
SPECTRAmax™ 250

Microplate Spectrophotometer

Operator's Manual



Molecular Devices Corporation
1311 Orleans Drive
Sunnyvale, California 94089

Part # 0112-0024

Rev. A



Molecular Devices Corporation

SPECTRAmax™ 250 Operator's Manual

Copyright

© Copyright 1994, Molecular Devices Corporation. All rights reserved. No part of this publication may be reproduced, transmitted, transcribed, stored in a retrieval system, or translated into any language or computer language, in any form or by any means, electronic, mechanical, magnetic, optical, chemical, manual, or otherwise, without the prior written permission of Molecular Devices Corporation, 1311 Orleans Drive, Sunnyvale, California, 94089, United States of America.

Patents

The SPECTRAmax 250 and methods are protected by the following patents:

U.S. Patents 4,958,148 and 5,112,134; Canadian Patent 1,305,334.

Other U.S. and International patents pending.

Trademarks

SPECTRAmax, SPECTRAplate, SPECTRAstrip, and AUTOMIX are trademarks and SOFTmax is a registered trademark of Molecular Devices Corporation.

Apple and Macintosh are registered trademarks of Apple Computer, Incorporated.

All other company and product names are trademarks or registered trademarks of their respective owners.

Disclaimer

Molecular Devices Corporation reserves the right to change its products and services at any time to incorporate technological developments. This manual is subject to change without notice.

Although this manual has been prepared with every precaution to ensure accuracy, Molecular Devices Corporation assumes no liability for any errors or omissions, nor for any damages resulting from the application or use of this information.

Molecular Devices Corporation Instrument Warranty

Molecular Devices Corporation warrants this product against defects in material or workmanship as follows:

- 1) All parts of the SPECTRAmax 250 Microplate Spectrophotometer are warranted for a period of one (1) year from the original date of delivery.
- 2) All labor charges to repair the product for a period of one (1) year from the original date of delivery will be paid by Molecular Devices Corporation.
- 3) This warranty covers the SPECTRAmax 250 instrument only, and does not extend to any computer, printer, software, reagents, or disposables used with the instrument.

Labor and Parts

To obtain warranty service during the applicable warranty period, you must take the product or deliver the product properly packaged in the original shipping materials and carton to an authorized Molecular Devices Corporation service facility. You must call or write to the nearest Molecular Devices Corporation service facility to schedule warranty service. You may call Molecular Devices Corporation at the telephone number or address below to locate the nearest service facility. You must schedule warranty service prior to bringing or shipping the product for servicing. At the time of requesting warranty service, you must present proof of purchase documentation which includes the date of purchase and Molecular Devices Corporation must have the enclosed Warranty Registration form completed, signed and returned by you within ten (10) working days of the date of delivery.

This warranty only covers defects arising under normal usage and does not cover malfunctions or failures resulting from misuse, abuse, neglect, alteration, modification, or repairs by other than an authorized Molecular Devices Corporation service facility.

Repair or replacement as provided under this warranty is the exclusive remedy of the purchaser (the "Buyer"). Molecular Devices Corporation (the "Seller") shall not be liable for any incidental or consequential damages for breach of any express or implied warranty on this product, except to the extent required by applicable law. The Seller specifically excludes all express and implied warranties including without limitation any implied warranty that the products sold under this agreement are merchantable or are fit for any particular purpose, except such warranties expressly identified as warranties and set forth in the Seller's current operating manual, catalog or written guarantee covering such product. The Seller also makes no warranty that the products sold under this agreement are delivered free of the rightful claim of any third party by way of patent infringement or the like. If the Buyer furnishes specifications to the Seller, the Buyer agrees to hold the Seller harmless against any claim which arises out of compliance with the specifications.

Any description of the products contained in this agreement is for the sole purpose of identifying them. Any such description is not part of the basis of the bargain, and does not constitute a warranty that the products shall conform to that description. Any sample or model used in connection with this agreement is for illustrative purposes only, is not part of the basis of the bargain, and is not to be construed as a warranty that the products will conform to the sample or model. No affirmation of fact or promise made by the Seller, whether or not in this agreement, shall constitute a warranty that the products will conform to the affirmation or the promise.

For the name of the nearest authorized Molecular Devices Corporation service facility please contact Molecular Devices at one of the following telephone numbers:

(408) 747-1700

(800) 635-5577 (U.S. and California)



Protected by US Patents 4,968,148 and 5,112,134

Protected by Canadian Patent 1,305,334

Other US and International patents pending



Contents

Figures	vii
Tables	vii
Conventions Used in this Manual	viii
Chapter 1	
Instrument Description	1-1
Introduction.....	1-3
Component Description.....	1-4
The Control Panel.....	1-4
Status Indicators.....	1-7
The Microplate Drawer.....	1-8
The Back Panel	1-9
Functional Description.....	1-10
Instrument Settings	1-10
Modes of Stand-Alone Operation.....	1-11
Wavelength Selection.....	1-13
Temperature Regulation	1-13
Auto Mix.....	1-14
Data Collection	1-14
Printed Data Output	1-15
Computer Control.....	1-15
Specifications.....	1-17
Chapter 2	
Installation.....	2-1
Installation Warnings.....	2-3
Installation Cautions	2-3
Unpacking	2-3
Setting Up for Stand-Alone Use.....	2-4
Chapter 3	
Stand-Alone Operation	3-1
Prepare for a Reading.....	3-3
Turn the Instrument and Printer On	3-3
Set the Temperature.....	3-3
Choose a Read Mode	3-3
Select the Wavelength(s)	3-5
Save/Recall Instrument Settings	3-5
Read the Microplate.....	3-6
Operation Overview.....	3-7



Chapter 4	Maintenance	4-1
	Technical Support	4-3
	Cleaning	4-3
	Installing Optional Optical Filters	4-4
	Entering Filter Wavelength Information	4-8
	Changing the Fuses	4-8
	Long-Term Shutdown	4-10
	Moving the SPECTRAmax 250	4-10
Chapter 5	Troubleshooting	5-1
	Error Codes and Probable Causes	5-3
	Warning Messages	5-5
	Opening the Drawer Manually	5-6
Appendix A	Printers and Cables	A-1
	Compatible Printers	A-3
	Cables	A-3
Appendix B	Accessories	B-1
	Items Available for Use with the SPECTRAmax 250	B-3
Index	I-1



Component Description

The main components of the SPECTRAmax 250 are:

- The control panel
- The microplate drawer
- The back panel (connections and power switch)

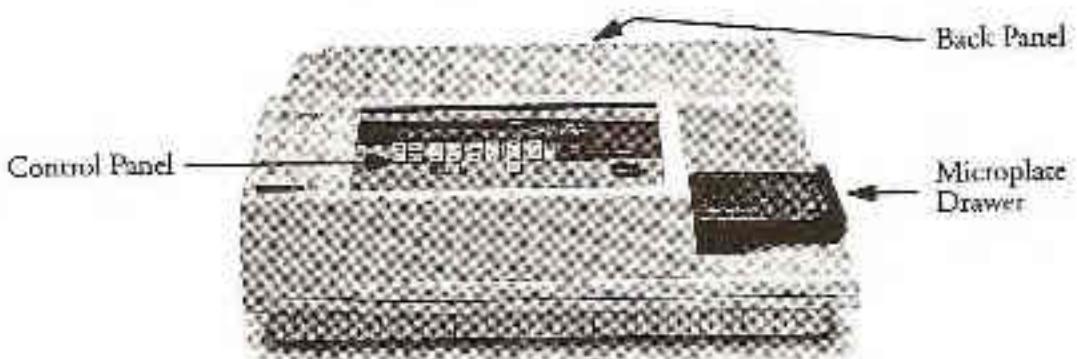


Figure 1.1: SPECTRAmax 250

The Control Panel

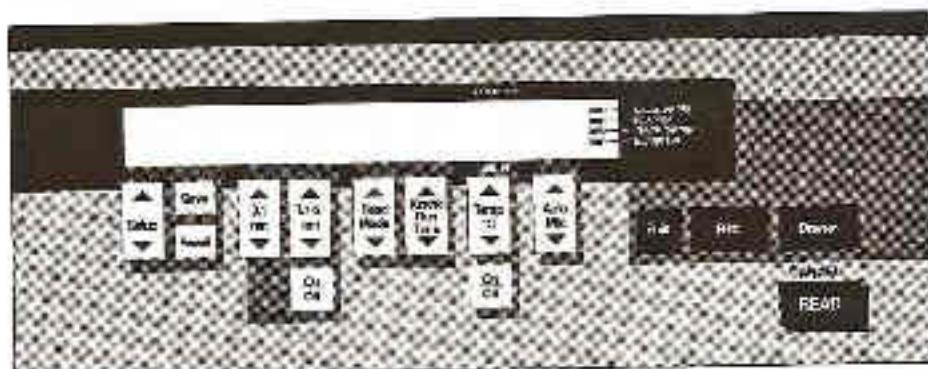


Figure 1.2: Control Panel

The control panel consists of an LCD and 15 pressure-sensitive membrane keys—all the controls necessary to perform stand-alone operation of the SPECTRAmax 250. The control panel is used to configure the instrument settings, store and recall assay protocols, and to initiate readings. When you press a control panel key, the SPECTRAmax 250 begins the desired action.

NOTE: When using Molecular Devices' SOFTmax PRO software to control the SPECTRAmax 250 from an external computer, the LCD will show "Remote Control" during the time SOFTmax PRO is running. During computer-controlled operation, the front control panel keys are disabled (except the [Drawer] key and the [Shift] and [Read] keys, which are used for the "Cancel" function). If you quit SOFTmax PRO, the LCD returns to normal and the instrument can be operated in stand-alone mode again.

LCD

A 2 × 40-character liquid crystal display which shows the current instrument settings. You can change the contrast of the display to appear darker or lighter as desired. Press and hold the [Shift] key and then press the up or down arrow on the [Auto Mix] key. Pressing the up (\blacktriangle) arrow makes the display lighter; the down (\blacktriangledown) arrow darkens the display.

Keys

Most stand-alone instrument functions can be performed by pressing a single key; a few others require that you press keys in combination. The functions of the control panel keys are described below.

**[Setup]**

Allows you to choose from a group of assay protocols that have been saved in non-volatile memory (stored by number from 0 through 9). The settings stored under "0" are factory preset defaults and cannot be modified or deleted. At the time of shipment, settings 1 through 9 also contain the same preset defaults.

NOTE: Non-volatile memory is retained even if the instrument is turned off.

**[Save]**

Saves the instrument settings you have chosen for the assay into memory under a specific number from 1 through 9 (0 is reserved for the default instrument protocol).

**[Recall]**

Recalls the instrument settings previously stored using the [Save] key.

**[λ 1 nm]**

Selects the *measurement* wavelength. Pressing this key scrolls up or down through the available wavelengths, starting at the previous setting. Pressing the up (\blacktriangle) or down (\blacktriangledown) arrow once increments or decrements the wavelength shown in the display by 1 nm; pressing and holding either arrow increments or decrements the wavelength shown in the display by 10 nm until it is released. If you increment the setting to the highest limit (750 nm) and continue pressing the up (\blacktriangle) arrow, the display returns to the lowest possible setting (250 nm) and begins incrementing from there. The inverse is true for decrementing by pressing the down (\blacktriangledown) arrow.

**[λ 1-2 nm]**

Selects the *reference* wavelength. This key will activate dual wavelength (if it was off) when pressed. When dual wavelength mode is selected (by pressing the [On/Off] key below this one), pressing this key scrolls up or down through the available wavelengths, starting from the previous setting. Pressing the up (\blacktriangle) or down (\blacktriangledown) arrow once increments or decrements the wavelength shown in the display by 1 nm; pressing and holding either arrow increments or decrements the wavelength shown in the display by 10 nm until it is released. If you increment the setting to the highest limit (750 nm) and continue pressing the up (\blacktriangle) arrow,



the display returns to the lowest possible setting (250 nm) and begins incrementing from there. The inverse is true for decrementing by pressing the down (▼) arrow.



[On/Off] (λ , 1-2 nm)

Enables/disables dual wavelength mode.



[Read Mode]

Selects the read mode by scrolling downward through the listed options. Choices are Blank, Endpoint, and Kinetic.



[Kinetic Run Time]

Allows you to choose the duration for a Kinetic run. (Kinetic read mode must have been chosen first for this key to be active.) Choices are 1, 2, 5, 10, and 20 minutes.



[Temp °C] (Incubator)

Allows you to enter a set point at which to regulate the microplate chamber temperature. Pressing this key scrolls up or down, starting at the previous temperature setting (or the default of 37.0°C, if no setting had been made). Pressing the up (▲) or down (▼) arrow once increments or decrements the temperature shown in the display by 0.1°C; pressing and holding either arrow increments or decrements the temperature shown in the display by 1°C until it is released. If you increment the setting to the highest limit (45°C) and continue to press the up (▲) arrow, the display will not change. If you decrement the setting to the lowest limit, 15°C, and continue to press the down (▼) arrow, the display will not change.

NOTE: The temperature set point must be at least 4°C above ambient. The ambient temperature must be greater than 20°C to achieve temperature regulation of 45°C.

CAUTION: If the incubator is disabled, pressing the [Temp °C] key will enable the incubator.



[On/Off] (Incubator)

Enables/disables the incubator function.



[Auto Mix]

Depending on the mode chosen, pressing this key selects automatic shaking of the microplate for a preset duration at one or more points before and/or during the read cycle. Choices are On, Once, and Off.



[Shift]

Activates secondary functions by first pressing and holding the [Shift] key followed by pressing the secondary key. Labels for secondary functions are printed in blue on the control panel.

Secondary Functions

- CANCEL—Stops the reading in progress. CANCEL is invoked by pressing the



[Shift/READ] key combination. CANCEL remains active when the instrument is in remote control mode.

- Filter Wavelength—This function allows you to select the wavelength corresponding to an optional specialty filter if one has been installed in the SPECTRAmax 250. Filters can be selected for the measurement (λ 1 nm) and/or the reference (λ 1-2 nm) wavelength. This function is invoked by pressing the [Shift] and [λ 1 nm] or the [Shift] and [λ 1-2 nm] key combination.

NOTE: Optional filters are available with 1-nm bandwidths. A narrow bandwidth filter may be required for some assays (the monochromator has a 5-nm bandwidth). For information regarding entering the filter wavelength information, refer to Chapter 4, "Maintenance."

- Display Contrast—To darken or lighten the display, press and hold the [Shift] key and then press either the up (\blacktriangle) or the down (∇) arrow on the [Auto MIX] key—the up (\blacktriangle) arrow lightens the display; the down (∇) arrow darkens it.

Print

Sends the data from the most recent reading to the printer (if it is connected directly to the SPECTRAmax 250).

Drawer

Opens or closes the microplate drawer. Whether or not the drawer will remain open depends on the incubator setting. If the incubator is off, the drawer will remain open; if the incubator is on, the drawer will close after approximately 10 seconds to assist in maintaining temperature control within the microplate chamber.

READ

Pressing this key causes the microplate drawer to close automatically, if it was open, after which the selected assay read mode begins.

Status Indicators

At the far right of the LCD are indicators as shown in Figure 1.3. These indicators will be illuminated when the SPECTRAmax 250 is performing certain actions as described below.



Figure 1.3: LCD Indicators

CALIBRATING Illuminated during the automatic calibration cycle before the instrument reads the microplate.

READING Illuminated while the instrument is reading a microplate.



TEMPERATURE This indicator flashes when the incubator is turned on and the set point has not yet been reached; it is illuminated continuously (no longer flashing) when the set temperature has been reached ($\pm 0.3^{\circ}\text{C}$).

BLANK ON Illuminated when a BLANK pattern is active.

The Microplate Drawer

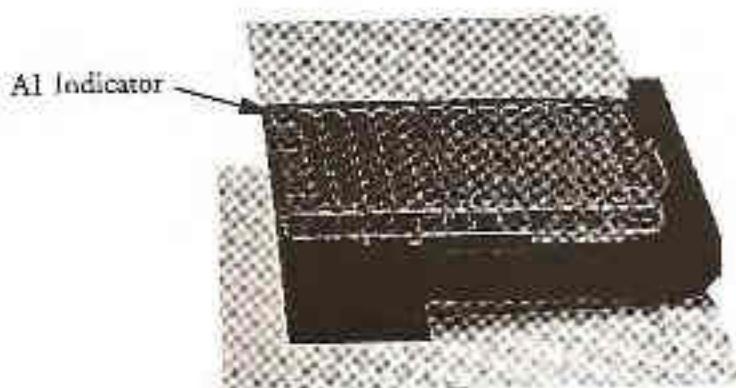


Figure 1.4: Microplate Drawer

The microplate drawer, located on the right side of the SPECTRAmax 250, holds microplates and blanking templates. The drawer slides in and out of the microplate chamber. Springs on two sides of the drawer automatically position and hold a microplate in the proper position. The drawer remains in the reading chamber during read cycles.

Microplate drawer operation varies, depending upon the incubator status. When the incubator is off, the microplate drawer remains open at power up and after a read. When the incubator is on, the drawer closes automatically to assist in controlling the temperature of the microplate chamber. To open the drawer, press the **[Drawer]** key. The drawer will remain open for approximately ten seconds, after which a beeping sound will alert you approximately two seconds before the drawer closes automatically.

NOTE: Do not obstruct the movement of the drawer. If you must retrieve a plate after an error condition or power outage and the drawer will not open, it is possible to open it manually (see Chapter 5, "Troubleshooting").

Microplates

The SPECTRAmax 250 can accommodate standard 96-well microplates, strip wells, and filter-bottom microplates. When using wavelengths below 340 nm, special UV-transparent, disposable or quartz microplates (SPECTRAPlate) allowing transmission of the deep UV spectra are available from Molecular Devices.

Not all manufacturers' microplates are the same with regard to design, materials, or configuration. Temperature uniformity within the microplate may vary depending on the type of microplate used.

Templates

Blank pattern templates supplied with the SPECTRAmax 250 can be used in stand-alone mode to select a blank set of wells. More information regarding the use of templates and creating blank patterns can be found Chapter 3, "Stand-Alone Operation."

The Back Panel

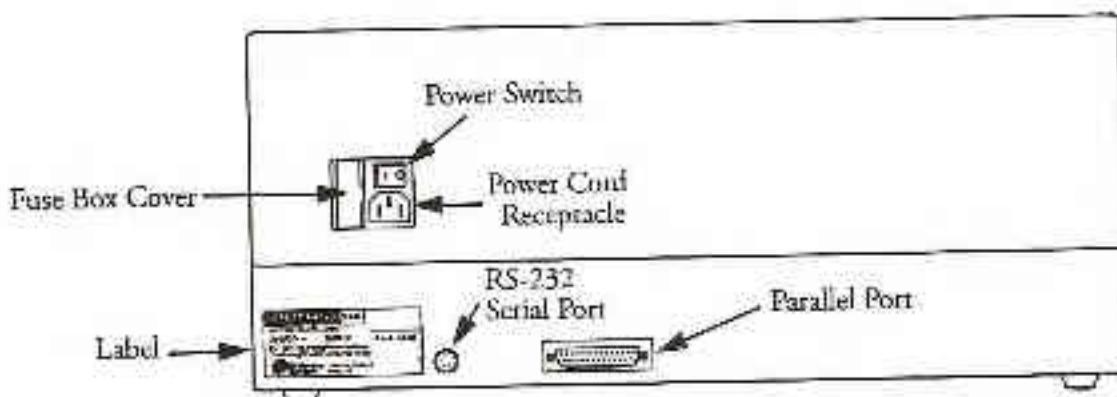


Figure 1.5: Components on the Back Panel of the SPECTRAmax 250

The following components are located on the back panel of the SPECTRAmax 250:

- **Power switch**—a rocker switch, labeled I/O (for on and off, respectively).
- **Power cord receptacle**—plug the power cord in here.
- **Fuse box cover**—cannot be opened while the power cord is plugged in. When opened, it provides access to the fuse box containing two fuses that are required for operation.
- **Printer port** (double-shielded, 25-pin parallel, for use in stand-alone operation)—plug the 25-pin end of the cable into this port; the other (Centronics) end attaches to a port on the printer.
- **Computer port** (double-shielded RS-232, for use with an external computer)—plug one end of an 8-pin DIN serial cable into this port; the other end attaches to the serial (modem) port of the computer.
- **Label**—provides information about the SPECTRAmax 250, such as line voltage rating, cautionary information, serial number, etc. Record the serial number shown on this label for use when contacting Molecular Devices Technical Services.



Functional Description

Instrument Settings

Up to nine user-definable assay protocols can be saved in non-volatile memory for future use. User-defined protocols are saved by number (1-9); the protocol labeled "0" contains default parameters, set at the factory, and cannot be altered. User-defined protocols can contain the following instrument settings:

- Read mode
- Wavelength(s) (up to two)
- Auto Mix state
- Blank pattern
- Temperature set point

Assay protocol settings that you save under numbers 1 through 9 are retained by the SPECTRAmax 250 in non-volatile memory—they are retained even when power to the instrument is turned off. At power up, the SPECTRAmax 250 always reverts to the default protocol 0, but the other saved settings are available.

Saving a Protocol

To store a protocol in memory, first define the instrument settings for the protocol by setting the parameters as desired. Then press the up or down arrows on the **[Setup]** key until the desired number (under which to save the protocol) is displayed. Then press the **[Save]** key to save the current protocol in non-volatile memory under that number.

NOTE: If any settings were already saved under that number, they will be overwritten by this process. Before choosing a number under which to save parameters, ensure first that it does not contain data you wish to retain.

Recalling a Saved Protocol

To recall a protocol that you saved previously, press the up or down arrows on the **[Setup]** key until the number of the saved protocol (from 1 to 9) appears in the display and then press **[Recall]**.

To restore the factory-default protocol and overwrite any settings in present memory, press **[Setup]**, choose "0," and then press **[Recall]**. To replace the settings for any protocol stored under numbers 1 through 9 with these factory default settings, use **[Setup]** to select the desired protocol number and then press **[Save]**.

Modes of Stand-Alone Operation

When operating the SPECTRAmax 250 as a stand-alone system, you can obtain readings using either Endpoint or Kinetic mode.

Endpoint

If you wish to obtain a single set of optical density (OD) readings for each well of a microplate, select either single- or dual-wavelength operation in Endpoint mode. OD data is printed in an 8- \times -12 microplate format.

When the instrument is set to Endpoint mode, the nine-second read cycle (for each wavelength selected) is automatically preceded by a calibration cycle requiring less than one second.

Dual wavelength readings are taken at a measurement wavelength (λ_1) as well as a reference wavelength (λ_2)—you may choose both settings. The difference between these readings ($\lambda_1-\lambda_2$) is displayed for each well.

If the Auto Mix function is selected for Endpoint readings, the plate is shaken for five seconds prior to the reading.

Kinetic

Kinetic analysis at a single wavelength (λ_1) can be performed for several pre-defined total reading times (1, 2, 5, 10, and 20 minutes) with preset read intervals. At the end of a reading, rates are reported as mOD/min for each well in an 8- \times -12 microplate format.

Kinetic analysis has many advantages when determining the relative activity of an enzyme in different types of microplate assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis is capable of providing improved dynamic range, precision, and sensitivity relative to Endpoint analysis.

In Kinetic mode, a calibration cycle requiring less than one second automatically precedes the first read cycle. During the Kinetic reading, the microplate remains in the isothermal microplate chamber. The interval between read cycles is determined by the instrument based on the Kinetic run time and the Auto Mix status.

If Auto Mix is selected for Kinetic analysis, the microplate is shaken for five seconds prior to the initial reading. Thereafter the microplate is shaken for three seconds before each read cycle. This ensures that the color developing in each well is uniformly distributed throughout the well prior to each reading. The Auto Mix function is strongly recommended for ELISAs and other solid-phase, enzyme-mediated reactions.

The SPECTRAmax 250 calculates the rate of reaction for each well, for either positive or negative Kinetic rates, using the first reading as the starting OD baseline value for each well and a limited OD excursion of 0.2 OD. The on-board, microprocessor-based, linear regression package reports the slope value for the line fit to each Kinetic plot as the Kinetic rate in mOD/min.



Kinetic Run Time

The total run time for a Kinetic reading is set using the up and down arrows on the [Kinetic Run Time] key. Choices are 1, 2, 5, 10, and 20 minutes. Table 1.1 shows the intervals (in seconds) and total number of readings that occur with each total run time setting, with and without Automix enabled.

Table 1.1: Intervals and Number of Readings for Total Kinetic Run Time

Kinetic Run Time (minutes)	No Mixing		Mixing	
	Interval (seconds)	Number of Plate Reads	Interval (seconds)	Number of Plate Reads
1	9	7	14	4
2	9	14	14	8
5	16	25	19	18
10	30	35	30	28
20	60	45	60	38

Blank Pattern

Selecting BLANK by pressing the arrows on the [Read Mode] key allows you to instruct the instrument regarding which wells should be treated as "blanks" or, taken together, as a blank pattern.

The active presence of a blank pattern is shown by the BLANK ON indicator on the LCD. The SPECTRAmax 250 retains blank pattern information in non-volatile memory; values are recalculated for each subsequent reading. A blank pattern may be cleared by setting the active protocol to the factory presets or by reading an empty drawer in the BLANK mode.

Each time a microplate is read, the average OD or milli-OD/minute reading of the wells in the current blank pattern will be computed. This mean value is then subtracted from all the readings, including those of the individual members of the blank pattern. For wells which are members of the blank pattern, the character "#" will replace the decimal point in printouts. This feature allows subtraction of values of substrate blanks or other special calibrations.

Blank pattern information is provided to the SPECTRAmax 250 by the use of a blank pattern template. (More information regarding the use of templates can be found in Chapter 3, "Stand-Alone Operation.") After reading the template, the instrument will print the well locations designated as members of the blank pattern.

Wavelength Selection

In Endpoint mode, you can select either single- or dual-wavelength mode during stand-alone operation of the SPECTRAmax 250. Dual wavelength mode is used when you wish data from both measurement and reference wavelengths to be acquired. For Kinetic readings, only single-wavelength mode is available.

Typically, you should select a measurement wavelength that is near the wavelength of maximum absorption (λ_{max}) for the chromophore/macromolecule of interest. The reference wavelength (if any) is usually set to a wavelength at which the chromophore/macromolecule shows relatively little absorption. The dual wavelength feature increases Endpoint accuracy—errors arising from optical imperfections, such as scratches and plastic irregularities in the microplate, can be effectively canceled out.

The display on the control panel shows the currently selected measurement (λ_1) and reference (λ_2) wavelength (if any). You can change the wavelength(s) by pressing the arrows on either wavelength selection key [$\lambda 1 \text{ nm}$] or [$\lambda 1-2 \text{ nm}$]) until the display shows the desired wavelength. The wavelength list will wrap around to the top when the last selectable wavelength is presented.

NOTE: If the measurement and reference wavelengths are the same, the instrument will beep and deselect dual wavelength.

Temperature Regulation

The SPECTRAmax 250 has been designed to regulate the temperature of the microplate chamber from 4°C above ambient to 45°C. Upon power up, when the incubator is off, the temperature in the SPECTRAmax 250 microplate chamber is ambient and isothermal. Pressing the incubator [On/Off] key below the [Temp °C] (incubator) key will cause the SPECTRAmax to begin warming the microplate chamber. The temperature set point defaults to 37.0°C at start-up. With the incubator on, the temperature of the microplate chamber can be set and regulated from 4°C above ambient to 45°C.

NOTE: Accuracy of the temperature set point is only guaranteed if the set point is at least 4°C above ambient. If the temperature set point is lower than the ambient temperature, the chamber temperature will remain at ambient. Temperature regulation is controlled by heaters only and, therefore, cannot cool the temperature to a setting lower than ambient. Additionally, the highest setting (45°C) can be achieved only if the ambient temperature is >20°C.

You can change the temperature set point by pressing the up (Δ) or the down (∇) arrow on the [Temp °C] (incubator) key until the desired set point is shown above the key in the display.

After activating the incubator, the TEMPERATURE indicator located at the right of the LCD will begin to flash and will continue flashing until the temperature within the microplate chamber reaches the set point ($\pm 0.3^\circ\text{C}$) when it will remain illuminated. Typically, the microplate chamber will reach 37.0°C in less



than 15 minutes. The indicator also flashes as a warning if the temperature within the microplate chamber deviates more than $\pm 0.3^{\circ}\text{C}$ from the set point.

The microplate chamber temperature is maintained at the set point until you press the incubator [On/Off] key again, turning temperature regulation off. The LCD indicator will go out, the drawer will open, and the temperature within the microplate chamber will begin returning to ambient.

NOTE: Should you turn the incubator back on after a momentary shutdown, allow about ten minutes for the control algorithm to fully stabilize the microplate chamber temperature.

Temperature regulation and control of the microplate chamber is achieved through electric heaters, a fan, efficient insulation, and temperature sensors. The heaters are located in the microplate chamber which is insulated to maintain the temperature set point. The sensors are mounted inside the chamber and measure the air temperature.

The temperature feedback closed-loop control algorithms measure the chamber air temperature, compares it to the temperature set point, and use the difference to calculate the heating cycles. This technique results in accurate, precise control of the microplate chamber temperature with a temperature variation of the air across the entire microplate of less than 0.3°C . (Temperature uniformity within the microplate itself will depend upon its design, materials, and/or configuration.)

Auto Mix

The Auto Mix function permits automatic shaking of the microplate at preset intervals, thereby mixing of the contents within each well. Auto Mix must be selected before beginning a reading.

Selectable Auto Mix settings are On, Once, or Off. The actions associated with these settings depend on the read mode chosen.

For Endpoint mode, setting Auto Mix to On or Once will shake the plate for five seconds and then read at all selected wavelengths.

When Kinetic mode is chosen, setting Auto Mix to On will shake the plate for five seconds before the initial reading and for three seconds before each subsequent reading. Setting Auto Mix to Once will shake the plate for five seconds only before the first reading, with no mixing between Kinetic readings.

When Auto Mix is enabled, either "On" or "Once" will be displayed above the [Auto MIX] key.

NOTE: Use of Auto Mix is strongly recommended for ELISAs and other solid-phase, enzyme-mediated reactions to enhance accuracy.

Data Collection

The SPECTRAmax 250 stores only the most recent Endpoint or Kinetic plate reading in a buffer memory.

CAUTION: Data in the buffer memory is lost when power to the SPECTRAmax 250 is turned off. This applies even to short power outages. Do not turn the instrument off while important data remains in the buffer memory.

Printed Data Output

During stand-alone operation, results are automatically printed as soon as a plate has been read. A new microplate can be loaded into the SPECTRAmax 250 while the results from the last reading are being printed.

NOTE: If you have performed a blank reading, the blank values will be subtracted from raw OD values, and the calculated result will be shown on the printout.

Computer Control

The SPECTRAmax 250 is equipped with an 8-pin DIN RS-232 serial port through which a computer can communicate with and control the instrument.

SOF^Tmax PRO[®]

Molecular Devices' SOF^Tmax PRO software is a highly integrated program that can be used to control and collect data from the SPECTRAmax 250. SOF^Tmax PRO is easy to use, yet is powerful and flexible, and expands the capabilities of the SPECTRAmax 250.

SOF^Tmax PRO allows you to:

- Expand the available read modes
 - Use up to six wavelengths for Endpoint and Kinetic readings
 - Perform Spectrum readings in the 250- to 750-nm range
 - Extend Kinetic run times up to 99 hours
 - Select your own read intervals for Kinetic runs
 - Specify the duration for Auto Mix before and between readings
 - Read a subset of microplate strips
- Design a microplate template to simplify data reduction
 - Identify groups of wells with labels of your choice
 - Identify individual wells with unique names
 - Blank individual wells
- Save instrument settings, template formats, and data analysis parameters as assay protocol files and recall them for later use
 - Rapid instrument and analysis set up for repeated microplate assays
 - Uniform analysis for equivalent microplates



- Acquire data from the SPECTRAmax 250
 - Save data files for in-depth analysis at a later time
 - Save multiple microplates with individual template and data analysis parameters in one data file
 - Pre-read microplates
 - Analyze Kinetic and Spectrum data as it is collected
- Display data on screen
 - Raw data is displayed in a microplate format
 - Ranged display presents the data as integers between 0 and 9 in a microplate format
 - Threshold display presents the data as being above, below, or between set limits in a microplate format
 - Gray scale display presents the data in seven shades of gray corresponding to high and low limits in a microplate format
 - Kinetic or Spectrum plots of all 96 microplate wells
 - Enlarge the display of individual well plots and overlay multiple well plots
- Perform data analysis using SOFTmax PRO features
 - Calculate maximum Kinetic rates on non-linear data
 - Assign plate, group, or sample blanks
 - Customize data analysis for each group in the template
 - Create graphs with multiple plots
 - Pick from five standard curve-fitting routines
 - Analyze unknown samples against a standard curve
- Multiple print formats
 - Print all or individual sections of the data file
 - Define and print a report containing only selected sections
 - Customize the order of data file sections
- Export data in tab-delimited ASCII format for use with Excel or other database programs

For a complete description of the features of SOFTmax PRO, refer to the *SOFTmax PRO User's Manual*.

Specifications

Thermal specifications for the SPECTRAmax 250 apply to flat-bottom microplates with isolated wells. All other specifications apply to standard 96-well polystyrene flat-bottom microplates.

NOTE: Technical specifications are subject to change without notice.

Photometric Performance

Wavelength range 250–750 nm

Wavelength selection Monochromator tunable in 1-nm increments

Wavelength bandwidth 5 nm

Wavelength accuracy <± 2.0 nm, referenced to Hoya V30 Didymium Multiband Calibration Filter

Wavelength repeatability <± 0.2 nm

OD indication range 0.000 to 4.000 OD

OD resolution 0.001 OD

OD accuracy (linearity) 0–2.5 OD: 250–750 nm <± 1.0% and ± 0.010 OD
2.5–3.0 OD: 250–750 nm <± 3.0% and ± 0.010 OD

OD precision (repeatability) 0–2.5 OD: 250–750 nm <± 1.0% and ± 0.005 OD
2.5–3.0 OD: 250–750 nm <± 3.0% and ± 0.005 OD

Photometric stabilization Instantaneous

Photometric drift None—continuous referencing of monochromatic output

Calibration Automatic before first Kinetic read and before every Endpoint reading

Optical alignment None required during lifetime of instrument

Light source Xenon flash lamp (10 watts maximum)

Average lamp lifetime > 5 years (1×10^6 plate readings)

Illumination Top-down

Interference filter capacity Two 1-nm narrow-bandwidth interference filters (optional for special applications)

Stray light control • Single-well sequential illumination
• Lenses above and below microplate
• Light-tight reading chamber

Photodetectors Silicon photodiode



Photometric Analysis Modes

- Single wavelength, optical density
- Multiple wavelength (λ_1 - λ_2 in stand-alone mode; up to six using SOFTmax PRO) optical density
- Kinetic; Kinetic graphics using SOFTmax PRO
- Spectral swoop using SOFTmax PRO (250–750 nm)

Measurement Time

Read time (Endpoint)	<ul style="list-style-type: none"> • 96 wells in 9 seconds (single wavelength) • 96 wells in 11* N seconds (N wavelengths)
Kinetic read intervals	<ul style="list-style-type: none"> • 96 wells, 9-second minimum interval between readings • 1 column, 2-second minimum interval between readings • M columns, 1 second * (M columns, M≥2) • Multiple wavelength, 9 seconds * (N wavelengths)
Calibration time	< 1 second per wavelength
Wavelength selection	< 2 seconds

Temperature regulation

Reading chamber	Isothermal when temperature regulation is not enabled; <1°C
Range	(Ambient + 4°C) to 45°C
Resolution	±0.1°C
Accuracy	±1.0°C
Well-to-well uniformity at equilibrium	±0.5°C
Chamber warm-up time	30 minutes (measured on air)
Temperature regulation	3 sensors
Variation	<0.3°C (regulated)
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling
Recommended microplate	Flat-bottom microplates with isolated wells and lid

Plate Mixing

Plate mixing modes	Selectable: off, once prior to any reading, and once prior to and between Kinetic readings
Plate mixing duration	Selectable: 1 to 999 seconds (three-second default) using external software

General Instrument

Display	2×40-character backlit LCD with adjustable contrast
Operating panel	15-key (plus [Shift] key functions) membrane keypad
Memory back-up	Stored protocols (nine maximum) and instrument calibration parameters
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism/reading chamber assembly is protected from accidental spillage by drainage ports
Calculated mean time between failures (MTBF)	> 20,000 hours
Data buffer	Memory downloading of data buffer (100-plate maximum)
Computer interface	8-pin DIN RS-232 serial (double shielding required)
Printer interface	Parallel 25-pin to Centronics (double shielding required)
Microplates supported	96-well and strip-well microplates including lids

Environmental

Operating temperature	15 to 40°C
Operating humidity	0 to 85%, non-condensing
Storage temperature	-20 to 65°C

Physical

Size (h × w × d)	8.6 in. (22 cm) × 22.8 in. (58 cm) × 15 in. (38 cm)
Weight	29 lb (13.2 kg)
Power consumption	< 250 watts
Line voltage	100–240 VAC, auto-ranging
Line frequency	50/60 Hz