured, the Standards screen (see “Using the Standards Screen” on page 94) shows the absorbance of each standard, along with the slope, intercept and correlation coefficient of the standard curve.

Using the Standards Screen

Here is an example of the Standards screen showing measurement results:

Standard Curve			15:50 4Feb02
Test Name: AMMONIA			Cell # 5
Std #	Conc. ppm	Abs 660.0nm	
1	0.120	0.037	
2	0.200	0.140	
3	0.250	0.152	
4	0.400	0.268	
5	0.500	0.312	
Curve Fit			Linear
Slope = 0.6091		Std Dev = 0.011	
Intercept = 0.01272		Corr Coeff = 0.995	
Page 1, Standards 1 - 5			
View Graph	Save Test	Edit Standards	Run Test

You can use this screen for:

- Displaying a graph of the standard curve data (press **View Graph**)
- [Saving a Test](#) (press **Save Test**)
- [Editing a Standard Curve](#) (press **Edit Standards**)
- [Measuring Samples](#) (press **Run Test**)

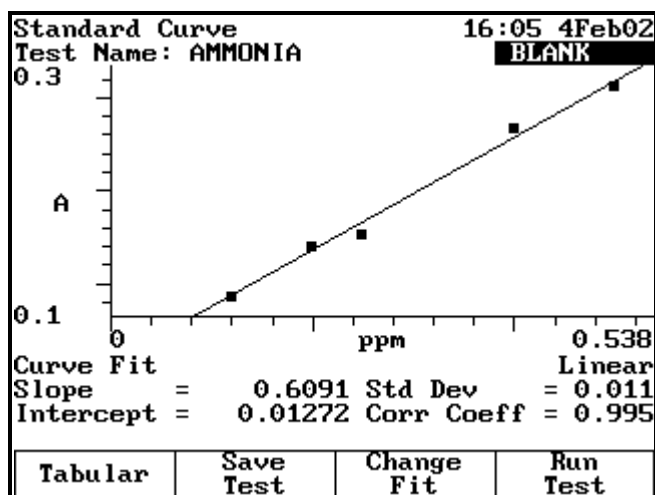
Measuring Samples

❖ To measure samples automatically using the calibration curve (using Auto 6 or Auto 3)

1. Press **Run Test**.
2. Install the blank and samples.
3. Press **Enter**.

The Standard Curve screen shows the absorbance and concentration of each sample.

To switch between tabular and graphical displays, press **View Graph/Tabular**.



❖ **To measure samples manually (using Manual 6 or Single Cell Holder)**

1. Press **Run Test**.
2. Install the blank and samples.
3. Press **Measure Blank**.

If a 6-Position Cell Holder is installed, it moves to the B position to measure the blank and returns to its previous position.

4. Press **Measure Samples**.

If a 6-Position Cell Holder is installed, press the cell position buttons to reposition the cell holder and measure the rest of the samples manually.

When the instrument has measured all the samples, the Standards screen shows the absorbance and concentration of each sample.

Editing a Standard Curve

You may edit the concentration of any standard on a standard curve. In addition, you may change the number of standards, select a different curve fit or delete points from the curve.

Std #	Conc. mg/L	
1	25.00	Edit Concentration
2	45.00	Add Standard
3	55.00	Delete
4	75.00	Clear Measurements
5	100.0	Reset Standards

Curve Fit

Slope = 0.002877	Std Dev = 0.017	Linear
Intercept = 0.002364	Corr Coeff = 0.985	

Page 1 of 0, Standards 1 - 5
Press ↑ or ↓ to view data

❖ **To edit the concentration of a standard**

1. With the standard curve displayed, highlight the standard to edit and press **Edit Standards**.
2. With **Edit Concentration** highlighted, press **Enter**.
3. Press **Edit Conc** or a number key.
4. Enter the concentration value in the **Entry** field.
5. Press **Enter**.

❖ **To add a standard**

1. With the standard curve displayed, press **Edit Standards**.
2. Highlight **Add Standard**.
3. Enter the concentration value of the additional standard in the **Entry** field.
4. Press **Enter**.
5. Press **Measure Standards** to remeasure all the standards.

❖ **To delete a standard**

1. With the standard curve displayed, highlight the standard to delete and press **Edit Standards**.
2. Highlight **Delete Standard** and press **Enter**.

❖ **To clear measurements**

1. With the standard curve displayed, press **Edit Standards**.
2. Highlight **Clear Measurements** and press **Enter**.

All absorbance measurements are removed from the screen.

❖ **To reset standards**

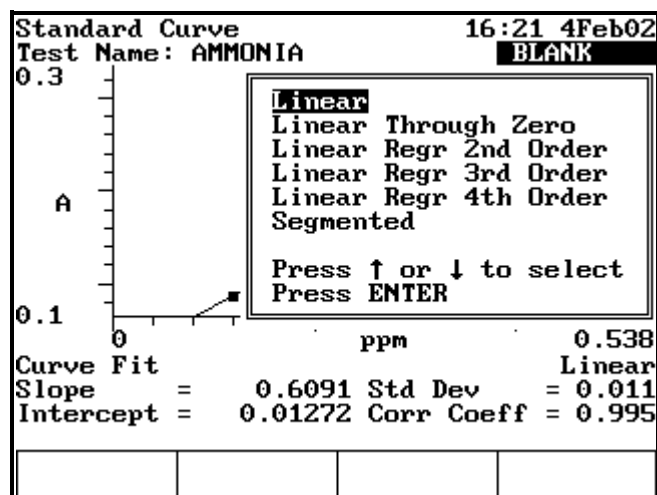
1. With the standard curve displayed, press **Edit Standards**.
2. Highlight **Reset Standards** and press **Enter**.

All standards and measurements are removed from the screen.

❖ **To select a different curve fit for a standard curve**

Note To change the curve fit for a standard curve, you must display the standard curve as a graph, not as a table.

1. With the standard curve you want to edit displayed as a graph, press **Change Fit**.



2. Highlight the curve fit to use for the standard curve and press **Enter**.

The instrument applies the selected curve fit to the data and displays the new fit.

20 Concentration Measurements—Standard Curve Application

Editing a Standard Curve

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Kinetics

The Kinetics application lets you measure the change in the sample absorbance as a function of time. The local control software allows the determination of a linear rate over a particular region, which can be defined after the data acquisition. Frequently used in enzymatic kinetics, a factor can be multiplied by the slope of the linear rate fit to determine activity.

Computer software control lets you greatly expand the kinetics capabilities of your instrument:

- Multicell parallel kinetics lets you monitor up to five reactions simultaneously. Extended kinetics data acquisition exceeds the 400 data point limit of the embedded software.
- Software control streamlines the use of more sophisticated computer applications to analyze kinetics data after collection.

Use Kinetics for:

- [Recalling a Test](#)
- [Setting Up Test Parameters](#)
- [Cell Correction](#)
- [Measuring a Blank](#)
- [Measuring Samples](#)
- [Recalling and Recalculating Graphical Kinetics Results](#)
- [Rescaling and Recalculating Tabular Kinetics Results](#)
- [Modifying the scale of the plot](#)

You can work with graphical or tabular data and perform the same functions with either. However, the location of the function keys depends on the display type.

Note The Kinetics application lets you measure only one sample at a time.

Note The Kinetics application lets you collect up to 400 data points per run. When setting test parameters, select the interval time and total run time accordingly.

To get started, press the **Test** button, highlight **Kinetics** and press **Enter**.

Recalling a Test

❖ To recall a test

1. In the Kinetics screen, press **Stored Tests**.
2. Highlight the test to recall and press **Enter**

Kinetics		4:17pm 5Aug09	
		BLANK	
Test Name -----			
Measurement Mode	Absorbance		
Wavelength	546.0nm		
Ref. Wavelength Correction	Off		
Delay Time (min:sec)	0:00		
Interval Time (min:sec)	0:01		
Total Run Time (hr:min:sec)	0:05:00		
Measure Blank	Once		
Display Result (Rate * Factor)	On		
More parameters...			
Press ↑ or ↓ to select item to change.			
	Save	Stored	Run
	Test	Tests	Test

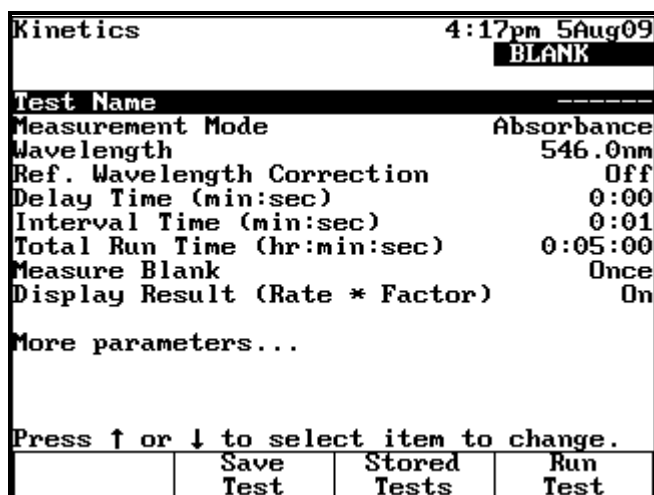
This screen provides these capabilities:

- [Setting Up Test Parameters](#)
- Setting up Cell Correction
- [Saving a Test](#)
- Viewing the list of stored tests
- [Measuring a Blank](#)
- [Measuring Samples](#)

Setting Up Test Parameters

❖ To set up test parameters

1. In the Kinetics screen, highlight the desired parameter.



Parameter	Description
Delay Time	Enters the time from Test Initiation to first measurement; allows for sample equilibration Adv.
Interval Time	Enters the time between repeated readings
Measure Blank (as function key)	Selects the frequency of zeroing the instrument Once or Every Reading

- When the parameters are set, press **Save Test** to save the test or **Run Test** to measure a blank or sample.

Note If Cell Correction is ON, you must run the Setup Correction application before you can access Run Test or Measure Samples.

Note If Auto Save Data is ON, you must enter a Data File Name before you can access Run Test or Measure Samples.

Measuring Samples

Note If a 6-Position Cell Changer is installed, place the blank in position B and the sample in cell position #1. The instrument always uses cell position #1 to scan the sample.

❖ To measure samples

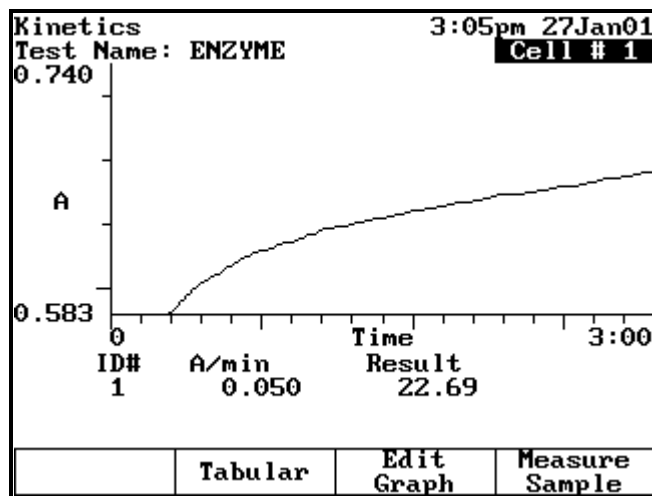
- In the Kinetics screen, press **Run Test**.
- If a 6-Position Cell Holder is installed, place the blank in position B and the sample in position #1.

3. Press **Measure Sample**.

After the measurement, the kinetics data and rate appear.

4. If a Single Cell Holder is installed, press **Measure Blank** to measure the blank, insert your sample and then press **Measure Sample**.

After the measurement, the kinetics data and rate appear.



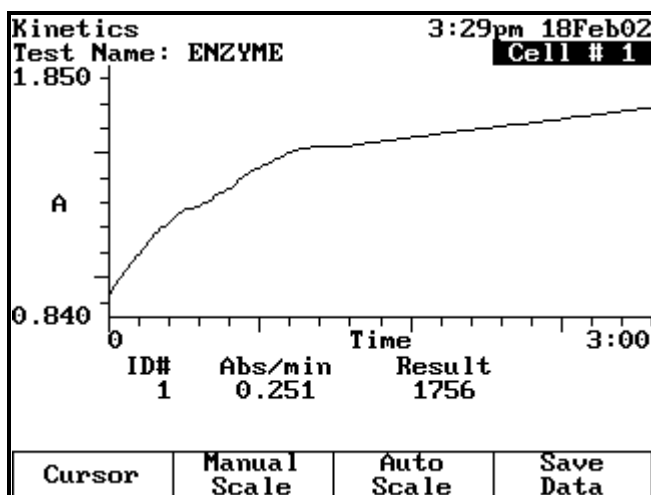
To switch between tabular and graphical displays, press **Graph/Tabular**.

On a graphical display you can press **Edit Graph** and then press **Cursor** to move the cursor line from one position to another on the plot. As the cursor moves, the rate and result values indicate the values for the point where the cursor is located.

Recalling and Recalculating Graphical Kinetics Results

The Kinetics application lets you view and manipulate results in graphical or tabular form. When your results are displayed, you can modify the range (start and stop time) and the instrument recalculates the reaction rate.

When working with graphical kinetics results, you need to press **Edit Graph** before you can rescale and recalculate.



You can modify the scale of your kinetics data plot automatically or manually. When you select **Auto Scale**, the instrument automatically scales the X- and Y-axes so all the data appears on the plot. When you select **Manual Scale**, you select specific minimum and maximum values for the X- and Y-axes. Whenever you modify the scale, the instrument recalculates and displays the new reaction rate and result.

The edit screen lets you:

- Use Auto Scale to change the scale, display the new graph and recalculate the results
- Use Manual Scale to change the scale, display the new graph and recalculate the results
- Use the cursor to select new minimum or maximum values for the X-axis and recalculate the results

❖ To use Auto Scale

With your kinetics data displayed on the Edit Graph screen, press **Auto Scale**.

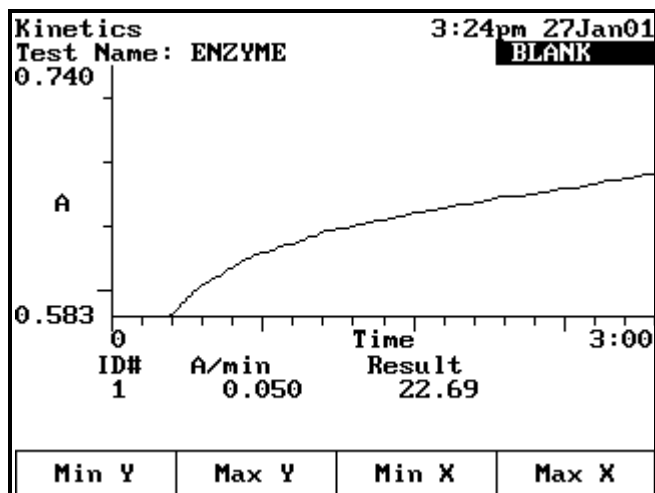
The instrument adjusts the minimum and maximum values for the X- and Y-axes so all the data appears on the plot. The instrument also recalculates the results, using all the data, and displays them.

❖ To use Manual Scale

1. With your kinetics data displayed on the edit screen, press **Manual Scale** to display the manual scale options.

21 Kinetics

Recalling and Recalculating Graphical Kinetics Results



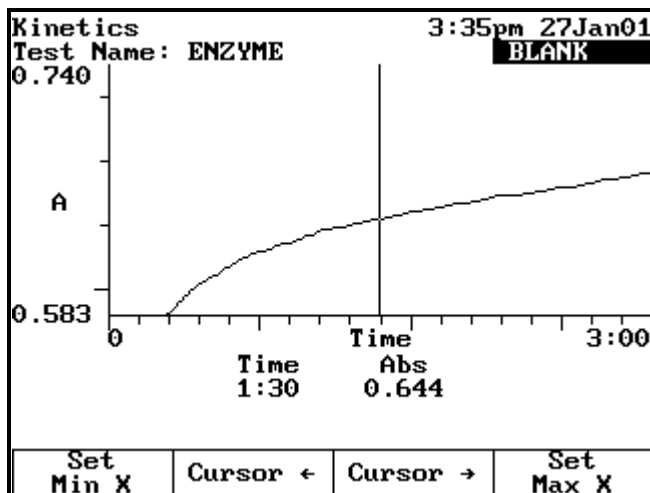
2. Enter the appropriate minimum or maximum value for the X- or Y-axis and press **Min Y**, **Max Y**, **Min X** or **Max X** to accept it.

The instrument redraws the plot using the entered minimum and maximum values and displays the recalculated rate and result.

3. Continue until you have entered all the values you want to change.

❖ To use the Cursor function

1. With your kinetics data displayed on the edit screen, press **Cursor** to display the cursor options.



2. Press **Cursor ←** or **Cursor →** to position the cursor line on the appropriate point on the graph.

The data for the selected point appears.

3. When the cursor line is in the correct position, press **Set Min X** or **Set Max X** to accept the selected point.

The instrument redraws the plot using the selected minimum and maximum values and displays the recalculated rate and result.

Rescaling and Recalculating Tabular Kinetics Results

When working with tabular kinetics results, you must press **Edit Data** before you can rescale and recalculate.

After collecting kinetics data, you can use all the data for the rate calculation or select specific start and end times. When you modify the start and end times or select all the data, the instrument recalculates and displays the new reaction rate and result.

The edit screen lets you:

- Use all the data to recalculate the results
- Select specific start and end times for the rate calculation and recalculate the results

Kinetics		12:57 30Jan01		
Test Name: KINETICS		Cell # 1		
HH:MM:SS	Abs	Delta	Lin	
→ 0:00	0.417	---	---	
0:10	0.471	0.054	---	
0:20	0.510	0.039	L	
0:30	0.534	0.024	L	
0:40	0.550	0.016	L	
0:50	0.559	0.010	L	
1:00	0.568	0.008	L	
1:10	0.574	0.007	L	
ID#	min/Abs	Result		
1	0.000	0.013		
Press ↑ or ↓ for more data...				
Use all data	Graph	Start Time	End Time	

❖ To use all the data to calculate the reaction rate

With your table of kinetics data displayed on the edit screen, press **Use All Data**.

The instrument calculates and displays the rate.

❖ To select specific start and end times for the rate calculation

1. With your kinetics data displayed on the edit screen, highlight the appropriate data point in the table.
2. Press **Start Time** or **End Time** to display the recalculated rate and result.

21 Kinetics

Rescaling and Recalculating Tabular Kinetics Results

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Performance Verification

Performance Verification lets you check the performance of your instrument with these tests:

- Wavelength Accuracy - Internal
- Wavelength Accuracy - Standard
- Wavelength Repeatability (UV-Vis only)
- Resolution (UV-Vis only)
- Photometric Accuracy
- Noise
- Stray Light

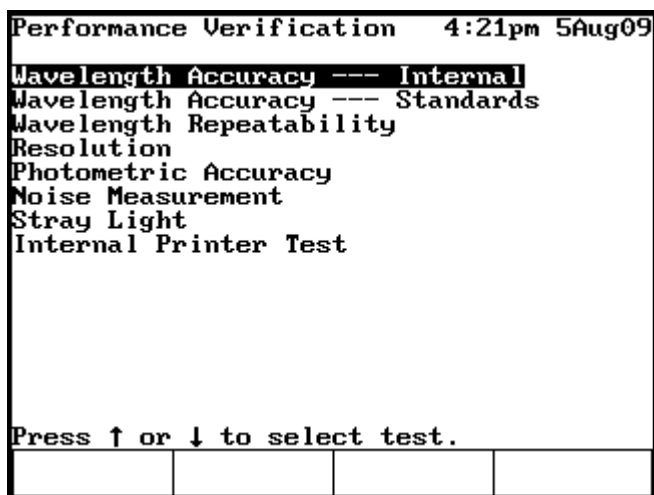
Run the appropriate tests regularly and maintain a log of results to help document the reliability of the instrument and indicate potential performance issues.

Note If a printer is installed and turned on, the instrument automatically prints test results. You can also press **Print** to print another copy of the results.

Accessing the Performance Verification Tests

❖ To access the Performance Verification tests

1. Press **Tests**.
2. Highlight **Performance Verification** and press **Enter**.



Troubleshooting Checklist

If a Performance Verification test fails, follow the instructions below to diagnose common problems.

If a test continues to fail after you have tried all these recommendations, follow the troubleshooting list for the test being run (included with the description of each test).

Make sure:

- You follow the instructions for the test properly.
- Filters and standards are clean.
- The sample compartment door is closed during the test.
- The sample compartment is clear of obstructions.
- The cell holder assembly is installed properly. If the 6-Position Cell Holder is installed, run the test once with the sample compartment door open to verify that the 6-Position Cell Holder is moving smoothly.
- No problems are indicated by the power-on diagnostics after you turn the instrument power OFF and then ON.
- The lamp is ON.
- The lamp compartment is clear of obstructions.

WARNING Do not open the lamp compartment unless the instrument power is OFF.

WARNING Do not turn the instrument power ON unless the lamp compartment is closed.

Wavelength Accuracy - Internal

This test locates the peaks of the internal xenon lamp (UV-Vis instruments) or internal didymium filter (Vis instruments) and displays the expected and measured wavelengths for the peaks.

- UV-Vis: A xenon lamp has strong, fundamental lines at 229 nm, 529 nm and 883 nm. These lines are an essential property of xenon and serve as a fundamental standard.
- Vis: Didymium glass has strong transmission peaks at 585nm and 807nm. These peaks are well characterized and suitable for use as wavelength standards.

When running the internal standard test, remember that:

- The wavelengths and tolerance values are preset and cannot be changed.
- The cell holder should be empty.

❖ To run the Wavelength Accuracy - Internal test

1. Highlight **Wavelength Accuracy - Internal** and press **Enter**.
2. Press **Start Test**.

The results indicate pass or fail for each wavelength.

Performance Verification		16:13 2Mar07	
Wavelength Accuracy --- Internal			
Expected	Tolerance	Measured	Result
nm	±nm	nm	
247.5	± 1	248.0	Pass
529.0	± 1	529.3	Pass
824.2	± 1	824.4	Pass
Make sure the Blank position is empty. Press Start Test			
			Start Test

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Contact technical support for more troubleshooting advice.

Wavelength Accuracy - Standard

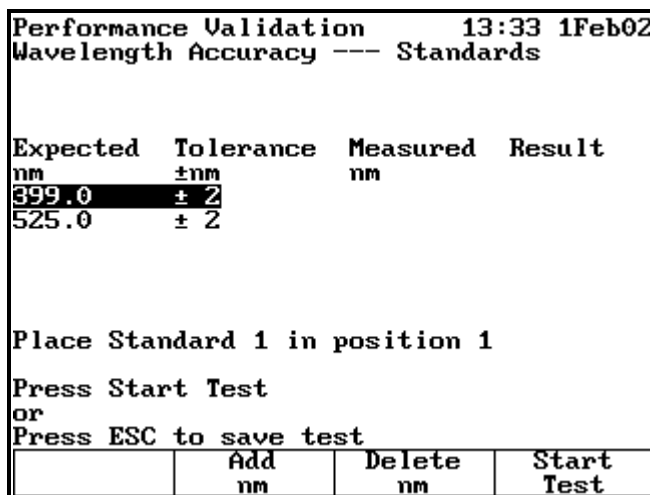
This test measures the absorbance of a wavelength accuracy standard and compares the location of the peaks with precisely known values at up to five wavelengths. By default, wavelength accuracy tests are performed in absorbance mode; however, this test may also be performed in %T mode. Typical wavelengths and tolerances are included in the firmware, but these values can be changed to match the calibration certificate included with your standards.

When running the Wavelength Accuracy test, remember:

- You need Wavelength Accuracy standards designed to measure the wavelength accuracy at the specified wavelengths.
- Use an empty cell holder as the blank.
- Measure the standards in the order they are listed on the test screen.
- You can measure the wavelength standard at only five wavelengths.

❖ To run the Wavelength Accuracy - Standards test

Highlight **Wavelength Accuracy - Standards** and press **Enter**.



❖ To add a wavelength

1. Press **Add nm** and enter the wavelength value in the **Entry** field.
2. Press **Add nm** again to add the wavelength to the list.
3. Enter the tolerance for the entered wavelength in the **Entry** field.
4. Press **Enter**.

❖ To delete a wavelength

1. Highlight the appropriate wavelength.

2. Press **Delete nm**.

❖ **To run the test**

1. Verify that the wavelengths and tolerances are set correctly.
2. Press **Start Test**.

The results indicate pass or fail for each wavelength.

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Make sure the target and tolerance values you entered for the calibrated wavelength are the same as the values on the calibration certificate for the standard.

Wavelength Repeatability (UV-Vis only)

This test measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner. The test uses the internal xenon lamp.

A xenon lamp has a strong, fundamental line 529 nm. This line is an essential property of xenon and serves as a fundamental standard.

When running the internal standard test, remember that:

- The wavelength and tolerance values are preset and cannot be changed.
- The cell holder should be empty.

❖ **To run the Wavelength Repeatability test**

1. Highlight **Wavelength Repeatability** and press **Enter**.
2. Press **Start Test**.

The results indicate pass or fail for each wavelength.

22 Performance Verification

Resolution (UV-Vis only)

Performance Verification		16:33 2Mar07	
Wavelength Repeatability			
Minimum	Maximum	Peak-to-Peak	Result
529.2	529.3	0.1	Pass
Make sure the Blank position is empty. Press Start Test			
			Start Test

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Contact technical support for more troubleshooting advice.

Resolution (UV-Vis only)

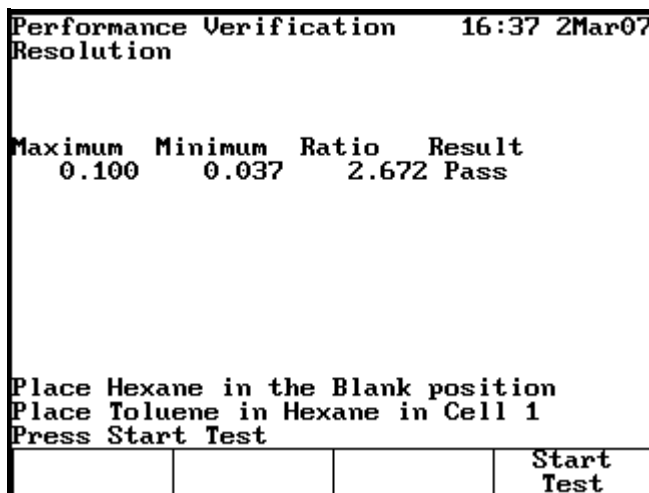
This test measures the ability of the spectrophotometer to resolve adjacent features in a spectrum. The test is performed using a 0.02% (v/v) solution of toluene in hexane and requires a hexane blank.

When running the internal standard test, remember that the wavelengths and tolerance values are preset and cannot be changed.

❖ To run the Resolution test

1. Highlight **Resolution** and press **Enter**.

Ensure that hexane is in the blank position and toluene in hexane is in cell 1.



2. Press **Start Test**.

The results indicate pass or fail for each wavelength.

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Contact technical support for more troubleshooting advice.

Photometric Accuracy

This test measures the absorbance (or %T) of a set of standards and compares the results with specified tolerances. The wavelength absorbencies and tolerances are preset, but you should change them to the values on the calibration certificate included with your standards.

Note You can display the tolerances for this test in either absorbance or %Transmittance.

When running the Photometric Accuracy test, remember:

- You need Photometric Accuracy standards calibrated to known absorbance values at specified wavelengths.
- Measure the standards in the order they are listed on the test screen.
- You can use one to five standards.

❖ To display the Photometric Accuracy screen

1. Highlight **Photometric Accuracy**.
2. Press **Enter**.

Performance Verification		16:46 2Mar07			
Photometric Accuracy					
#	nm	Expected %T	Tol. ±%T	Meas. %T	Result
1	590.0	50.000	± 1.200		
2	590.0	10.000	± 1.200		
Place Standards 1 - 2 in positions 1 - 2					
Change to Abs	Add Std	Delete Std	Start Test		

Selecting the Mode

To switch between absorbance and %Transmittance, press **Change to %T** (or **Change to ABS**) until the appropriate mode appears.

Adding Standards

You will need to set three values whenever you add a standard: the wavelength, the absorbance (or %Transmittance) and the tolerance value.

❖ To add a standard

1. Press **Add Std** and enter the wavelength value in the **Entry** field.
2. Press **Enter** or **Add nm** to add the wavelength to the list.
3. Enter the absorbance or %T value for the entered wavelength in the **Entry** field.
4. Press **Enter**.
5. Enter the tolerance for the entered wavelength in the **Entry** field.
6. Press **Enter**.

The test screen displays the values you just entered for that standard.

7. Press **Start Test** to begin the measurement or **Press Esc** to save the test.

Deleting Standards

❖ To delete a standard

1. Highlight the appropriate standard.

2. Press **Delete Std.**

Running the Test

In the Photometric Accuracy screen, make sure the wavelengths, absorbance (or %T) values and tolerances are set correctly.

❖ To run the Photometric Accuracy test

Press **Start Test.**

The results indicate pass or fail for each wavelength.

Performance Validation		11:44 15Feb02			
Photometric Accuracy					
#	nm	Expected %T	Tol. ±%T	Meas. %T	Result
1	590.0	10.1	± 1.2	10.1	Pass
2	590.0	9.4	± 1.2	9.4	Pass

Place Standards 1 - 2 in positions 1 - 2

Press Start Test
or
Press ESC to save test

Change to Abs	Add Std	Delete Std	Start Test
------------------	------------	---------------	---------------

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Follow the guidelines provided with the standard reference materials.
- For more troubleshooting advice contact our sales or service representative in your area or use the information at the beginning of this document to contact us.

Noise

This test measures the amount of noise. The Vis instrument measures only at 340 nm.

All test parameters are determined by instrument specifications and cannot be changed by the user. When running the noise test, remember:

- Perform the 0A measurement with the cell holder empty. Optionally you can perform the 2A measurement with a 2A filter.

❖ **To run the Noise Measurement test**

1. Highlight **Noise Measurement**.
2. Press **Enter**.

Performance Validation		11:54 15Feb02	
Noise Measurement			
@0A	Peak-to-Peak Tolerance	Meas. Abs	Result
230.0nm	≤0.002	0.000	Pass
340.0nm	≤0.001	0.000	Pass
@2A	Peak-to-Peak Tolerance	Meas. Abs	Result
230.0nm	≤0.003	0.000	Pass
340.0nm	≤0.002	0.000	Pass
Make sure the Blank position is empty. Place 2A filter in Cell Position 1 Press Start Test			
			Start Test

3. With the Blank position empty, insert the 2A filter in position #1.
Ignore the test results at 2A if you do not have a filter installed in position #1.
4. Press **Start Test**.

The results of the test indicate pass or fail for each wavelength.

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Make sure the instrument is warmed up for at least 30 minutes, with the standby mode feature turned off.
- For more troubleshooting advice contact our sales or service representative in your area or use the information at the beginning of this document to contact us.

Stray Light

This test measures the stray light at selected wavelengths and compares the measurements with expected values. The wavelengths and expected values are preset and cannot be changed. Running the stray light test takes about 30 seconds.

When running the stray light test, remember:

- You need Stray Light standards designed to measure stray light at 220 nm (UV-Vis only), 340 nm and 400 nm (i.e., must have ≤0.1 %T at the wavelength of interest).

For UV-Vis instruments:

- Position B should be empty.
- Use position #1 for the 220 nm stray light standard (SRE 220 or equivalent).
- Use position #2 for the 340 nm stray light standard (SRE 340 or equivalent).
- Use position #3 for the 400 nm stray light standard (SRE 400 or equivalent).

For Vis-only instruments:

- Position B should be empty.
- Use position #1 for the 340 nm stray light standard (SRE 340 or equivalent).
- Use position #2 for the 400 nm stray light standard (SRE 400 or equivalent).

Running the Test

In the Stray Light screen, make sure the wavelengths and tolerances are set correctly.

❖ To run the Stray Light test

1. Highlight **Stray Light**.
2. Press **Enter**.
3. Press **Start Test**.

The results indicate pass or fail for each wavelength.

If the test fails, follow these guidelines:

- Repeat the test twice to verify that the test is failing consistently.
- Make sure all the filters being used are specifically designed to measure stray light at the specific wavelengths.
- Verify that the filters are placed in the correct cell positions.
- For more troubleshooting advice contact our sales or service representative in your area or use the information at the beginning of this document to contact us.

22 Performance Verification

Stray Light

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Maintenance

The spectrophotometer is durable and reliable, so routine maintenance is minimal. This section explains:

- [Routine Care](#)
- [Changing the Fuse](#)
- [Replacing the Tungsten Lamp](#)



WARNING Operating the instrument with the cover off exposes the operator to potentially dangerous voltages and ultraviolet (UV) radiation. Therefore, we recommend that only authorized service representatives perform procedures requiring removal of the instrument cover and replacement of electrical components. To protect both yourself and the instrument, be sure to contact an authorized service representative to perform any service procedure you do not feel comfortable performing.

Routine Care

Routine care for the spectrophotometer does not require a lot of time. To help minimize maintenance time and increase the life and performance of your instrument, please follow these guidelines:

- Always replace the dust cover when the instrument is not turned on to prevent dust from accumulating in and on the instrument.
- Do not use or store the instrument in a corrosive environment.
- Gently wipe the outside of the instrument, including the keypad, with a soft cloth to remove any dust or spills. Water, isopropyl alcohol and other common laboratory cleaning agents may be used if necessary.
- Always clean up spills as soon as they occur to prevent or minimize damage to the instrument. If concentrated acids or bases, or any hydrocarbon materials, are spilled on the instrument, clean up the affected area immediately.

Cleaning and Maintaining Cells

Carefully check the condition of the cuvettes and other cells used to measure samples. If they are chipped, cracked or scratched, it is important to discard the damaged cell(s) and replace them with new ones.

Ensuring your cuvettes are clean both inside and out is important to the quality of your results for two reasons: 1) contaminating material may absorb light resulting in falsely high absorbance readings; and 2) contaminants in the cell may react chemically with subsequent reagents or standards introduced into the cell.

Cleaning methods depend to some extent on the nature of the contaminating material. It is important to identify the residual material in the cell that needs to be removed. Refer to the following table for suggestions on cleaning methods, solvents and material.

Solvents	Examples	Suggested Cleaning Methods
Aqueous	Protein, Biologics, DNA	<ul style="list-style-type: none"> • Warm water with detergent • Dilute nitric acid (<10%) rinse • Copious water rinse
Aqueous	Salt solutions	<ul style="list-style-type: none"> • Dilute nitric acid (<10%) rinse • Copious water rinse
Aqueous	Basic solutions	<ul style="list-style-type: none"> • Warm water with detergent • Dilute nitric acid (<10%) rinse • Copious water rinse
Organic	Hydrocarbons, small molecules, oils	<ul style="list-style-type: none"> • Rinse with organic solvent • Warm water with detergent • Dilute nitric acid (<10%) rinse • Copious water rinse
Organic	Alcohol solutions	<ul style="list-style-type: none"> • Rinse with similar alcohol, acetone, or other solvent • Copious water rinse

Solvents	Examples	Suggested Cleaning Methods
Organic	Acidic solutions	<ul style="list-style-type: none"> • Rinse with organic solvent • Warm water with detergent • Dilute nitric acid (<10%) rinse • Copious water rinse
Organic	Hydrocarbons, small molecules, oils	<ul style="list-style-type: none"> • Rinse with organic solvent • Warm water with detergent • Dilute nitric acid (<10%) rinse • Copious water rinse

IMPORTANT Keeping the cell clean is very important for long cell life.

- Never store cuvettes long term in a water or solvent bath between uses. If the solvent you are using dries, impurities in the water or solvent may be deposited on the inside of the cell, causing permanent damage.
- Use only lens cleaning tissue/paper or fine soft cloth to wipe optical surfaces. Most paper products (such as facial tissues, paper towels, etc.) contain wood fibers that can damage the cell material.
- At the end of the day, ensure that all cells are well cleaned and stored in a suitable container after drying.

Term	Definition
Dilute acid	Dilute nitric acid (<10%)
Acid	Hydrochloric (5M) acid or nitric acid (5M) (see the Note below)
Solvent rinse	Rinse with the solvent that was originally used to solvate your analyte
Copious water rinse	Use a pure water (e.g., deionized, distilled, RO) and rinse at least 10 times
Detergent	Use a neutral pH detergent (Triton [®] X-100), if available, to dilute acid wash; water rinse to remove residue

Note Do not use 5M nitric acid on an Anti-reflection mirror coated cell.

IMPORTANT Using an ultrasonic cleaning bath for your cells is not recommended. Each bath generates a different frequency; therefore, if your bath operates at the resonant frequency of a cell, the cell will break. If a cell was cleaned in an ultrasonic bath, the warranty is void by the manufacturer.

IMPORTANT Do not dry cells in an oven.

Micro flowcells can be kept clean by:

- Flushing well with a solvent after use.
- Aspirating dilute acid, base, non-filming detergent or Clorox® through the cell in short bursts.
- Storing with distilled water in the cell.

Cleaning the Windows of the Sample Compartment

Do not use acetone or abrasive materials to clean the windows of the sample compartment. Instead, use a non-abrasive laboratory cleaning solution (such as a commercial cell cleaning solution), distilled water or alcohol.

Use the liquid and a soft, lint-free cloth to clean the windows. Do not apply too much pressure or the surface of the windows may be damaged. Be sure to remove all fingerprints.

Changing the Fuse

The fuse is located in the power entry module located at the center of the back panel of the instrument.

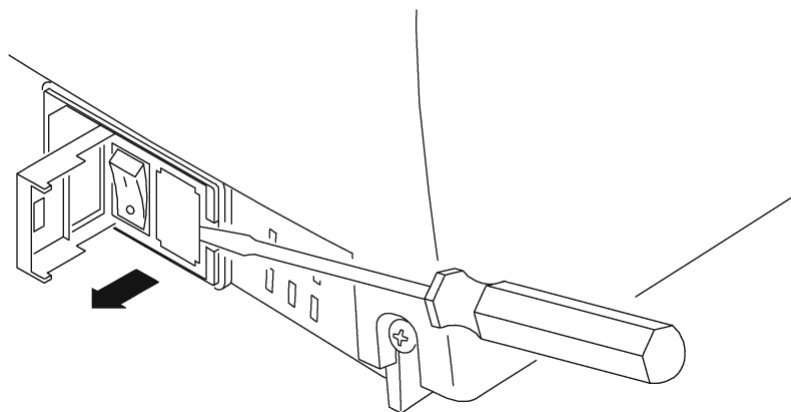
- 120 VAC, 2.5 A, Slo Blo
- 240 VAC, 1.25 A, Slo Blo (2 required)

IMPORTANT The instrument fuse must be replaced with the same type and rate.

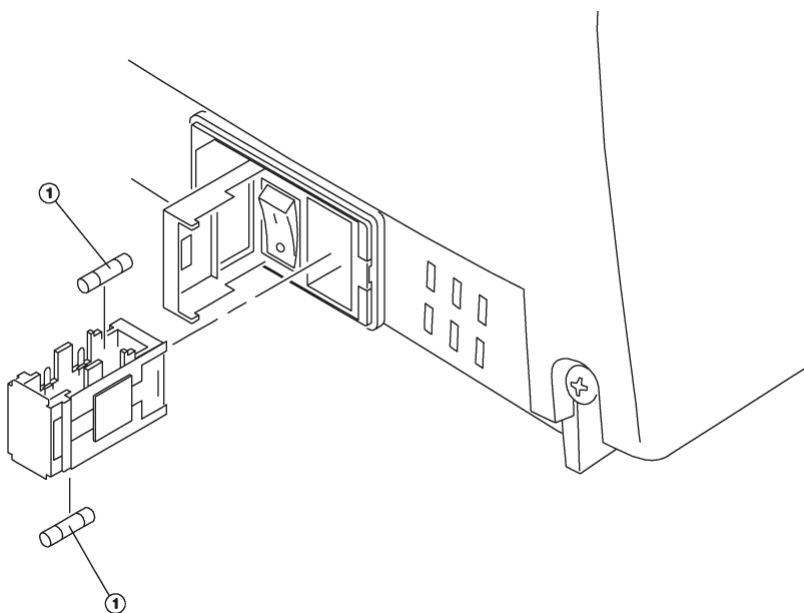
IMPORTANT If the fuse fails repeatedly, it may indicate a serious problem with the instrument. Contact technical support as soon as possible.

❖ To change the fuse

1. Turn off and unplug the instrument from the wall outlet or power strip.
2. Position the instrument so you can access the power entry module on the back of the instrument.
3. Remove the power cord.
4. Insert a flat-blade screwdriver into the notch on the fuse cover and pry off the cover.



5. Use a flat-blade screwdriver to remove the fuse holder.



6. Unsnap both fuses to remove them.
7. Insert the new fuses, pushing them in so they snap into place.
8. Replace the fuse cover.
9. Replace the power cord.
10. Plug the instrument back in to the wall outlet or power strip and turn on the power.

Note If the fuse blows again, contact your distributor or technical support.

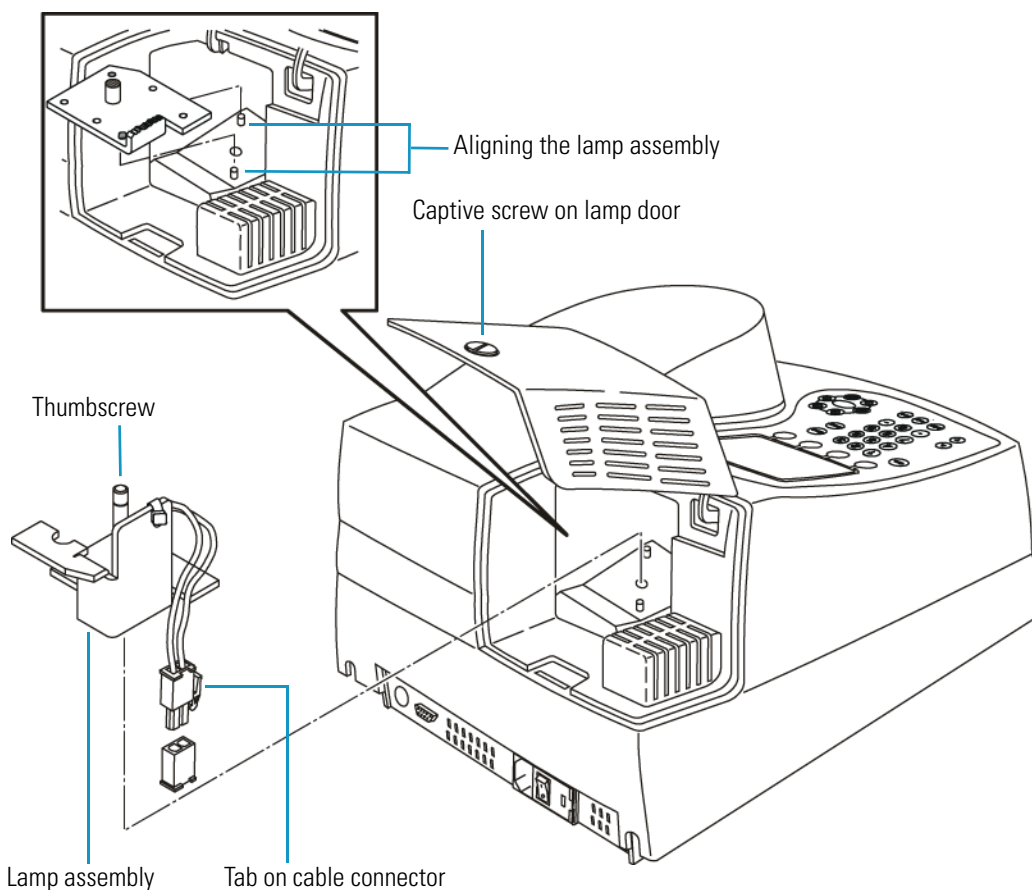
Replacing the Tungsten Lamp

WARNING This lamp gets very hot during operation. Before removing the lamp, turn off the instrument and allow the lamp to cool for 10 minutes.

WARNING Turn off and unplug the instrument before removing the cover of the lamp compartment.

❖ **To replace the tungsten lamp**

1. Turn off and unplug the instrument from the wall outlet or power strip.
2. Make sure the lamp has cooled (for at least 10 minutes).
3. Loosen the captive screw on the lamp door by rotating it counterclockwise about $\frac{1}{4}$ turn.



4. Raise the lamp door.
5. Unscrew the thumbscrew while holding the lamp assembly in place.
6. Carefully pull the assembly out until you can reach the connector.
7. Release the tab and disconnect the cable.
8. Unpack the new lamp assembly.
9. Use a soft, lint-free cloth to wipe off any fingerprints on the lamp.
10. Connect the cable to the connector.

11. Position the new assembly as shown above, making sure to align the pins correctly.
12. Tighten the thumbscrew.
13. Close the lamp door.
14. Turn on the instrument and press the **UTILITY** key.
15. Press the arrow keys to highlight **Lamp Hours** and press **ENTER**.
16. Press **ENTER** to reset the lamp hours to zero.

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Parameters

Parameter	Description
+ - x ÷	Enters math operators when in Calculator mode (Utility)
% of lamp life used	Displays the estimated percentage of lamp life used (based on a typical lamp life of five years) (Utility)
3-Pt Net	Lets you calculate peak height from the tangential baseline in the graph (Scanning)
Absorbance	Enters the absorbance value
Accept Name	Accepts the displayed Name entry (Test Name and Edit [Units])
Add Character	Adds a highlighted character to the Name entry (Test Name and Edit [Units])
Add nm	Lets you add a wavelength and factor to the list in Multiwavelength tests and some Performance Verification tests
Area	Lets you calculate area under the peak in the graph (Scanning)
AutoPrint	Turns the automatic printout on or off
Autoscale	Rescales the graph to the original ranges of the X- and Y-axes (Kinetics, Scanning)
Baseline Expiration	Enters the time when the baseline for scan tests will need to be collected again (Utility)
Beeper	Turns the audible signal for key presses on and off (Utility)
Calculation Baseline	Selects the zero baseline or the tangential baseline to calculate area under the peak in the graph (Scanning)

Parameter	Description
Calculator	Enables the calculator mode (Utility)
Cell Correction	Selects the option to automatically correct for variances in absorption between the cuvettes (all test types)
Cell Position #	Displays the position placed in the light path (only with auto turret)
Change Mode Change to Abs Change to %T	Switches measurement modes (Basic A-%T-C and some Performance Verification tests)
Collect Baseline	Starts the collection of the baseline (Scanning only)
Concentration	Sets the concentration value
Conc of Standard	Displays the entered concentration value (Adv A-%T-C)
Correction Mode	Selects the mode for cell correction (Discrete nms or Scan)
Cursor	Goes to Cursor tracking mode to view data points in the graph (Kinetics, Scanning)
Cursor → ← Cursor	Moves the cursor right or left on the graph and displays the data of each point (Kinetics, Scanning)
Curve Fit	Selects the type of line fit calculation (Standard Curve tests)
Data File Name	Allows entry of a name for the data file when AutoSave = ON
Date Cell Correction	Displays the date when cell correction data on cuvettes was last collected
Date Standards Measured	Displays the date when standards were last measured with this instrument (Standard Curve tests)
Date/Time Setup	Enters the current date and time settings for the instrument (Utility)
Delay Time	Enters the time from Test Initiation to first measurement; allows for sample equilibration (ADV. A-%T-C and Kinetics)
Delete Character	Deletes the last character of Name entry (Test Name and Edit [Units])

Parameter	Description
Delete File	Deletes a test or data file from the Stored Tests Directory (Utility)
Delete Name	Deletes the entire name to allow a new entry (Test Name and Edit [Units])
Delete nm	Removes a wavelength and factor from the list (Multiwavelength and some Performance Verification tests)
Diluent Volume	Enters the volume of diluent added before measurement (Dilution Multiplier in some Bio Tests)
Dilution Multiplier	Displays the factor used to correct for sample dilution
Display Activity	Indicates whether results should include protein concentration
DNA ϵ (260)	Calculates the extinction coefficient
DNA Factor	Enter the factor to calculate DNA concentration (DNA Bio Tests)
Edit	Lets you change a wavelength or factor in the list (Multiwavelength and some Performance Verification tests)
Edit Curve	Lets you manipulate the graph (Kinetics)
Edit Data	Lets you select a portion of the data in a table for recalculation of a result (Kinetics and Scanning)
Edit Graph	Lets you manipulate the graph (Scanning)
Edit Scale	Lets you change the graph axis scales and view individual data points (Scanning)
Factor	Enters a factor to convert a datum to a result Abs x Factor 1 = Concentration Result Abs/min x Factor 2 = Kinetics Result Can be entered or calculated from concentration and absorbance values in ADV A-%T-C
Factor 1	Enters a factor to convert a datum to a result Abs(WL1) x Factor = Result (Abs Ratio, Abs Diff, Multiwavelength tests)

Parameter	Description
Factor 2	Enters a factor to convert a datum to a result Abs(WL2) x Factor = Result (Abs Ratio, Abs Diff, Multiwavelength tests)
Factor 3-31	Enters a factor to convert a datum to a result Abs (WL3-31) x Factor = Result (Multiwavelength tests)
Graph	Displays the graph of collected data (Kinetics, Scanning)
ID #	Enters the numeric identifier for measurement; autoincrements during the test until turned off (set to 0)
Instrument Serial Number	Displays the serial number of the instrument (Utility)
Intercept	Enters where the line crosses the Y-axis (Abs where conc=0)
Interval	Enters the wavelength range between data points (Scanning tests only)
Interval Time	Enters the time between repeated readings (Kinetics only)
Linearity Value	Enters a linearity value (Kinetics only)

To help determine the linearity of the reaction during the measurement, the instrument offers a linearity parameter. This is the difference between the changes in the absorbance of two measurements as shown in the following example:

Time	Abs	?A	Linearity
1	.1	--	---
2	.2	.1	---
3	.29	.09	P
4	.38	.09	P
5	.46	.08	P
6	.52	.06	F

Linearity is the ?A between ?A calculations.

P=Pass and F=Fail

Parameter	Description
Load Test	Loads the highlighted test from the Stored Tests Directory into active memory and sets the instrument to the test parameters (Utility)
Lock/Unlock	Used to protect stored tests from accidental deletion or alteration; asks for a password to allow the user to lock or unlock the file (Utility)
Low/High Limits	Enters the lowest and highest acceptable results, outside of which the result is flagged as “Low” or “High” (Adv. A-%T-C, Std Curve, Abs Ratio, Abs Diff, Kinetics, 3-Pt Net and some Bio Tests)
Math	Accesses manipulation functions of the graph (Scanning)
Measure Blank (as function key)	Initiates measurement of the blank
Measure Blank (as test parameter)	Selects the frequency of zeroing the instrument Once or Every Reading (Kinetics)
Measurement Mode	Selects the type of photometric data reported for a measurement (Abs, %T, Conc) (in A-%T-C, Kinetics, Scanning, Multiwavelength)
Measure Samples	Initiates measurement of samples
Max, X	Enters a maximum X-value to manually rescale the graph (Kinetics, Scanning)
Max, Y	Enters a maximum Y-value to manually rescale the graph (Kinetics, Scanning)
Min, X	Enters a minimum X-value to manually rescale the graph (Kinetics, Scanning)
Min, Y	Enters a minimum Y-value to manually rescale the graph (Kinetics, Scanning)
Next Cursor	Selects a cursor point in functions using more than one cursor setting: Scan-Area and Scan-3-Pt Net calculations in the graph (Scanning)
Number of Matched Cuvettes	Enters the number of cuvettes that will be run in the Correction Program (maximum of 8)
Number of Samples	Enters the number of samples to be measured in the test (Not available in Kinetics or Scanning)

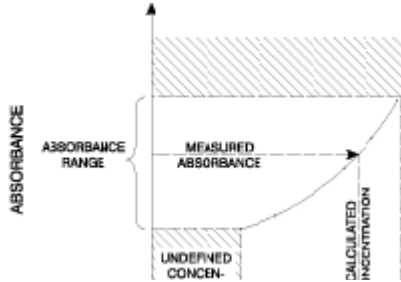
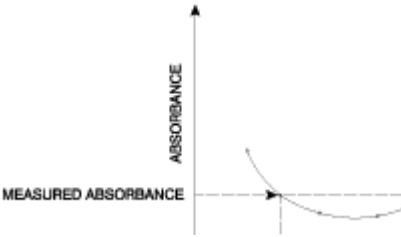
Parameter	Description
Number of Standards	Enters the number of standards to be measured for the standard curve
Printer	Selects the output mode (RS-232, Parallel) (Utility)
Protein Factor	Enters the factor to calculate Protein concentration (DNA Bio Tests)
Ref. Wavelength	Enters a reference wavelength value; for each reported measurement, measures the analytical wavelength and reference wavelength. Reported measurement = $Abs@Analytical\ WL - Abs@Reference\ WL$
Ref. Wavelength Correction	Turns reference wavelength correction on or off
Run Corr.	Initiates the collection of cuvette data for cell correction
Run Standard	Goes to the Standards entry screen
Run Test	Goes to the data collection screen (all tests)
Sample Positioner	Selects the type of Positioner 1 Cell = no movement (zeros and measures sample in same position) (Not available in Kinetics and Scanning) Manual 6 = cell changer moved by buttons (always zeros on position B, then returns to set position to start measurement) (Not available in Kinetics and Scanning) Auto 3 = turret auto moved - B, 2, 4 (always zeros on position B, then goes to position 2 to start measurement) (Not available in Kinetics and Scanning) Auto 6 = turret auto moved - B,1,2,3,4,5 (always zeros on position B, then goes to position 1 to start measurement)
Sample Volume	Enters the total volume of sample (under Dilution Multiplier in some Bio Tests)
Save Test	Saves all parameters of the current test in internal memory for later recall (all tests)
Scan speed	Selects the speed (nm/min) for a scan 4-8 Slow, Medium, Fast (Scanning tests only)
Screen Contrast	Lets you improve visibility of the display by changing the contrast between the background and text (Utility)

Parameter	Description
Select Test	Tags the highlighted test name with “>” to include the test in the SmartStart menu (Utility Stored Tests Directory)
Set Max. X	Sets the cursor position in the graph as the minimum X and the maximum X values for recalculating rate (Kinetics)
Set Min. X	
Set nms	Lets you enter and edit the wavelength and factor values
Set Options	Selects the factor entry or the baseline to calculate area under the peak in the graph (Scanning)
Setup correction	Initiates the procedure to collect the data necessary to correct for absorbance differences between cuvettes (all test types)
Slope	Enters ?Abs/?Concentration value (Standard Curve test type)
Smoothing	Turns data smoothing on and off (Scanning)
Software Revision	Displays the version of firmware in the instrument (Utility)
SRE tolerance	Acceptable minimum stray light
Standard Concentrations	Enters the concentration of standards used to generate the standard curve for the test
Standby	Selects the time since the last keystroke or instrument activity; powers down the unit to save lamp life (Utility)
Start wavelength	Enters the beginning wavelength for a scan (Scanning tests only)
Statistics	Turns stats on or off; if ON, calculates the average and Std Dev of results; Statistics registers are cleared when Statistics = OFF and/or when instrument is OFF, and/or when test parameters are changed, and/or when test is saved (or resaved) (in all test types except Kinetics, Scanning, Multiwavelength)
Std Concentration	Enters the concentration of the analyte in the standard solution
Stop wavelength	Enters the ending wavelength for a scan (Scanning tests only)
Stored Tests Directory	Displays the list of tests stored in the instrument (Utility)

Parameter	Description
Tabular	Displays the list of collected data (Kinetics, Scanning)
Test Name	Lets the user enter an alphanumeric name (maximum of 16 characters) for the test; the name will be included on the data printout and, if the test is saved, will be displayed in the Utility Test Directory screen (available in all tests)
Total Run Time	Enters the time from the Run initiation to the end of the test; equals Delay Time + \sum Interval Times + \sum Measurement Times (Kinetics)
Units	Selects or creates units labels for results (all stored tests except Abs Ratio, Scanning, Cell Growth)
Unselect Test	Removes the ">" tag of the highlighted test name to remove the test from the SmartStart menu (Utility Stored Tests Directory)
Wavelength	Enters values for the analytical wavelengths

Calculations for Software

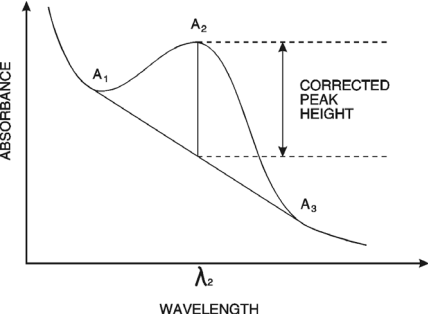
Calculation	Calculation(s)	Graphs
Standard Curves		
Partial sums	$SX = \sum x_i$ $SY = \sum y_i$ $SXX = \sum x_i^2$ $SYY = \sum y_i^2$ $SXY = \sum x_i y_i$ $SQX = \sum (x_i - \bar{x})^2 = N * SXX - SX^2$ $SQY = \sum (y_i - \bar{y})^2 = N * SYY - SY^2$ $SSXY = \sum (x_i - \bar{x})(y_i - \bar{y}) = N * SXY - SX * SY$ <p>Where: x_i = Concentration of i^{th} standard y_i = Absorbance of i^{th} standard N = number of standards</p>	
Linear regression (general case)	$A = A(c)$ <p>Where: A = absorbance c = concentration A(c) is defined by an equation of the form:</p> $A(c) = a_4 c^4 + a_3 c^3 + a_2 c^2 + a_1 c + a_0$ <p>Where: a_0 = Y-axis intercept $a_1 \dots a_4$ = coefficients</p> <p>(The coefficients are computed using the least squares method.)</p>	

Calculation	Calculation(s)	Graphs
Linear regression through zero	$A = a_1 \cdot (c)$ Where: A = absorbance c = concentration $a_1 = \text{slope}$ The slope is calculated as: $a_1 = SXY/SXX$ This model requires: <ul style="list-style-type: none"> • Slope is not equal to zero or infinity • At least one standard data point with concentration >0 • The absorbance of the 0 concentration blank = 0A 	
Segmented model	The segmented model requires: <ul style="list-style-type: none"> • Data for at least two standard data points with different concentrations and absorbances • Slopes of all segments must be ascending (positive) or descending (negative) 	
Validity of standard curves	$A(c_1) > A(c_2)$ for all $c_1 > c_2$ or $A(c_1) < A(c_2)$ for all $c_1 > c_2$ Where: A = absorbance $c_1, c_2 = \text{concentration}$	 <p>The graph shows a coordinate system with 'ABSORBANCE' on the vertical axis. A curve starts at the origin and increases monotonically. A horizontal dashed line labeled 'MEASURED ABSORBANCE' intersects the curve at a single point. A vertical dashed line from this intersection point to the horizontal axis is labeled 'CALCULATED CONCENTRATION'. A bracket on the vertical axis indicates the 'ABSORBANCE RANGE'. A shaded area at the top of the graph is labeled 'UNDEFINED CONCEN-'.</p>
	If this is not the case, there will be more than one solution within the specified domain, and the message “Curve cannot be used to determine sample concentrations – it may produce ambiguous results” will appear when the curve is viewed.	 <p>The graph shows a coordinate system with 'ABSORBANCE' on the vertical axis. The curve is non-monotonic, forming a loop. A horizontal dashed line labeled 'MEASURED ABSORBANCE' intersects the curve at two points. A vertical dashed line from the lower intersection point to the horizontal axis is shown, indicating ambiguity in the calculated concentration.</p>

Valid nonlinear standard curve

Invalid nonlinear standard curve

Calculation	Calculation(s)	Graphs
Statistics (Linear regression general case)	$\sigma = \sqrt{\frac{\sum (y_i - \bar{y}_i)^2}{N - n - 1}}$ <p>Where: N = Degree of polynomial</p> $r = \frac{ SSXY }{\sqrt{SQX * SQY}}$ <p>The calculation for the correlation coefficient applies only to first order linear regression curves (first degree polynomials).</p>	
Linear regression through zero model	$\sigma = \sqrt{\frac{SYY - (a_1 * SXY)}{N - 1}}$	
Absorbance ratio	$\frac{Abs\lambda_1}{Abs\lambda_2} \quad \text{or} \quad \frac{Abs\lambda_1 - Abs_{ref}}{Abs\lambda_2 - Abs_{ref}}$	
Absorbance Difference	<p>Result =</p> $Abs\lambda_1 * factor \quad 1 - Abs\lambda_2 * factor \quad 2$ <p>or</p> $(Abs\lambda_1 - Abs\lambda_{ref}) * factor \quad 1 - (Abs\lambda_2 - Abs\lambda_{ref}) * factor \quad 2$	

Calculation	Calculation(s)	Graphs
3-Point Net	Baseline corrected absorbance = $A_2 - \left(A_3 + \left([A_1 - A_2] * \frac{\lambda_3 - \lambda_2}{\lambda_3 - \lambda_1} \right) \right)$	 <p data-bbox="1120 640 1396 709">3-Point Net Absorbance sample curve</p>
3-Point Net (ASTM E169-04)	Baseline corrected absorbance = $A_1 - \left(A_3 + \left([A_2 - A_3] * \frac{\lambda_3 - \lambda_1}{\lambda_3 - \lambda_2} \right) \right)$	