
**SmartSpec™ Plus
Spectrophotometer
Instruction Manual**

**Catalog Number
170-2525**



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For technical service call your local Bio-Rad office. In the U.S., call 1-800-4BIORAD (1-800-424-6723).

Warranty



The SmartSpec Plus Spectrophotometer and accessories are warranted against defects in materials and workmanship for one year. If any defects occur in the instruments or accessories during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than Bio-Rad Laboratories.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent[†] or sample.



For any inquiry or request for repair service, contact Bio-Rad Laboratories. Inform Bio-Rad of the model and serial number of your instrument.

Important: This Bio-Rad instrument is designed and certified to meet EN61010* safety standards. Certified products are safe to use when operated in accordance with the instrument manual. This instrument should not be modified or altered in any way.

Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the EN61010 safety certification
- Create a potential safety hazard

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent.

[†] SmartSpec Plus Spectrophotometer is not compatible with chlorinated hydrocarbons (*e.g.*, chloroform), aromatic hydrocarbons (*e.g.*, toluene, benzene) or acetone. Use of organic solvents voids all warranties.

* EN61010 is an internationally accepted electrical safety standard for laboratory instruments.

Section 1 General Safety Information

This instrument is intended for laboratory use only.

This product conforms to the "Class A" standards for electromagnetic emissions intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as those applications. The user should be aware of this potential and take appropriate measures to avoid interference.

1.1 Caution Symbol



Read the manual before using the SmartSpec Plus Spectrophotometer. For technical assistance, contact your local Bio-Rad office or, in the U.S., call technical services at 1-800-4BIORAD (1-800-424-6723).

Definition of Symbols



Caution (refer to accompanying documents)

1.2 Precautions

1. Do not use near flammable materials.
2. Always inspect the SmartSpec Plus for damaged components before use.
3. Always connect the system to the correct AC power source.
4. Always connect the correct instrument (printer or computer only) via the serial port connector.
5. Do not pour liquid into the sample chamber. Thorough clean-up is needed after each spill (Section 9.1).
6. Do not place objects on the SmartSpec Plus.
7. The SmartSpec Plus has no customer-serviceable components, **do not open the unit.**

Section 2 Packing List

The SmartSpec Plus Spectrophotometer (catalog number 170-2525) is shipped with the following components. Upon receiving your instrument, please check that all items listed below were shipped. If any items are missing or damaged, contact your local Bio-Rad office.

| Item | Quantity |
|--|----------|
| Instruction manual | 1 |
| Warranty card (please complete and return) | 1 |
| SmartSpec Plus Spectrophotometer | 1 |
| Power cable | 1 |
| Cuvette spring | 1 |
| Thermal paper roll | 1 |

Section 3 Installation

3.1 Environmental Requirements

To ensure correct operation and stable performance over an extended period of time, install the SmartSpec Plus in a location which meets the following conditions:

- Room temperature between 15 and 35°C. Not recommended for cold room use (4°C).
- Not exposed to direct sun light.
- Not subject to direct or continuous vibration.
- Not subject to intense magnetic or electromagnetic fields.
- Relative humidity between 0–95%.
- Area free from corrosive gases or other corrosive substances.
- Area with very little dust or other airborne particles.
- Allow a 10 cm minimum space around the instrument for proper air flow.

3.2 Power On and Initial Display

1. **Power up SmartSpec.** When you turn on SmartSpec Plus, it will go through a self-diagnostic routine during which it tests the lamp, optical components, diode array, powersupply and other components. If a problem is detected, an error message will be displayed and you should call Bio-Rad Technical Support. If boot up is successful and no errors are detected, the Home screens will be displayed.

| |
|-------------------------------|
| CHOOSE ANY SMARTSPEC ASSAY |
|-------------------------------|

alternating with

| | |
|-------------|--------------|
| 04:23:11 PM | SmartSpec |
| Fri | Apr 23, 1999 |

Section 4 Introduction

4.1 Identification of System Components

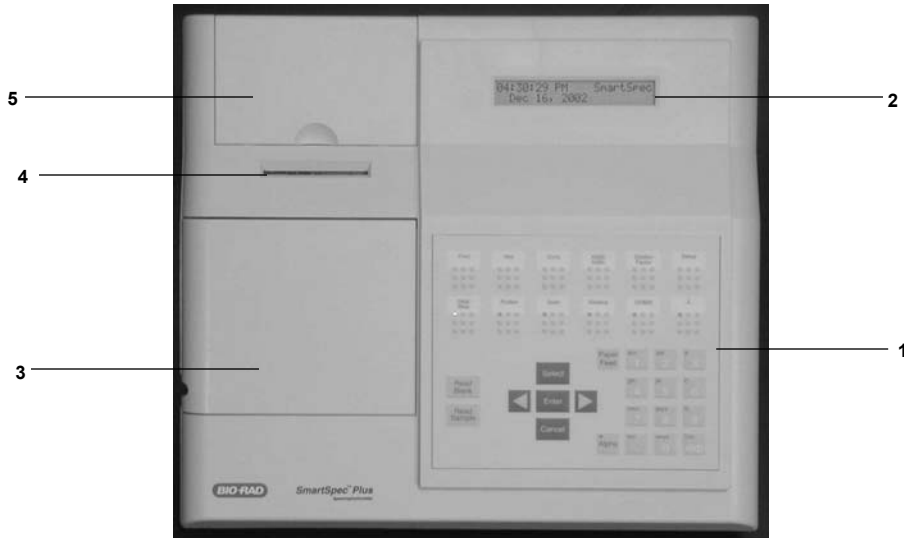


Fig. 1. View of the SmartSpec Plus Spectrophotometer.

Instrument

- | | |
|-----------------------------------|--|
| 1. Keypad interface | Change menu and select options by pressing these buttons. Press a single button to perform action described on the menu display. |
| 2. Display | 2 x 24 alphanumeric backlit LCD display. Displays various menus and options available. |
| 3. Sample compartment door | Lift door to insert cuvette into the cuvette chamber. |
| 4. Integrated thermal printer | Built-in alphanumeric/graphic printer. Press print button to record results immediately. |
| 5. Printer paper compartment door | Remove door to install new roll of printer paper |



Fig. 2. Rear view of the SmartSpec Plus Spectrophotometer.

- | | |
|---|---|
| 6. Fan Guard | Exhaust air leaves cabinet through this opening. |
| 7. Universal Power Input Module and ON/OFF Switch | Appropriate power cord is connected to the power input module. Instrument power switch. |
| 8. Serial Output Port | RS-232 connection to serial printer or computer. |

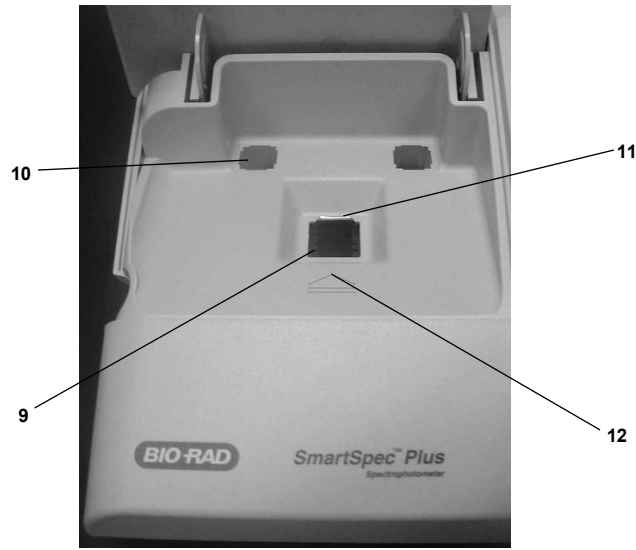


Fig. 3. View of the SmartSpec Plus sample compartment.

- | | |
|---------------------------------|--|
| 9. Cuvette chamber | Insert cuvette into the cuvette chamber. Ensure that the cuvette is placed in the proper orientation with respect to the light path. |
| 10. Cuvette storage compartment | Storage space for cuvette. |
| 11. Cuvette spring | Aligns cuvette. Accommodates various cuvette configurations. |
| 12. Light path | Light path axis. |

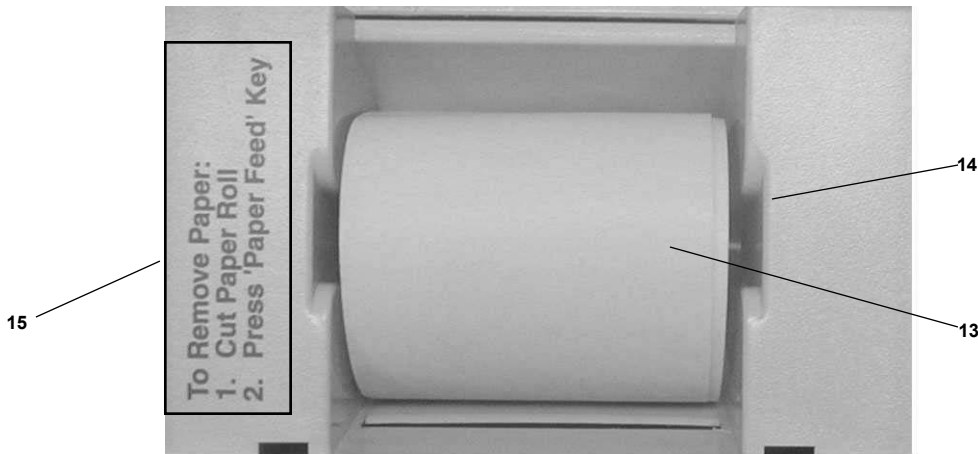


Fig. 4. View of the SmartSpec Plus integrated thermal printer paper with door removed.

- | |
|--------------------------------|
| 13. Thermal printer paper |
| 14. Printer paper shaft |
| 15. Paper removal instructions |

UV/Visible Spectroscopy is based on the absorption of light as a function of wavelength. All spectrophotometers have a light source that generates light of specific wavelengths. The SmartSpec Plus uses a xenon flash lamp as its light source. Xenon flash lamps have several advantages over the conventional light sources in that they reduce warm up time, do not heat up the instrument and have a lamp life of 10 to 15 years. Additionally, xenon flash lamps emit both UV and visible wavelengths, in contrast to deuterium lamps which emit only UV wavelengths or tungsten lamps which primarily emit visible wavelengths. In the SmartSpec Plus, the xenon flash lamp pulses light only when measuring absorbance of a sample.

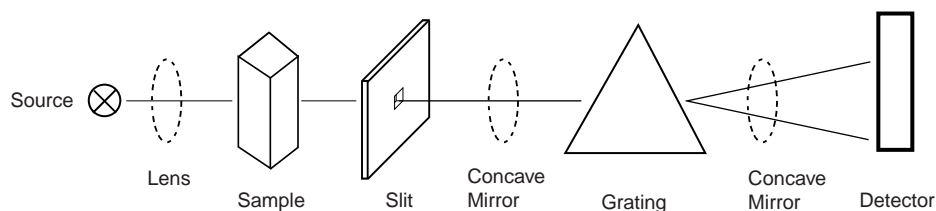
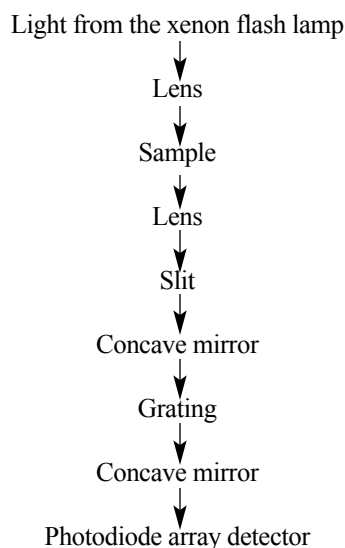


Fig. 5. A symbolic representation of the SmartSpec Plus optics module.

The path of light in the SmartSpec Plus is as follows:



The advantages of using a grating as a dispersion device are that:

1. They eliminate nonlinear dispersion.
2. Are not temperature sensitive.

Photodiode arrays have several advantages:

1. They acquire spectral data simultaneously without mechanically moving parts;
2. Have excellent wavelength reproducibility and,
3. A wide dynamic range.

It is also important to note here that in an array, several photodiode detectors are arranged on a silicon crystal. The added benefit of an array is the ability to do side-by-side readings, thus increasing speed.

The Lambert-Beer's law identifies the relation between sample concentration and the intensity of transmitted light. Absorbances and % transmittances are defined by the following equations:

% Transmittance (%T)

$$\%T = I_t/I_0 * 100 = 10^{-\epsilon cl} * 100$$

Absorbance or optical density (A or O.D.)

$$A = \log_{10} 1/T = \log_{10} I_0/I_t = \log_{10} 10^{\epsilon cl} = \epsilon cl$$

- Where I_0 = Intensity of incident light
 I_t = Intensity of transmitted light
 ϵ = Molar extinction coefficient (liter/mole/cm)
 c = Sample concentration (mol/liter)
 l = Optical pathlength

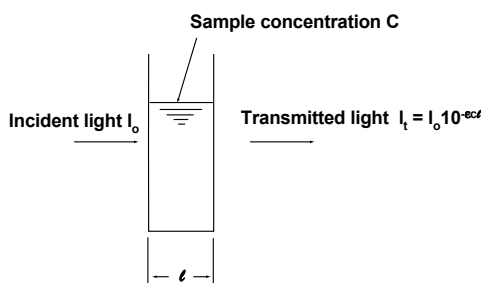


Fig. 6. Lambert-Beer's law.

$$A = \log 1/T$$

$$A = \epsilon cl$$

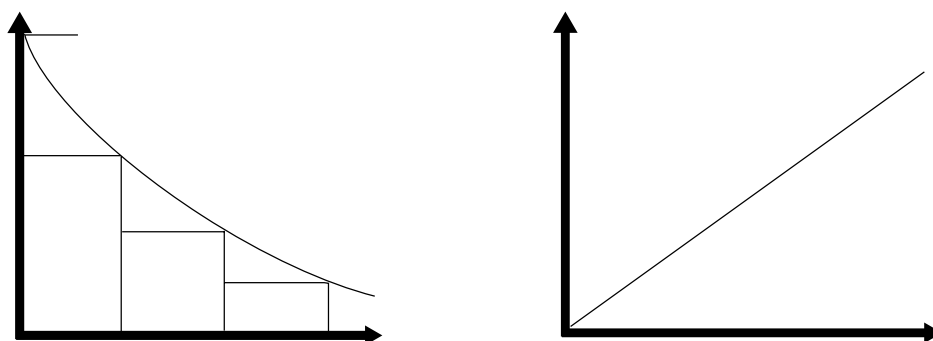


Fig. 7. Relationship between Transmittance and Concentration and Absorbance and Concentration.

4.2 Setup

When the SmartSpec Plus is not carrying out an assay, you can enter the Setup routine by pressing **Setup**. To proceed through Setup without changing the current setting, press **Enter**. Most of the current settings can be changed by pressing **Select** until you see your desired choice; sometimes you have to use the number pad to change an entry. In either case, press **Enter** after the selection is made. When you have gone all the way through Setup, SmartSpec Plus will automatically return to Home screen. You can exit Setup at any time by pressing **Cancel**. When you exit Setup, all changes will be saved.

In Setup you can specify the following:

1. Language
2. Pathlength
3. Background compensation for DNA/RNA measurements.
4. Default type of nucleic acid.
5. Default method to determine DNA and RNA oligonucleotide molar extinction coefficient and molecular weight.
6. Default number of sample replicates.
7. New operator name.
8. Current operator.
9. Serial port export.
10. Current time.
11. Current date.
12. Time display mode.
13. Date display mode.
14. Performance validation

1. **Language.** SmartSpec Plus may display one of six different languages on its LCD screen. After pressing the Setup key, you can choose from: English(Eng), French(Fra), German(Deu), Italian(Ita), Spanish(Esp) or Japanese.

English is the default language and will be automatically selected if no choice is made. If another language is selected, once you exit Setup all changes will be saved and the instrument will display the selected language until changed in the Setup.

2. **Pathlength.** The pathlength describes the distance that the light travels as it passes through the sample, and is the same as the width of the cuvette. By using a cuvette with a shorter pathlength, you can measure the absorbance of more concentrated solutions. The relationship between absorbance, path length and sample concentration is described by Beer's law where

$$A = \epsilon bc$$

A is the absorbance, ϵ the molar extinction coefficient in liter/mole/cm, b the pathlength in cm and c the concentration in moles/liter.

```
Select Pathlength:
>=Help           10 mm
```

Pressing **Select** once will change the screen to

```
Select Pathlength:
>=Help           5 mm
```

As you continue pressing **Select**, the choices change to 2 mm and then 1 mm.

3. **Background compensation.** When using the DNA/RNA assay, you can choose to have SmartSpec Plus automatically subtract background absorbance readings collected at 320 nm from the A260 and A280 measurements. If the background subtraction option is invoked, then the A320 is subtracted from the A260 and A280 readings before these measurements are displayed and before they are used in the calculation of nucleic acid concentration. The default condition is to not subtract the A320 background. You can toggle background subtraction on and off by pressing **Select**.

```
Substract A320 Background
for DNA/RNA? Yes  >=Help
```

4. **Default type of Nucleic Acid.** SmartSpec Plus can convert absorbance reading of nucleic acid solutions into concentration. The conversion factor is determined by the type of nucleic acid being measured. You can select the default type of nucleic acid in Setup, so that when you press **DNA/RNA**, this type of nucleic acid will be shown first on the display. You can choose another type of nucleic acid at any time by pressing **Select**. The choices are double-stranded DNA (**dsDNA**), single-stranded DNA (**ssDNA**), **RNA**, **DNA oligo** and **RNA oligo**. The factory default choice is dsDNA.

```
Select default type of
DNA/RNA:    dsDNA  >=Help
```

5. **Default method to determine oligo molar extinction coefficient and molecular weight.** SmartSpec Plus uses the molecular weight and molar extinction coefficients of oligonucleotides to convert the absorbance readings into mass ($\mu\text{g/ml}$) and molar ($\text{pmol}/\mu\text{l}$) concentrations. You can input the molecular weight and molar extinction coefficient directly if you know them, otherwise SmartSpec Plus will use one of three methods to estimate them for you: Length, Composition or Sequence. The simplest method to estimate either the molecular weight or the molar extinction coefficient is by the length of the oligonucleotide. This method assumes an equivalent distribution of all four nucleotides among the oligo. More accurate estimates can be calculated if you specify the composition (*i.e.*, the number of A's, C's, G's and T's or U's) of the oligo and the best estimate of molar extinction coefficient is calculated with the sequence of the oligonucleotide.

```
Select default method of
find oligo conc:    length
```

Press **Select** to change the default to Composition (**Compstn**) and press **Select** again to set the default to **Sequence**.

6. **Default number of sample replicates.** SmartSpec Plus has the ability to accept samples (and standards) in replicate groups and to calculate a mean and standard deviation for each sample (or standard) replicate group. The factory default number of sample replicates is 1, but you can change the number of replicates to any number between 1 and 9. Use the keypad to choose the number of sample replicates and press **Enter** or **Setup** when finished. During most assays you can change the number of sample replicates by choosing *Options* from the Ready Screen.

```
Enter default number of
sample replicates:_____
```

7. **New Operator.** When SmartSpec Plus prints a report, it includes the name of the operator (if specified). You can add a new operators name to the list of operators in Setup.

```
Want to add the name of
a new operator? No
```

Press **Select** to toggle from No to Yes and the screen will change to

```
Enter new operator's
name: (8 char): _____
```

The keyboard will automatically switch to the alpha mode and you can use the keys to enter a name with as many as eight characters. To enter an **A** in the first space, press the 2 key once. To enter a **B**, press the 2 key twice, and to enter a **C**, press it three times. To move from the first space to the second space, press the right arrow key. When the last letter of the name has been specified, press **Enter**. If you want to mix numerals with letters, you can toggle back and forth between the alpha mode and number mode by pressing **Alpha**. When the LED on the Alpha key is lit, then the keyboard is in alpha mode. You can enter a space by pressing the right arrow key twice.

8. **Current operator.** Press **Select** until the desired name is displayed on the screen and then press **Enter**. This name will be printed on the final report.
9. **Serial Port or Printer Data Export.** This feature allows the user to export data using either the printer, serial port or both. The screen will prompt for the desired configuration. Use the Select key to toggle between the data export options.

```
Printer: On
Serial port: Off
```

10. **Current time.** The time will be displayed in the present format.

```
Current time: 1:12 PM
is this OK? Yes
```

If the displayed time is incorrect, press **Select** and then **Enter**.

```
Enter new time. Select
AM or PM: ____ : ____ AM
```

- Use the number pad to enter the hour and press **Enter**, then enter the minutes and press **Enter** again. After setting the time, press **Select** to change from **AM** to **PM** if you are using the 12-hour format. Press **Enter** when complete.

11. **Current date.** The date will be displayed in the present format.

```
Current date: (MM/DD/YY)
11/25/98 Is this OK? Yes
```

If the date is not correct, press **Select** to toggle from **Yes** to **No** and **Enter**.

```
Enter new date in format
MM/DD/YY: ____/____/YY
```

- Use the number pad to input the new date. Press **Enter** to move to the next field. Press **Select** to set the day of the week. Press **Enter** when you are finished.

12. **Time convention.** You may choose to print time in 12-hour or 24-hour mode. Toggle between the choices by pressing **Select**, and then press **Enter**.

```
Select time display mode
 24-hr e.g. 13:12
```

Press **Select** for

```
Select time display mode
12-hr e.g. 1:12
```

13. **Date convention.** You can choose to print the date in either MM/DD/YY or DD-MM-YY mode. Toggle between the choices by pressing **Select** and then press **Enter**.

```
Select date display mode
MM/DD/YY e.g. 11/25/98
```

Press **Select** for

```
Select date display mode
DD-MM-YY e.g. 25-11-98
```

14. **Performance Validation.** *The Performance Validation function requires the purchase of the SmartSpec Plus with Performance Validation Package (170-2520) or the separate Performance Validation Package (170-2509). SmartSpec Plus will automatically perform absorbance and wavelength tests and create a printable report.*

Press **Enter** to access the Performance Validation program. Enter the four digit Validation Cuvette number found on the Cuvette's certificate of analysis.

After entering absorbance information found on the Validation Cuvette's certificate of analysis, the SmartSpec Plus will automatically perform wavelength and absorbance accuracy tests. This process can take up to 5 minutes to complete. When finished, a report may be printed.

15. **Exit Setup.** After going through the entire Setup menu, the display will return to the Home screen. All changes will be saved. You can also exit Setup at any time by pressing **Cancel** and all changes up to that point will be saved.

4.3 Serial Port Functions

The SmartSpec Plus has the ability to communicate with an external printer or computer via the serial port located at the back of the instrument. The SmartSpec Plus sends data to these peripherals as ASCII text. When connecting the SmartSpec Plus to an external printer or computer, a "standard serial" cable should be used. The correct pin-out for this cable is described in the table below. Check the printer or computer for the correct serial port connector end (male or female).

| DB9F Connector to SmartSpec Plus (male) | Signal and Direction | DB9M Connector to serial device |
|--|-------------------------|------------------------------------|
| 2 | TX --> | 3 |
| 3 | <-- RX | 2 |
| 5 | Common | 7 |

(other pins are open in SmartSpec Plus)

The serial port parameters are: 9600 baud rate, 8 data bits, no parity, 1 stop, and XON/XOFF flow control. These parameters are pre-set in the unit. When using a serial printer set the printer to the serial port parameters specified above. Please note that the data are sent from the SmartSpec Plus to a computer or serial printer as hex characters. If your computer or printer is not set to accept these characters please refer to the instrument's instruction manual for correct settings. (For detailed instructions see Section 4.4.)

4.4 PC Serial Connection Instructions

1. Connect RS232 9-pin serial cable from SmartSpec Plus to computer.
2. Using a terminal program (e.g. HyperTerminal), set the connection to 9600 baud, 8 bits-no-parity-1 stop bit (9600-8-N-1), no flow control.
3. In addition to the actual data transmission/reception parameters, other parameters in HyperTerminal may have to be set. The correct settings may vary from computer to computer, but these are settings currently used and offered as an example. It may not be necessary to implement all parameters. **Only the Connect Direct to Com Port is essential.**

From the main HyperTerminal menu

File

Properties

Open the properties dialog box. Using the tabbed HyperTerminal properties box:

Connect To

Connect using Direct to Com [n] (*n = serial port number*). **This must be set.**

Settings

Terminal Keys

Del

Emulation

Tel net terminal

Underline

Blink

Backscroll buffer line

Echo typed characters locally

Wrap lines that exceed terminal width

4. When the SmartSpec Plus sends data, either when requested from **Scan**, or **Kinetics**, or when the user activates the data echo toggle in **Setup**, the data streams to the terminal window.
5. Capturing data in HyperTerminal and saving the data in a file:
In the main HyperTerminal window menu, first click Transfer and then click Capture Text.

Transfer

Capture Text . . .

[give file name and path]

e.g. c:\program files\accessories\hyperterminal\capture.txt

A dialog box with the default file name, file extension and file path will appear. Accept the default, or type in a new path and/or file name.

Use the SmartSpec and send data from the SmartSpec to the computer.

When finished:

Transfer

Capture Text . . .

Stop (closes file)

6. Start Excel (or Wordpad, or MS Word, etc.)

If using Excel click

File

Open

Open the SmartSpec/HyperTerminal data file.

e.g. c:\program files\accessories\hyperterminal\scan.txt

make sure files of types: (e.g. *.txt) is set to correct extension

Select and Open desired file

Text Import Wizard:

Delimited

Colon (:) is used as a delimiter for Scan and Kinetics

General data type should be sufficient for expressing numerical data. The system selects the appropriate data type.

4.5 Keypad Descriptions

| | |
|------------------------|--|
| Print | SmartSpec Plus will print data on the current sample, a user-selectable range of samples or all samples in the current assay. |
| Abs | SmartSpec Plus will automatically display the absorbance reading of the current sample and the wavelength being read. If more than one wavelength is being read, press Abs to toggle among the different wavelengths. If you are reading one of a replicate group, you can choose to see the absorbance of the current replicate or you can see the mean and standard deviation of all members of the replicate group by first pressing Abs. |
| Conc | The Conc key is used in the DNA Oligo and RNA Oligo assays to toggle the display of concentration from units of $\mu\text{g/ml}$ to $\text{pmoles}/\mu\text{l}$. This key has no function for the Scan, Kinetics and λ assays. |
| A260:A280 | SmartSpec Plus will calculate and display the ratio of A260 to A280. This is a method of approximating the purity of nucleic acid preparations; pure preparations of DNA have ratios of about 1.8 and pure RNA preparations have a ratio of about 2.0. If you are using background compensation (in Setup), then the ratio is calculated after the background reading of A320 is subtracted from the A260 and A280. |
| Dilution Factor | The dilution factor is used as a final multiplier in calculation of concentration from absorbance data. The default dilution factor is 1.0 and the dilution factor is reset to 1.0 each time that one of the Assay buttons is pressed. The adjusted concentration will be printed on the final report only if the dilution factor is changed after measuring each unknown sample. |
| Setup | SmartSpec Plus will enter the setup routine where you can specify the pathlength, defaults for certain options, time and date convention and time and date. You exit Setup either by proceeding all the way through the routine, or by pressing Cancel at any time. When you exit the Setup routine, all changes are saved. |
| Assay Buttons | The six assay buttons are in the second row of function keys on the face of the SmartSpec Plus. Press one of these to begin an assay with SmartSpec Plus. When any assay button is pushed, SmartSpec Plus resets the sample number to 1, and checks the memory for unprinted data. |
| DNA/RNA | SmartSpec Plus will automatically collect absorbance data at 260 and 280 nm, and use those readings to calculate nucleic acid concentration. If selected in Setup, background will automatically be subtracted at 320 nm. The calculation of concentration depends on the type of nucleic acid specified. |

| | |
|-----------------------------|---|
| Protein | SmartSpec Plus will automatically read one or more wavelengths depending on the type of assay specified (Bradford, Lowry, BCA or UV Protein) or the user can specify a wavelength. SmartSpec Plus will convert absorbance data to concentration by use of a standard curve, except for the case of UV Protein in which the absorbance readings are multiplied by user-selectable constants. The standard curve can be one recalled from memory, or a new one constructed by SmartSpec Plus. |
| Scan | SmartSpec Plus will collect and print the absorbance of a sample over a user-defined range of wavelengths from 200 to 800 nm. An optional background wavelength may be specified and its absorbance will be subtracted from all absorbances in the scan range before the scan is displayed. SmartSpec Plus will identify and quantitate the points of maximum and minimum absorbance over the scanned range. These data can be printed or downloaded via the serial port to an external computer. |
| Kinetics | SmartSpec Plus will collect data from a single cuvette at fixed periodic intervals over a certain time period. Both the interval of data collection and length of data collection are specified by the user. An optional background wavelength may be specified. The kinetic readings may be printed or exported to external software for analysis. |
| OD600 | SmartSpec Plus will collect absorbance data at 600 nm. This assay is used for monitoring the growth of bacterial cultures. The user may specify a conversion factor which is multiplied by the dilution factor and the absorbance to calculate the concentration in cells per ml. |
| λ | SmartSpec Plus will collect data at up to three different user-specified wavelengths. An optional background wavelength may also be specified and its absorbance will be subtracted from the other wavelengths before they are reported. |
| Read Blank | SmartSpec Plus will zero the detector in preparation for reading samples. It is important to zero the instrument at each new assay. Insert a cuvette that contains all assay components except the analyte and press Read Blank . |
| Read Sample | SmartSpec Plus will collect absorbance data from the cuvette in the instrument. |
| Select | Used to toggle between or among choices. Once the choice is made, its selection is confirmed by pressing Enter . |
| Enter | Indicates to SmartSpec Plus that a choice has been made. |

| | |
|----------------------|--|
| Cancel | Cancels the last entry or causes an exit from Setup. When editing sequence information, press Cancel to delete a base. When Options are displayed, press Cancel to return to the assay. |
| < and > | Used to access other menus or screens. Also used to move the cursor. |
| Paper Feed | Feeds paper through the printer. Used when removing or installing a paper roll. |
| Alpha | The Alpha key allows the user to toggle between alpha and numeric input for naming stored standard curves or naming operators. When in alpha mode, an LED is illuminated on the key. To type an alphabetic character when in the alpha mode, press one of the number keys in the left or center columns. To type an A , press the 1 key once; to type a B , press the 1 key twice, and to type a C , press the 1 key three times. These keys also enable the user to enter sequence data. Sequence data are input using the last column of keys on the number pad: to type an A , press the 3 key; to type a C , press the 6 key; to type a G , press the 9 key and to type a T or U , press the exp key. |
| Number pad | Used to input numeric values for SmartSpec Plus calculations. The exp key is used for making entries in exponential notation, and when it is pressed, an e is displayed by SmartSpec Plus. It refers to the base 10 and not to the base of the natural logarithm. |

Section 5 Quick Guide

1. Power up the instrument. It will go through system status check and display an error message if there are any problems.
2. Press one of the Assay buttons.
 - A. DNA/RNA.
 - i. Choose type of nucleic acid
 - a. dsDNA, ssDNA or RNA: accept or modify the conversion factor
 - b. DNA oligo or RNA oligo: input molar extinction coefficient and molecular weight; otherwise choose method for SmartSpec Plus to estimate these values.
 - ii. Choose whether to subtract background and, if so, specify background wavelength.
 - B. Protein
 - i. Choose type of assay.
 - a. Bradford. Measure absorbance at 595 nm.
 - b. Lowry. Measure absorbance at 750 nm.
 - c. BCA. Measure absorbance at 562 nm.
 - d. UV. Measure absorbance at 260, 280, and 320 nm.
 - e. Other. User specifies the wavelength to read.

- ii. Choose standard curve option.
 - a. Create a new standard curve.
 - b. Recall a standard curve from memory.
 - c. No standard curve. SmartSpec Plus will not be able to convert absorbance to concentration.
- C. Scan
- i. Set upper and lower limits of scan. (200 nm to 800 nm)
 - ii. Choose whether to subtract background and, if so, specify background wavelength.
 - iii. Choose fast or slow scan.
 - iv. For the fast scan, choose number of successive scans.
- D. Kinetic
- i. Choose wavelength to read.
 - ii. Choose duration of data collection.
 - iii. Choose interval between successive readings.
 - iv. Choose whether to subtract background and, if so, specify background wavelength.
- E. OD600
- i. Accept or modify the conversion factor.
- F. λ
- i. Choose number of wavelengths to read.
 - ii. Specify the wavelengths to read.
 - iii. Choose whether to subtract background and, if so, specify background wavelength.
3. If your dilution factor is not 1.0, set the dilution factor.
 4. Zero the instrument. Place a cuvette containing the blank solution into SmartSpec Plus and press **Read Blank**.
 5. Collect absorbance data. Place cuvette containing sample solution into SmartSpec Plus and press **Read Sample**. Continue collecting absorbance data until all samples are read.
 6. Absorbance and/or concentration data are displayed automatically as they are collected. If there are absorbance data at other wavelengths, press **Abs** to see them. DNA or RNA oligo concentrations are available in units of $\mu\text{g/ml}$ and $\text{pmole}/\mu\text{l}$; press **Conc** to toggle between them. If absorbance or concentration data are displayed, press Enter to return the Ready screen, or simply put the next sample cuvette into SmartSpec Plus and press **Read Sample**.
 7. After last sample, press left arrow key to exit assay.
 8. For protein assays, save the standard curve (if you made a new one) and print the full report.

Section 6 Operating Instructions

Note: It is Good Laboratory Practice (GLP) to use the SmartSpec Plus spectrophotometer to measure absorbances in the range of 0.100 to 1.500 AU.

- 1. Choose an assay.** SmartSpec Plus has six different assay keys in the second row of buttons. When you press one of them, it signals SmartSpec Plus to clear its memory of absorbance readings from the previous assay, to reset the sample number to 1, and to reset the dilution factor to 1. Before the data buffer is cleared, SmartSpec Plus will offer to print any unprinted data. All of the assays collect absorbance data and some of the assays are designed to convert those readings into concentrations or other useful information. The user interface of each assay is described in detail in the next section. The available assays are:

- A. DNA/RNA.** In this assay, SmartSpec Plus will automatically collect absorbance readings at 260 and 280 nm. If you choose to subtract the background at 320 nm (in Setup), then the A320 reading is subtracted from the A260 and A280 readings before the A260 and A280 absorbances are displayed or used in any calculations of nucleic acid concentration or in calculation of the A260:A280 ratio.

Calculations. Nucleic acid concentrations of dsDNA, ssDNA and RNA are determined by multiplying the A260 absorbance reading by a mass/absorbtion conversion factor and by the dilution factor, or by multiplying the difference between the A260 and A320 by the conversion and dilution factors.

$$\text{Concentration} = \text{A260} * \text{Conversion Factor} * \text{Dilution Factor}$$

or

$$\text{Concentration} = (\text{A260} - \text{A320}) * \text{Conversion Factor} * \text{Dilution Factor}$$

SmartSpec Plus is programmed with default conversion factors for dsDNA, ssDNA and RNA. These factors can be modified at any time.

| <u>Species</u> | <u>A260 of 1.000</u> |
|----------------|----------------------|
| dsDNA | 50.0 µg/ml |
| ssDNA | 37.0 µg/ml |
| RNA | 40.0 µg/ml |

DNA and RNA oligonucleotide concentrations are reported in two different ways: by mass (µg/ml) and moles (pmol/µl); you can toggle between the two values by pressing the **Conc** key. In order to make these determinations, SmartSpec Plus requires the molecular weight of the oligo and either the molar extinction coefficient or the mass/absorbance conversion factor (as shown in the table above for dsDNA, etc.). Frequently the molar extinction coefficient and/or the mass/absorbance conversion factor is provided when you order an oligonucleotide from a custom synthesis laboratory. However, if you don't know this factor, SmartSpec Plus can estimate them for you in one of three different ways. In order of increasing accuracy, by length of the oligo, by composition of the oligo, (*i.e.*, how many A's, C's, G's and T/U's) or by the primary sequence of the oligo. See Section 7.1 for a complete explanation of the calculations (pg. 30).

- B. Protein.** The Protein function will automatically select the proper wavelength for the Bradford (595 nm), Lowry (750 nm), BCA (562 nm) or UV Protein (260, 280, and 320 nm) assays, or you can select a different wavelength for another assay. The Bradford, Lowry and BCA are all well-characterized colorimetric assays for protein concentration. The UV protein assay can determine protein concentration in a nucleic acid background.

The SmartSpec Plus Bradford, Lowry, BCA and Other Protein assays use standard curves created by measuring the absorbances of solutions of known concentration to determine the concentration of unknown samples. If you do not want to construct a new standard curve, you may recall one from the on-board memory. SmartSpec Plus has the capability of storing up to ten standard curves in memory. The stored curves are numbered automatically from 0 to 9, or you can overwrite the number. Stored curves may also be assigned alphabetic names using the Alpha function of the number pad.

You also have the option of declining construction or recall of a standard curve, but without the standard curve, the SmartSpec Plus cannot convert absorbance readings to concentrations.

The UV Protein assay, developed to estimate protein concentration in a nucleic acid background, as in a crude cell extract, calculates protein concentration by the equation

$$\text{Concentration} = C1 * (A_{280} - A_{320}) - C2 * (A_{260} - A_{320})$$

where C1 and C2 are constants. If you select the UV Protein assay, SmartSpec Plus will first show you the current values for C1 and C2 and ask you to accept or modify them. The preset values for C1 and C2 are 1.55 and 0.76 respectively.

Standard Curve Construction. SmartSpec Plus will calculate a mean and standard deviation for each standard replicate group (if replicates are specified) and then use those data to fit a linear standard curve. The instrument will report the slope and intercept of the standard curve and the square of the correlation coefficient for the curve (r^2).

The correlation coefficient (r) provides an indication of how well the linear polynomial regression model fits the data. In effect, the correlation coefficient is the square root of the proportion of explained variation to total variation of the regression.

$$r = (\text{explained variation} / \text{total variation})^{1/2}$$

The amount of explained variation increases as the goodness of fit improves. In a perfect fit, all of the variation is explained, the explained variation equals the total variation, and the ratio of the explained variation to total variation becomes 1.000. Or, more simply, the closer the correlation coefficient is to 1.000, the better the fit of the regression, and the better the estimate of concentration.

Storing Standard Curves. As many as ten standard curves may be stored in SmartSpec Plus's on-board memory. These may be recalled for use in determining concentrations in subsequent assays. A new curve will be assigned a number from 0 to 9 by the firmware and you can then give it an optional name of up to eight characters in length.

Printing Standard Curves. If you choose to print a full report at the end of an assay, the standard curve will be printed along with the information about the curve. This will include the concentrations and absorbances of the standard and the slope, intercept and coefficient of the curve.

You can choose to print the same information about a standard curve when it is first created or when it is recalled from memory.

Limitations of the methods. The linear regression, when fit properly, generally produces reliable results at the extremes of the range of the standard curve. Results calculated from mean absorbance data outside the range of the standard curve may be unreliable. (For the purposes of this discussion, the range of the standard curve is defined as the absorbance range between the highest and lowest mean absorbances of the standards used to form the standard curve.) Calculated results that are determined from mean absorbances outside the range of the standard curve are extrapolations, and should be treated as such when determining the final results of your analysis.

The SmartSpec Plus will not be able to give a unique concentration for a given absorbance if the calibration data are not monotonic (*i.e.*, two standards of differing concentration have the same absorbance reading).

- C. **Scan.** SmartSpec Plus can read any subset of the interval between 200 nm and 800 nm and then print the scan; the interval can be further subdivided into as many as four regions, and for each region the local minimum and maximum are identified. You may select a background wavelength and its absorbance will be subtracted from the absorbance at all other wavelengths in the interval of the scan. There are two scanning speeds: fast and slow. If you choose a slow scan, you will get the best data, but will take several minutes to complete, depending on the sample and the range being read. You may not specify more than one repetition for slow scanning. Scan data may be downloaded to an external computer via the serial port, or may be printed by SmartSpec Plus.
- D. **Kinetic.** SmartSpec Plus can collect absorbance readings at periodic intervals for a specified length of time so that you can collect kinetic data. You may specify a wavelength for background subtraction; background absorbances are collected at the same interval and are subtracted from the reading wavelength. You may then print out the data and/or download the data to another program for analysis. Permissible reading intervals range from 2 seconds to 999 seconds and data may be collected for as long as 9999 seconds.
- E. **OD600.** This function is useful for monitoring growth of bacterial cultures. SmartSpec Plus can also convert the absorbance reading into a concentration with units of cells/ml if provided with a conversion factor. The conversion factor is multiplied by the absorbance reading and the dilution factor to estimate the concentration of cells in cells per ml. For example, if for certain *E. coli* an OD of 1.0 indicates a concentration of 5×10^8 cells/ml, then the conversion factor would be 5×10^8 . On the SmartSpec Plus 5×10^8 is entered in exponential notation using the **exp** key (press 5, **exp**, 8 and **Enter**) and displayed as **5 e8**. On the display, e refers to the base 10 and not to the base of the natural logarithm.
- F. **λ .** The wavelength function allows you to choose up to three wavelengths for measurement and any other wavelength for optional background subtraction. It is a rapid and simple way to collect absorbance data without constructing standard curves or otherwise calculating concentrations.

- 2. Choose sample replicates.** Samples may be read in replicate and you do not need to have the same number of replicates for each different sample. For each sample replicate group, SmartSpec Plus will calculate a mean absorbance and standard deviation. When the full report is printed, each individual absorbance is reported along with the mean and standard deviation of the replicate group. If the default number of sample replicates has been set to 2–9 (in Setup), then SmartSpec Plus will ask if any of the samples in this assay are to be read in replicate. The default answer is No.

```
Are any samples to be
read in replicate?  No:
```

- If you want to read samples in replicate, press **Select** to toggle from **No** to **Yes** and press **Enter**. You do not need to have the same number of replicates for each sample.

```
Enter number of sample
replicates # 3
```

- Use the number keys to enter the number of sample replicates. The default number is the number chosen in Setup.

The default number of sample replicates can be changed during the course of the assay by choosing Options from the Ready screen.

- 3. Set the dilution factor.** The dilution factor is reset to 1 each time an assay button is pushed. The dilution factor is always a number greater than or equal to 1; if one volume of sample has been diluted with nine volumes of buffer, the dilution factor is entered as 10.

```
Enter a dilution factor:
```

- Use the number keys to input the dilution factor and press **Enter**.

- 4. Zero the spectrophotometer.** Zero (blank) SmartSpec Plus by placing a cuvette containing the sample solution without analyte into the instrument and pressing **Read Blank**. You can zero the instrument at any time during an assay.

- 5. Collect absorbance data.** SmartSpec Plus is now ready to collect absorbance data and convert them to concentration. Irrespective of whether you are going to read your samples in replicate or not, the Ready screen will be displayed.

```
Ready to Read Absorbance
<=Exit Assay    >=Options
```

A. No sample replicates.

- Place your first sample in the cuvette chamber and press **Read Sample**. After the absorbance data are collected, they will be displayed as described below. At this point, you can put your second sample into the cuvette chamber and press **Read Sample**.
- If you wish to begin reading samples in replicate, first return to the Ready screen by pressing **Enter**

```
Ready to Read Absorbance
<=Exit Assay    >=Options
```

and then press the right arrow key to choose Options.

```
<=Change next sample #
>=Change # of replicates
```

- Press the right arrow key to change the number of sample replicates.

```
Enter number of sample
replicates: _____
```

B. Sample replicates.

- Place the first replicate of your first sample in the cuvette chamber and press **Read Sample**. Before it collects the absorbance data, SmartSpec Plus will ask for the number of replicates for the first sample.

```
Sample #1   Enter number
of replicates: 3 >=Option
```

- Use the number keys to input the number of replicates and press **Enter**. The absorbance data for the first replicate will be collected and the SmartSpec Plus will direct you to insert the second replicate

```
Insert Rep #2/3   Samp #1
and press Read Sample
```

This will continue until the last replicate of the first sample has been read. SmartSpec Plus will then display the mean absorbance of all replicates of the first sample as described below. When the absorbance data are displayed, you can insert the first replicate of the second sample and press **Read Sample**.

- You can change the number of sample replicates at any time in the assay by first going to the Ready screen (if absorbance data are displayed, you can return to the Ready screen by pressing **Enter**).

```
Ready to Read absorbance
<=Exit Assay   >=Options
```

- Press the right arrow for Options.

```
<=Change next sample #
>=Change # of replicates
```

- Press the right arrow key to change the number of sample replicates.

```
Enter number of sample
replicates: _____
```

- Use the number keys to input the new number of sample replicates and press **Enter**.

6. **View the absorbance and concentration data.** Absorbance data are automatically displayed after they are collected. In the case that you are reading samples in replicate groups, the mean of the replicate group is displayed. The standard deviation of the replicate group is available in the printed report.

- A. **DNA/RNA.** Immediately after the sample is read, the data are displayed in the form

```
A280=  x.xxx   A320=  x.xxx
Conc = xxx.xxx   µg/ml
```

- Press **Abs** to toggle the display to view the other absorbance data

```
A260=  x.xxx           Samp #1
Conc=  xxx.xxx           µg/ml
```


- Press **A260:A280** to see the ratio displayed

```
A260=  x.xxx  A280= x.xxx
A260:A280  =  x.xxxx
```

- For DNA oligos and RNA oligos, press **Conc** to see the concentration displayed in units of pmoles/ μ l.

```
A260=  x.xxx      Samp #1
Conc=  xxx.xxx    pmoles/ $\mu$ l
```

- To read the next sample, insert the cuvette in the chamber and press **Read Sample**. From any of the above screens you can press **Enter** to return to the Ready screen.

```
Ready to Read absorbance
<=Exit Assay    >=Options
```

- B. Protein.** Immediately after the sample is read, the data are displayed in the form

```
A595=  x.xxx      Samp #1
Conc=  xxx.xxx     $\mu$ g/ml
```

- To read the next sample, insert the cuvette in the chamber and press **Read Sample**. From any of the above screens you can press Enter to return to the Ready screen.

```
Ready to Read Absorbance
<=Exit Assay    >=Options
```

The available options from the Ready screen are Change next sample number and Change number of sample replicates.

- C. Scan.** Immediately after the absorbances are calculated the screen will display the message

```
Press <Print> when ready
<=nm scale     >=AU scale
```

- Press **Print** to begin printing the scan. If you do not want to print the scan, press **Cancel**. From this screen, you can press the left arrow key to select nm scale and direct SmartSpec Plus to print only a subset of the data collected.

```
Begin scanning at ____ nm
End scanning at ____ nm
```

You can also change the minimum and maximum values on the AU scale, by pressing the right arrow key.

```
Min AU scale:
Max AU scale:
```

- Enter the lower limit. If you want the lower limit to be a negative value, enter the absolute value of the lower limit and press **Enter** and then press **Select**. For example, if you want the lower limit to be -0.500, press the decimal key and the 5 key and then **Enter**. The display will show

```
Min AU scale: +/-    0.500
Max AU scale:
```

- Now press **Select** and the negative sign will be displayed in front of 0.500.

```
Min AU scale: +/- -0.500
Max AU scale:
```

- Press **Enter** again and now define the upper limit. The upper limit must be a positive number.

After the scan data are printed, or if you choose not to print the scan data, you are next offered the opportunity to download the data via the serial port to a computer.

```
Press <Enter> when ready
to accept serial data.
```

- If you want to download the data to a computer via the serial port, make sure the proper connections are made and press **Enter**. If you do not want to download the data, press **Cancel**. After the data are downloaded, or after you press **Cancel**, the instrument will reset for the next scan and the Ready screen will be displayed.

```
Ready to Read Absorbance
<=Exit Assay    >=Options
```

- Press the right arrow key to see the available options. You can change the number of repetitions (for fast scans), change the range, choose a background, define a subregion of interest and choose smoothing of the curve.

```
1. Repetitions    2. Range
3. Background     >=More
```

and

```
4. Regions        5. Smooth
<= Back, Cancel to return
```

- Press 4 to choose regions. You will be prompted for the number of regions and then the limits for each region. When the data are printed, the local minimum and maximum in each region are printed.
- Smoothing will take a running average of five consecutive data points, two on either side of the current wavelength, and plot those. For example, the absorbances at 455, 456, 457, 458, and 459 nm are averaged and plotted at 457 nm. Then the absorbances at 456, 457, 458, 459, and 460 are averaged and plotted at 458 nm.

Note: After the data are printed, you can go back to the Ready screen and turn on or off the smoothing feature and then plot the same data again. First go to the Ready screen, choose Options, reset the Smoothing and then press Print.

- D. Kinetics.** Immediately after the data are collected, you will be asked if you want to print the data

```
Press <Print> for
kinetics report.
```

- Press **Print** to print the report. If you do not wish to print the report, press **Cancel**. In either case, you will next be offered the chance to download the data into a computer via the serial port.

```
Press <Enter> when ready
to accept serial data.
```

- If you want to download the data, make sure the connections are made to the computer and then press **Enter**. If you do not want to download the data, press **Cancel**.

```
Ready to Read Absorbance
<=Exit Assay   >=Options
```

After the data are downloaded SmartSpec Plus will reset for the next kinetic data collection. From the Ready screen, you can change any of the parameters for the kinetic data collection.

- E. OD600.** Immediately after the absorbance data are collected, they are displayed along with the cellular concentration.

```
OD600 = x.xxx
Conc = xxxe xxx cell/ml
```

- You can read a second sample by placing the cuvette in the chamber and pressing **Read Sample**. You can also return to the Ready screen by pressing **Enter**.

```
Ready to Read Absorbance
<=Exit Assay   >=Options
```

Available options are Change next sample number and Change number of sample replicates.

- F. λ.** Immediately after data are collected they are displayed

```
A480=x.xxx  A580=x.xxx
A680=x.xxx  Samp #1
```

- You can read a second sample by placing the cuvette in the chamber and pressing Read Sample. You can also return to the Ready screen by pressing **Enter**.

```
Ready to Read Absorbance
<=Exit Assay   >=Options
```

Available options are Change next sample number and Change number of sample replicates.

- 7. Print the data.** You can print data as they are collected, but it is more efficient to print absorbance and concentration data at the end of an assay. For assays other than Scan and Kinetics, if you press **Print**, the display will show

```
1.Print unprinted Samp #s
2.Select # 3.Full Report
```

- Press 3 to print the full report which includes the absorbances and concentrations (if available) for each sample in the current assay.
- Press 1 to print all data collected since the last time you printed.
- press 2 to print only one sample

```
Enter Sample # _____ to
print sample report.
```

8. **Exit the Assay.** You can exit the assay by pressing the left arrow key when the Ready screen is displayed.

```
Ready to read absorbance
<=Exit Assay    >=Options
```

SmartSpec Plus will prompt you to save a newly created standard curve (for protein assays) and then prompt you to print a complete report of absorbances, concentration and A260:A280 ratios. The report will also show means and standard deviations for each standard or unknown sample replicate group. If you do not print the assay report before another Assay key is pressed, the data will be lost.

Section 7

Examples of Assays

7.1 DNA/RNA ASSAY. Press DNA/RNA.

1. Select the type of nucleic acid.

```
Select type of nucleic
acid: dsDNA
```

Press **Select** to scroll through the other choices and press **Enter** to signify your selection.

```
Select type of nucleic
acid: RNA
```

```
Select type of nucleic
acid: ssDNA
```

```
Select type of nucleic
acid: DNA oligo
```

```
Select type of nucleic
acid: RNA oligo
```

2. **Determining the conversion factor.**

A. **dsDNA, ssDNA or RNA**

- If you choose dsDNA, ssDNA or RNA, the next screens display the conversion factor and allow you to modify it if you want. The conversion factor is used in calculating nucleic acid concentrations from absorbance data. The factor represents the concentration of a solution with an A260 of 1.000. *E.g.*, for ds DNA

```
A260 1.0=50.0 µg/ml. Is
conversion factor OK? Yes
```

- If this factor is acceptable, press **Enter**. If this is not an acceptable conversion factor, press **Select** to toggle from **Yes** to **No** and press **Enter**.

```
Enter conversion factor:
A260 1.0= _____ µg/ml
```

- After the default conversion factor is accepted or changed, SmartSpec Plus is ready to measure nucleic acid absorbance and convert that absorbance to concentration.

```
Ready to read absorbance
<=Exit Assay    >=Options
```

B. DNA oligonucleotides and RNA oligonucleotides.

If you choose DNA oligo or RNA oligo, you must provide the molecular weight of the oligo and either the molar extinction coefficient or the mass/absorbance conversion factor. If you don't know them, then SmartSpec Plus can estimate them for you.

```
Do you know oligo molar
extnctn coefficient? No
```

- If you know the molar extinction coefficient, press **Select** to toggle from **No** to **Yes** and press **Enter**. If you do not know the molar extinction coefficient, press **Enter** and skip to **ii** below.

i. If you know the molar extinction coefficient.

```
Enter molar extnctn coef
in L/mole/cm: _____
```

- Use the number keys to input the extinction coefficient and press **Enter**.
Note: You must enter the molar extinction coefficient in units of liter mole⁻¹ cm⁻¹.

SmartSpec Plus will next prompt for the oligo molecular weight.

```
Do you know molecular
weight of oligo? No
```

- If you know the molecular weight, press **Select** to toggle from **No** to **Yes** and press **Enter**. If you do not know the molecular weight of the oligo, press **Enter** and skip to **b** below.

a. If you know the molecular weight.

```
Enter oligo molecular
weight: _____ g/mol
```

- Use the number keys to input the molecular weight and press **Enter**.

After you have entered the molar extinction coefficient and the molecular weight, SmartSpec Plus will display them.

```
Mol Ext Coef=xxxxxxx
MW=xxxxx    >=Continue
```

- Press **>** to continue the assay, and the screen will display

```
Ready to read absorbance
<=Exit Assay    >=Options
```

SmartSpec Plus is now ready to collect absorbance data.

b. If you do not know the molecular weight.

```
Enter oligo composition:  
#A:___#C:___#G:___#T:___
```

- Use the number keys to input the number of A's, then press **Enter** to move the cursor to the next field and enter the number of C's. After entering the number of T's (or U's for RNA oligos), press **Enter**. SmartSpec Plus will calculate the molecular weight and display it along with the molar extinction coefficient.

```
Mol Ext Coef=xxxxxxx  
MW=xxxxx      >=Continue
```

- Press the right arrow key to continue the assay. SmartSpec Plus is now ready to collect absorbance measurements and to convert those to concentrations.

- ii. If you do not know the molar extinction coefficient, but you know the mass/absorbance conversion factor.** If you do not know the molar extinction coefficient, SmartSpec Plus will ask for the mass/absorbance conversion factor. This factor would be multiplied by the absorbance to calculate mass concentration (e.g., for dsDNA the mass/absorbance conversion factor is 50.0 $\mu\text{g/ml}$ for an A260 of 1.0). If you do not know either the molar extinction coefficient or the mass/absorbance conversion factor, SmartSpec Plus will estimate them for you.

```
Do you know conv factor  
from A260 to conc? No
```

- If you know the conversion factor, then press **Select** to toggle from **No** to **Yes** and press **Enter**. If you do not know the conversion factor, press **Enter** and skip down to **iii**.

```
Enter conversion factor:  
A260 1.0 = _____  $\mu\text{g/ml}$ 
```

- Use the number keys to input the conversion factor and press **Enter**. SmartSpec Plus will now need the molecular weight of the oligonucleotide in order to convert absorbance measurements into concentrations of $\mu\text{g/ml}$ and $\text{pmol}/\mu\text{l}$.

```
Do you know molecular  
weight of oligo? No
```

- If you know the molecular weight, press **Select** to toggle from **No** to **Yes** and press **Enter**. If you do not know the molecular weight of the oligo, press **Enter** and skip to **b** below.

a. If you know the molecular weight.

```
Enter oligo molecular  
weight: _____  $\text{g/mol}$ 
```

- Use the number keys to input the molecular weight and press **Enter**.

After you have entered the molar extinction coefficient and the molecular weight, SmartSpec Plus will display them.

```
Mole Ext Coef=xxxxxxx  
MW=xxxxx      >=Continue
```

- Press > to continue the assay, and the screen will display

```
Ready to read absorbance
<=Exit Assay    >=Options
```

- SmartSpec Plus is now ready to collect absorbance data.

b. If you do not know the molecular weight.

```
Enter oligo composition:
#A:___#C:___#G:___#T:___
```

- Use the number keys to input the number of A's, then press **Enter** to move the cursor to the next field and enter the number of C's. After entering the number of T's (or U's for RNA oligos), press **Enter**. SmartSpec Plus will calculate the molecular weight and display it along with the molar extinction coefficient.

```
Mole Ext Coef=xxxxxxx
NW=xxxxx    >=Continue
```

- Press the right arrow key to continue the assay. SmartSpec Plus is now ready to collect absorbance measurements and to convert those to concentrations.

```
Ready to read absorbance
<=Exit Assay    >=Options
```

- iii. If you do not know the molar extinction coefficient or the mass/absorbance conversion factor.** SmartSpec Plus will estimate the molar extinction coefficient and the molecular weight. You must choose which of the three methods it will use: length, composition or sequence. The calculated molar extinction coefficient and molecular weight will be displayed at the end. A fourth option of **Do not need** may be selected if you do not want to convert the absorbance data to concentration.

Caution: Estimations based on oligo length assume an equivalent distribution of the four bases. If your oligo composition differs significantly from this ratio, use the composition or sequence options for more accurate estimates of molar extinction coefficient and molecular weight.

```
Select a method to find
mol ext coef: Length
```

- Press **Select** to scroll through the other options.

```
Select a method to find
mol ext coef: Composition
```

```
Select a method to find
mol ext coef: Sequence
```

```
Select a method to find
mol ext coef: Do not need
```

- Press **Enter** when your preference is displayed.

a. Length

```
Enter oligo length: ___
```

- Use the number keys to input the length of the oligo and press Enter. You can enter a length of up to 99 nucleotides. After you press **Enter**, SmartSpec Plus will calculate and display the extinction coefficient and molecular weight.

```
Mol ext coef= _____
MW= _____ >=Continue
```

The molar extinction coefficient is displayed with units of liter mole⁻¹ cm⁻¹

- Press the right arrow to continue the assay. SmartSpec Plus will now be ready to measure the absorbance of your oligonucleotide and to convert those absorbance data into concentrations of µg/ml and pmol/µl.

```
Ready to read absorbance
<=Exit Assay   >=Options
```

b. Composition.

```
Enter oligo composition:
#A:___#C:___#G:___#T:___
```

- Use the number keys to input the number of each type of nucleotide. Use the arrow keys to move from field to field. Press **Enter** when all four have been specified. SmartSpec Plus will calculate and display the extinction coefficient and the molecular weight.

```
Mol ext coef= _____
MW= _____ >=Continue
```

The molar extinction coefficient is displayed with units of liter mole⁻¹ cm⁻¹

- Press the right arrow to continue the assay. SmartSpec Plus will now be ready to measure the absorbance of your oligonucleotide and to convert those absorbance data into concentrations of µg/ml and pmol/µl.

```
Ready to read absorbance
<=Exit Assay   >=Options
```

c. Sequence

```
Enter oligo sequence:
_____
```

- SmartSpec Plus will automatically shift to the Alpha mode on the keypad so that you enter an A by pressing the **3** key, a C by pressing the **6** key, a G by pressing the **9** key and a T or U by pressing the **exp** key. You may enter a sequence of up to 60 nucleotides. As you enter sequence, it will scroll to the left. You can move around in the sequence with the arrow keys. Put the cursor under a nucleotide and press **Cancel** to delete it. Put the cursor under a nucleotide in front of which you want to make an insertion, then type the base to be inserted. Press **Enter** when the sequence has been completely specified. SmartSpec Plus will calculate and display the extinction coefficient and molecular weight of the oligo.

```
Mol ext coef= _____
MW= _____ >=Continue
```

The molar extinction coefficient is displayed with units of liter mole⁻¹ cm⁻¹

- Press the right arrow to continue the assay. SmartSpec Plus will now be ready to measure the absorbance of your oligonucleotide and to convert those absorbance data into concentrations of $\mu\text{g/ml}$ and $\text{pmol}/\mu\text{l}$.

| |
|----------------------|
| Mol ext coef= _____ |
| MW= _____ >=Continue |

Nucleic Acid Calculations

Molecular Weight (MW):

- Length (L): $\text{MW} \approx \text{L} * 310 - 61$
- Sequence or Composition:
DNA: $\text{MW} = (\#A * 312.2) + (\#C * 288.2) + (\#G * 328.2) + (\#T * 303.2) - 61$
RNA: $\text{MW} = (\#A * 312.2) + (\#C * 288.2) + (\#G * 328.2) + (\#U * 289.2) - 61$

Molar Extinction Coefficient (ϵ):

- Length: $\epsilon \approx \text{L} * 10,000 \text{ liter/mole/cm}$
- Composition:
DNA: $\epsilon \approx (\#A * 15480) + (\#C * 7340) + (\#G * 11760) + (\#T * 8850) \text{ liter/mole/cm}$
RNA: $\epsilon \approx (\#A * 15480) + (\#C * 7340) + (\#G * 11760) + (\#U * 9000) \text{ liter/mole/cm}$
- Sequence: See Optical properties of nucleic acids, absorption, and circular dichroism spectra, p. 589, CRC Handbook of Biochemistry and Molecular Biology 3rd Edition, Nucleic Acids - Volume I. Gerald D. Fasman, Ed. CRC Press 1975.

III. DNA and RNA Oligonucleotide Concentration:

- Concentration = $A/(\epsilon b) * 10^6 \text{ pmole}/\mu\text{l}$
- Concentration = $A/(\epsilon b) * \text{MW} * 10^3 \mu\text{g/ml}$
 where A = absorbance, ϵ = molar extinction coefficient in liter/mole/cm,
 b = pathlength in cm and MW = molecular weight.

For cuvettes with a 10 mm (1 cm) pathlength, these equations simplify to:

- Concentration = $A/\epsilon * 10^6 \text{ pmole}/\mu\text{l} = (\mu\text{g/ml}) * (1000)/\text{MW}$
- Concentration = $A/\epsilon * \text{MW} * 10^3 \mu\text{g/ml} = (\text{pmol}/\mu\text{l}) * (\text{MW})/1000$

IV. dsDNA, RNA, ssDNA concentration:

- $A_{260} * \text{Conversion Factor} * \text{Dilution Factor} = \mu\text{g/ml concentration}$

OR

- $(A_{260} - A_{320}) * \text{Conversion Factor} * \text{Dilution Factor} = \mu\text{g/ml concentration}$

Default conversion factors are:

1.0 O.D. unit = 50 $\mu\text{g/ml}$ dsDNA concentration

1.0 O.D. unit = 40 $\mu\text{g/ml}$ RNA concentration

1.0 O.D. unit = 37 $\mu\text{g/ml}$ ssDNA concentration

A_{260}/A_{280} ratios are 1.8 and 2.0 for pure DNA and RNA preparations, respectively.

7.2 Protein Assay

1. **Choosing the type of assay.** Press **Protein** and the display becomes

```
Select assay:  Bradford
```

As you press **Select**, other options are displayed:

```
Select assay:  Lowry
```

```
Select assay:  UV Protein
```

```
Select assay:  BCA
```

```
Select assay:  Other
```

Press **Enter** when the correct type of assay is listed. SmartSpec Plus knows what wavelength to read for the first four assays, but if you select **Other**, then you must enter the wavelength to be read.

```
Enter wavelength:  ____ nm
```

Use the number keys to enter the desired reading wavelength and press **Enter**.

2. **Background subtraction.** You will next be offered a chance to choose a wavelength for a background reading.

```
Do you want to subtract  
background reading?  No
```

If you do not wish to subtract a background reading, press **Enter**. Skip to 3 below. If you do want to subtract a background reading, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
Enter wavelength for the  
background reading:  __ nm
```

- Use the number keys to enter the desired reading wavelength and press **Enter**.

3. **Standard Curves.** Once you have selected the type of assay, SmartSpec Plus will ask if you want to make a new standard curve. You can decline to make a standard curve, in which case it will not be possible for SmartSpec Plus to convert absorbance readings to concentration; you can recall a saved standard curve from memory, or you can create a new standard curve.

```
Do you want to make a
new standard curve? Yes
```

If you want to make a new standard curve, press **Enter** and skip ahead to part B below. If you do not want to make a new standard curve, press **Select** to toggle from **Yes** to **No** and press **Enter**.

- A. **If you choose not to create a new standard curve.** SmartSpec Plus will offer you the opportunity to recall a standard curve from memory.

```
Do you want to recall a
stored std curve? Yes
```

- If you want to recall a stored standard curve, press **Enter** and skip ahead to part ii below. If you don't want to recall a standard curve from memory, press **Select** to toggle from **Yes** to **No** and press **Enter**.

- i. **If you do not recall a standard curve from memory.** SmartSpec Plus will alert you that it will not be able to convert absorbance readings into concentrations.

```
Cannot calculate conc
without std curve. OK? Yes
```

- If you want SmartSpec Plus to convert absorbance to concentration, press **Select** to toggle from **Yes** to **No** and press **Enter**. You will be prompted again to create a new standard curve. If you don't mind that concentrations cannot be calculated, press **Enter** and SmartSpec Plus will be ready to collect absorbance data.

```
Ready to read absorbance
<=Exit Assay    >=Options
```

- ii. **If you want to recall a stored standard curve from memory.**

```
Select Curve:   Bradford
Std Curve #1   N A M E
```

- Press **Select** to scroll through the list of stored standard curves until you see the one you want to recall, and then press **Enter**.

Note that the type of assay being performed when the standard curve was calculated is also displayed.

After you choose the standard curve, SmartSpec Plus will offer you a chance to view the slope, intercept, correlation coefficient and date of creation for the curve. These data will be printed on the final report.

```
Want to view info on std
curve #1 now? No >=Help
```

- In order to see the information about the standard curve, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
r2=x.xxx  CONC=ABS*M +I
0>Show more  >=Continue
```

r² refers to the square of the correlation coefficient and is a value between 0 and 1, with 1 representing a perfectly straight line (see discussion above). The general equation of the curve fit is presented in the top right part of the display

$$\text{Concentration} = \text{Absorbance} * \text{Slope} + \text{Intercept}$$

- Press 0 to see the slope. Return to the assay by pressing the right arrow key or go back to the previous screen by pressing the left arrow key.

```
Slope (M) =  xx.xx  exx
<=Back  0=More  >=Continue
```

If the slope or intercept has a value greater than 9999 or less than 0.0001, it will be shown in exponential notation. In all cases with SmartSpec Plus, e represents the base 10, not the base of the natural logarithm. For example, 100,000 would be represented as 1.000 e 05 and 0.00001 would be represented as 1.000 e-05.

- Press 0 to see the intercept. Return to the assay by pressing the right arrow key or go back to the previous screen by pressing the left arrow key.

```
Intercept (I)=xxx.xx e xx
<=Back 0=More  >=Continue
```

- Press 0 to see the name of the standard curve and the date that it was created. Return to the assay by pressing the right arrow key or go back to the previous screen by pressing the left arrow key.

```
Name: _____  11/25/98
<=Back  >=Continue
```

- Press the right arrow key to return to the assay. SmartSpec Plus is now ready to collect absorbance data and to convert the absorbances into concentrations using the standard curve recalled from memory.

```
Ready to read absorbance
<=Exit Assay  >=Options
```

- B. If you choose Yes, to construct a Standard Curve.** The first step in construction of a standard curve is to blank SmartSpec Plus. The instrument will prompt for the number of blank replicates. You may have as many as 9 blank replicates, or as few as 1.

```
Enter number of blank
replicates (1-9): ____
```

- Use the number keys to input the number of blank replicates and press **Enter**.

SmartSpec Plus will now direct you to put the blanks into the instrument, one after the other.

```
Insert Blank #1/3 and
Press Read Blank
```

- Each time you place a new blank into SmartSpec Plus, press **Read Blank**. After the absorbance reading is collected, the instrument is zeroed and the display will show

```

Blanking
Spectrophotometer

```

You can check the absorbance reading at any time by pressing **Abs**.

```

A750 = 0.000
=>Continue

```

- Press the right arrow key to return to the assay.

After all the blanks are read, SmartSpec Plus will prompt you for the number of standards, the units of concentration for those standards, and then the number of standard replicates. If you do have replicates, then it will ask you if you have the same number of replicates for each standard. SmartSpec Plus allows you to have differing numbers of replicates for each standard.

```

Enter the number of
standards (2-9): ____

```

- Use the number keys to input the number of standards and press **Enter**. Next SmartSpec Plus will prompt for the units of concentration. Your standards must all have the same units.

```

Select concentration
units: mg/ml

```

- Press **Select** until you see the desired units of concentration and then press **Enter**. As you press **Select**, other choices of units are displayed, each new choice representing a reduction of one order of magnitude in concentration, or you can choose to have no units.

```

Select concentration
units: ug/ml

```

```

Select concentration
units: ng/ml

```

```

Select concentration
units: none

```

Next SmartSpec Plus will ask if any of the standards are to be read in replicate.

```

Select concentration
units: ug/ml

```

- If you have prepared any of your standards in replicate, press **Select** to toggle from **No** to **Yes** and press **Enter**. Skip ahead to ii. If none of the standards are prepared in replicate, press **Enter**.

- i. **If there are no standard replicates.** SmartSpec Plus will direct you to enter the concentration of each standard and then to measure the absorbance of each standard.

```
Enter concentration of
standard #1: _____
```

- Use the number keys to input the concentration of standard #1 and press **Enter**.

You may enter concentrations with as many as eight significant digits, but all results are reported with four significant digits because SmartSpec Plus absorbance data are measured to four significant digits.

```
Insert Standard #1 and
press Read Sample
```

- Place the cuvette with standard #1 in SmartSpec Plus and press **Read Sample**.

SmartSpec Plus will continue to prompt for all subsequent standards until the last one is read. Before each standard is measured, you will be asked to specify the concentration of the standard. The units of concentration for all standards must be the same as those specified before the first standard was read.

```
Enter concentration of
Standard #2: _____
```

```
Insert Standard #2 and
press Read Sample
```

•
•
•

```
Enter concentration of
Standard #9: _____
```

```
Insert Standard #9 and
press Read Sample
```

When collecting data for construction of the standard curve, SmartSpec Plus does not display the absorbance data, but you may view it at any time by pressing **Abs**.

```
A750 = x.xxx
Standard #1 >=Continue
```

- Press the right arrow key to return to the assay.

Once all the standards are read, SmartSpec Plus will calculate a standard curve. Skip ahead to C.

- ii. **If there are standard replicates.** If you indicate that one or more of the standards are read in replicate, then you will be asked if there is the same number of replicates for each standard.

```
Same number replicates
for each standard? Yes
```

- If you have varying numbers of replicates for the different standards, press **Select** to toggle from **Yes** to **No** and press **Enter**. Skip ahead to **b**. If you have the same number of replicates for each standard, press **Enter**.

- a. If there are the same number of replicates for each standard.** You can have as many as nine replicates for each standard

Enter the number of
standard replicates: ____

- Use the number keys to input the number of standard replicates and press **Enter**.

Now SmartSpec Plus will prompt for the concentration of the first standard and then for units of concentration.

Enter concentration of
standard #1: _____

- Use the number keys to input the concentration of standard #1 and press **Enter**.

You may enter concentrations with as many as eight significant digits, but all results are reported with four significant digits because SmartSpec Plus absorbance data are measured to four significant digits.

Once you have selected the units of concentration, SmartSpec Plus will begin to collect absorbance measurements on the replicates of standard #1. After all replicates of standard #1 are read, then you will be asked for the concentration of standard #2, and then directed to input the replicates of standard #2. SmartSpec Plus will continue in this manner until all replicates of all standards are measured.

Insert Rep 1 of Std #1
and PRESS Read Sample

- Insert the cuvette with the first replicate of standard #1 into SmartSpec Plus and press **Read Sample**.

SmartSpec Plus will continue to prompt for each additional replicate of standard #1 until all are read.

Insert Rep #2 of Std #1
and PRESS Read Sample

•
•
•

Insert Rep #9 of Std #2
and PRESS Read Sample

After all replicates of standard #1 are measured, SmartSpec Plus asks for the concentration of standard #2 and directs you to put the replicates into SmartSpec Plus one at a time until all are read.

```
Enter concentration of
Standard #2: _____

Insert Rep #1 of Std #2
and PRESS Read Sample

.

.

.

Insert Rep #9 of Std #1
and PRESS Read Sample
```

This process continues until all replicates of all standards have been read.

When collecting data for construction of the standard curve, SmartSpec Plus does not display the absorbance data, but you may view by pressing **Abs**. If you press **Abs**, you must indicate if you want the reading of the current replicate or if you want the mean and standard deviation of the current replicate group. All standard absorbance data can also be printed at the end of the assay.

```
<=Show last replicate
>=Show mean and std dev
```

- Press the left arrow key to view the absorbance of the last replicate.
- or
- Press the right arrow key to view the mean and standard deviation of the absorbance of current replicate group.

Next SmartSpec Plus will prompt for the units of concentration. Your standards must all have the same units. You will only be prompted for units after the first standard.

```
A750 = x.xxx
Std #1 Rep #2 >=Continue
```

or

```
A750 = x.xxx +- x.xxx
Std #1 Mean >=Continue
```

- Press the right arrow key to return to the assay.

After the last replicate of the last standard is measured, SmartSpec Plus will construct the standard curve. Skip ahead to C.

b. If there are varying numbers of standard replicates. Each time SmartSpec Plus prepares to read a standard, you will be prompted for the number of replicates for that standard and then for the concentration of the standard. After all replicates of the first standard are read, then SmartSpec Plus will prompt for the number of replicates of the second standard and then for the concentration of the second standard. This will continue until the last replicate of the last standard is read.

```
Enter number replicates
for Standard #1 (1-9): __
```

- Use the number keys to input the number of replicates of standard #1 and press **Enter**.

Now SmartSpec Plus will prompt for the concentration of the first standard and then for units of concentration.

```
Enter concentration of
Standard #1: _____
```

- Use the number keys to input the concentration of standard #1 and press **Enter**.

You may enter concentrations with as many as eight significant digits, but all results are reported with four significant digits because SmartSpec Plus absorbance data are measured to four significant digits.

SmartSpec Plus will begin to collect absorbance measurements on the replicates of standard #1.

```
Insert Rep #1 of Std #1
and press Read Sample
```

- Insert the cuvette with the first replicate of standard #1 into SmartSpec Plus and press **Read Sample**.

SmartSpec Plus will continue to prompt for each additional replicate of standard #1 until all are read.

```
Insert Rep #2 of Std #1
and press Read Sample
```

•
•
•

```
Insert Rep #9 of Std #1
and press Read Sample
```

After all replicates of standard #1 are measured, SmartSpec Plus asks for the number of replicates of standard #2 and then the concentration of standard #2 before prompting you to insert each of the replicates of standard #2.

Enter number replicates
for Standard #2 (1-9): __

Enter concentration of
Standard #2: _____

Insert Rep #1 of Std #2
and press Read Sample

•
•
•

Insert Rep #5 of Std #2
and press Read Sample

•
•
•

Enter number replicates
for Standard #9 (1-9): __

Enter concentration of
Standard #9: _____

Insert Rep #1 of Std #9
and press Read Sample

•
•
•

Insert Rep #9 of Std #9
and press Read Sample

When collecting data for construction of the standard curve, SmartSpec Plus does not display the absorbance data, but you may view it by pressing **Abs**. If you press **Abs**, you must indicate if you want the reading of the last replicate or if you want the mean and standard deviation of the current replicate group. All standard absorbance data can also be printed at the end of the assay.

<=Show last replicate
>=Show mean and std dev

- Press the left arrow key to view the absorbance of the last replicate.
- or
- Press the right arrow key to view the mean and standard deviation of the absorbance of current replicate group.

The display will show either

```
A750 = X.XXX  
Std #1 Rep #2 >= Continue
```

or

```
A750 = X.XXX = - X.XXX  
Std #1 Mean >= Continue
```

- Press the right arrow key to return to the assay.

After the last replicate of the last standard is measured, SmartSpec Plus will construct the standard curve.

- C. Editing standard curve data.** After the absorbance of the last replicate of the last standard has been read, SmartSpec Plus will offer you a chance to edit the data set before constructing the standard curve. You can re-read a standard or any replicate of a standard group, or you can edit the concentration information for any standard or standard replicate group.

```
Do you wish to modify  
the Standard Curve? No
```

- If you do not want to edit the data, press **Enter** and skip to D below. If you need to edit the data or to re-read one or more samples, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
1. Edit Concentrations  
2. Reread a Std <=Back
```

- To edit a concentration, press 1.

```
Select Standard to edit:  
Standard #1: 123.4567
```

- Press **Select** until the standard you want to edit is displayed and then press **Enter**.

```
Change Conc Std #1 from  
123.45678 to _____
```

- Use the number keys to input the correct concentration and press **Enter**.

```
1. Edit Concentrations  
2. Reread a Std <=Back
```

- Press the left arrow key to return to the assay and complete construction of the standard curve. If you want to re-read a standard, press 2.

```
Enter Standard# _____  
Enter Rep# _____
```

- Use the number keys to input the number of the standard that you want to re-read, press **Enter**, input the replicate number and press **Enter** again.

```
Do you want to reread  
blanks each time? Yes
```

- You can choose to blank the instrument again before you re-read your sample by pressing **Enter** at this screen. If you do not want to re-zero SmartSpec Plus, press **Select** to toggle from **Yes** to **No** and press **Enter**.

SmartSpec Plus will now direct you to insert blank (if you chose that option above) and then to insert the specified standard. When the absorbance data have been collected, SmartSpec Plus will offer you an opportunity to continue re-reading other samples.

```
Continue rereading
Standards? Yes
```

- If you want to re-read another sample, press **Enter**. Otherwise press **Select** to toggle from **Yes** to **No** and press **Enter**.

```
1. Edit Concentrations
2. Reread a Std <=Back
```

- If you have completed the editing, press the left arrow key to return to the assay and SmartSpec Plus will complete the calculation of the standard curve.

D. Completing the standard curve. SmartSpec Plus will now calculate the equation of the new standard curve and offer you a chance to see the slope, intercept, and correlation coefficient of the curve. All this information will be printed at the end of the assay if you choose to print the full report. You may want to view the correlation coefficient before completing the assay; if it is too far from 1.000, you may have constructed a poor standard curve and the concentration calculations may not be very accurate. At the end of the assay, you will be offered a chance to save the standard curve into memory for future use, either as a standard curve or to compare to another standard curve.

After the last standard is read, you will see

```
Calculating
Standard Curve
```

and then once the calculations are completed.

```
Want to view info on new
std curve now? No >=Help
```

- To view the correlation coefficient of the new standard curve, press **Select** to toggle from **No** to **Yes** and then press **Enter**.

```
r2=x.xxx  CONC=ABS*M +I
0>Show more  >=Continue
```

r² refers to the square of the correlation coefficient and is a value between 0 and 1 with 1 representing a perfectly straight line (see discussion above). The general equation of the curve fit is presented in the top right part of the display

$$\text{Concentration} = \text{Absorbance} * \text{Slope} + \text{Intercept}$$

- Press 0 to see the slope. Return to the assay by pressing the right arrow key.

```
Slope (M) = xx.xx exx
<=Back 0=More >=Continue
```

- Press 0 to see the intercept. Press the right arrow to return to the assay or press the left arrow key to see the slope displayed again.

```
Intercept (I)=xx.xx e xx
<=Back  0=More  >=Continue
```

If the slope or intercept has a value greater than 9999 or less than 0.0001, it will be shown in exponential notation. In all cases with SmartSpec Plus, e represents the base 10, not the base of the natural logarithm. For example, 100,000 would be represented as 1.000 e 05 and 0.00001 would be represented as 1.000 e-05.

- Press 0 to see the creation date of the standard curve.

```
Name:                11/25/98
<=Back              >=Continue
```

- From this display, press the left arrow to see the intercept again, or press the right arrow to return to the assay.
- When you return to the assay, SmartSpec Plus will be ready to collect absorbance data and to convert the absorbances into concentrations using the new standard curve.

```
Ready to read absorbance
<=Exit Assay      >=Options
```

- E. Printing Standard Curves.** If you choose to print a full report at the end of an assay, the standard curve will be printed along with the information about the curve, including the concentrations and absorbances of the standards, and the slope, intercept and correlation coefficient of the curve.

You can choose to print the same information about a standard curve when it is first created or when it is recalled from memory. When a standard curve is newly created the following prompt is displayed:

```
Want to view info on new
STD CURVE now? No  >=HELP
```

Press **Select** to toggle from **No** to **Yes** and press **Enter**. Now you can press **Print** to begin printing the standard curve. First the printer is initialized and then you are offered the choice of printing just the graph, just the data used to create the graph, or all the information.

```
Print:  1=Data only
        2=Plot  only 3=both
```

If you choose to print both the plot and the data, the data are first printed and then you are offered a chance to rescale the axes of the plot. You may choose to rescale the Y (absorbance) or the X (concentration) axis, and the other axis will be automatically rescaled at the same time.

```
Rescale standard curve?
NO
```

If you wish to rescale the axes, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
Rescale standard curve?
Absorbance  (Y-axis)
```

If you want to rescale the Y axis, press **Enter**. If you want to rescale the X axis, press **Select** to toggle the display and press **Enter**.

```
Rescale standard curve?  
Absorbance      (X-axis)
```

When you rescale the X axis, you must input the new maximum X value and press **Enter**. A new maximum Y value is automatically calculated and the graph is plotted.

```
Max  conc=150.0000  
Enter new max:
```

When you rescale the Y axis, you must input the new maximum Y value and press **Enter**. A new maximum X value is automatically calculated and the graph is plotted.

```
Max  abs=1.234  
Enter new max:
```

Quantitating UV-proteins:

$$\text{Protein (mg/ml)} = C1 * (A_{280} - A_{320}) - C2 * (A_{260} - A_{320})$$

Coefficients 1 and 2 (defaults 1.55 and 0.76, respectively) can be changed for different proteins.

7.3 Spectral Scanning

SmartSpec Plus can collect data over a range of wavelengths and then plot those absorbances. There are both Fast and Slow scans: the fast scan generally takes a minute or less to accomplish while the slow will take several minutes, but results in more accurate absorbance data and a smoother curve. The scanning time is dependent on the range scanned and the absorbance of the sample. You can improve the accuracy of fast scans by specifying more than one repetition of the scan. The scan can be repeated as many as five times and the readings are averaged before plotting; five fast scans can be carried out more rapidly than one slow scan.

SmartSpec Plus will identify the local maximum and minimum over the scanned region, or you can specify as many as four different subregions within the scanned area, and SmartSpec Plus will identify the maximum and minimum in each subregion. This is available through the Options menu.

SmartSpec Plus can smooth the curve by taking a running average of absorbance readings at 5 consecutive wavelengths. For example, it will average the absorbance readings at 255, 256, 257, 258, and 259 nm and plot this average at 257. Then it will average the absorbance readings at 256, 257, 258, 259, and 260 and plot this average at 258 nm. This is available through the Options menu.

When you press **Scan**, SmartSpec Plus will prompt for upper and lower limits of the scan.

```
Begin scanning at ____ nm
End scanning at ____ nm
```

- Use the number keys to input the starting wavelength and press **Enter**. Then use the number keys to input the ending wavelength and press **Enter**. You can move inside the number fields with the right and left arrow keys.

```
Do you want to subtract
a background reading? No
```

- If you do not want to subtract a background reading, press **Enter** and SmartSpec Plus is ready to begin the scan. If you do want to subtract a background, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
Enter wavelength for
background: _____ nm
```

- Use the number keys to input the background wavelength and press **Enter**.

SmartSpec Plus next lets you choose between the Fast and Slow scans.

```
Select type of scan: Fast
```

- If you prefer the slow scan, press **Select** to toggle from **Fast** to **Slow** and press **Enter**. Press **Enter** to accept a Fast scan.

If you choose the Fast scan, then you can specify the number of repetitions.

```
Enter the number of
successive scans (1-5):__
```

- Use the number keys to input the number of successive scans and press **Enter**.

SmartSpec Plus will now display the Ready screen. If you want to invoke the smoothing option or the subregion option, choose Options from the Ready screen.

```
Ready to read absorbance
<=Exit Assay    >=Options

1. Repetitions  2. Range
3. Background   >=More
```

The first three options allow you to change the conditions already specified above. Press the right arrow key to see the other options.

```
4. Subregions  5. Smooth
<=Back,  Cancel = return
```

If you choose Regions, SmartSpec Plus will prompt for the number of regions.

```
Enter number of
subregions (0-4): ____
```

- Use the number keys to input the number of regions. Choose 0 if you don't want any subregions. If you specify 1 or more subregions, then you are next prompted to define them.

```
Subregion #1:
Start:____ nm.  End:____nm
```

- Use the number keys to input the beginning wavelength and press **Enter**. Then use the number keys to input the ending wavelength for the first subregion and press **Enter** again. After you define the last subregion, the Option screen is displayed again.

```
4. Subregions  5. Smooth
<=Back,  Cancel = return
```

- To turn smoothing on, press 5.

```
Select Smoothing: Off
```

- Press **Select** to toggle from **Off** to **On** and press **Enter**.

7.4 Kinetics

When you press Kinetic, you begin by specifying the reading wavelength.

```
Enter reading wavelength
for kinetic assay: ___ nm
```

- Use the number keys to input the wavelength to be read and press **Enter**.

```
Enter duration of timed
assay : _____ seconds
```

- Use the number keys to input the duration of the assay in seconds and press **Enter**. You may collect data for as long as 9999 seconds.

```
Enter interval of data
collection: _____ sec
```

- Use the number keys to input the interval between successive readings and press **Enter**. The shortest interval is 1 second and the longest is 999 seconds.

```
Do you want to subtract
a background reading? No
```

- If you do not want to subtract a background reading, press **Enter** and SmartSpec Plus will be ready to begin collecting kinetic data. If you want to subtract a background reading, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
Enter wavelength for the
background reading: __ nm
```

- Use the number keys to input the background wavelength and press **Enter**.

SmartSpec Plus is now ready to collect kinetic absorbance data at the specified wavelength. For best results, you should first zero the SmartSpec Plus by inserting a cuvette containing blank solution and pressing **Read Blank**.

```
Ready to read absorbance
<=Exit Assay   >=Options
```

After the kinetic data are collected, SmartSpec Plus will reset for the next scan and then display the Ready screen. If you want to change any of the kinetic parameters, you can do that without exiting the assay if you choose Options from the Ready screen.

7.5 OD600

This assay is useful for monitoring growth of bacterial cultures. When you press **OD600**, SmartSpec Plus will display the current conversion factor and offer you the choice of accepting or modifying it.

```
A600 1.0=xx.xxxx cell/ml  
Is this factor OK? Yes
```

- If the displayed conversion factor is acceptable, press **Enter**. Otherwise, press **Select** to toggle from **Yes** to **No** and press **Enter**.

```
Enter new conversion  
factor; xx.xxxx cell/ml
```

- Use the number keys and the **exp** key to input the new conversion factor and press **Enter**.

SmartSpec Plus is now ready to collect OD600 readings. For best results, you should first zero the SmartSpec Plus by inserting a cuvette with media (without cells) and pressing **Read Blank**.

```
Ready to read absorbance  
<=Exit Assay    >=Options
```

Cells/ml:

Cells/ml = A600 * Conversion Factor * dilution factor

Default conversion factor is 5×10^8 cells/ml

7.6 Lambda λ

This assay allows you to collect absorbance data from up to three different wavelengths simultaneously. No concentrations are calculated. When you press λ , SmartSpec Plus will prompt you first for the number of wavelengths to read.

```
Enter number (1-3) of
wavelengths to read: 3
```

- Use the number keys to input the number of wavelengths to be read and press **Enter**. SmartSpec Plus will then ask for each individual wavelength.

```
Enter wavelength #1:
_____ nm
```

- Use the number keys to input the wavelength and press **Enter**.

After all wavelengths are entered, choose if you want to subtract a background reading from each wavelength. (The same background measurement is subtracted from all readings.)

```
Do you want to subtract
a background reading? No
```

- If you do not want to subtract a background reading, press **Enter** and SmartSpec Plus will be ready to collect absorbance data. If you want to subtract a background reading, press **Select** to toggle from **No** to **Yes** and press **Enter**.

```
Enter wavelength for the
background reading: ___ nm
```

- Use the number keys to input the wavelength and press **Enter**.

SmartSpec Plus is now ready to collect absorbance data at the specified wavelengths. For best results, you should first zero the SmartSpec Plus at specified wavelengths by inserting a blank solution and pressing **Read Blank**.

```
Ready to read absorbance
<=Exit Assay    >=Options
```

Section 8 Troubleshooting

Troubleshooting Table

| Problem | Cause | Solution |
|--|--|---|
| Unit does not power up | Power switch in off position. | 1. Check power switch on back of unit. |
| | No power from outlet. Bad power cord. | 2. Check power source. 3. Replace. |
| LCD display difficult to read | Back lighting burned out. | 1. Call Bio-Rad service. |
| | Contrast setting out of adjustment. | |
| Printer jam | Thermal paper caught between print head and thermal plate when tearing off paper. | 1. Remove excess paper between printer head and plate using fine tip tweezers. |
| | | 2. Power down unit, remove thermal paper roll and re-load paper in printer. |
| Printer paper would not self feed or advance | Printer paper inserted incorrectly, leading edge of paper uneven or folded over. | 1. Cut a straight clean leading edge on paper with a pair of scissors. |
| | Paper feed button keyboard is faulty. | 2. Call Bio-Rad service. |
| Print out text too dark | Printer paper damaged caused from excessive heat, moisture. | 1. Replace with new paper roll. |
| | Paper loaded incorrectly. | 2. Be sure the paper is installed in the proper orientation. (refer to illustration in Section 9.3) |
| | Thermal print head/plate damaged. | 3. Replace printer. Call Bio-Rad service. |
| Print out text too light or not visible | 1. Paper loaded incorrectly (incorrect orientation). | 1. Be sure the paper is installed in the proper orientation (refer to illustration in Section 9.3) |
| | 2. Printer paper damaged by excessive heat, moisture. | 2. Replace the printer paper roll. |
| | 3. Thermal print head/plate damaged. | 3. Call Bio-Rad Technical Service. |
| Keyboard buttons not responding | Incomplete system initialization during power up. Pressed wrong button for operation. | 1. Power down unit/Restart unit. |

| Problem | Cause | Solution |
|---|--|--|
| RS232 serial port/no response | Incomplete system initialization during power up. PC setup incorrect/wrong instruct mode. Cable's not fully connected/wrong adaptor. Serial Port not selected in Setup | 1. Power down unit/Restart unit. 2. Check spreadsheet program setup. 3. Check all connections. 4. Enter Setup function and ensure that the serial port is activated. |
| Cuvette does not fit in cuvette chamber | Cuvette wrong size. Cuvette spring in wrong location. Cuvette spring missing. | 1. Chamber accommodates cuvettes up to 12.5 mm width. 2. Re-insert spring to correct location. 3. Obtain new cuvette spring. |
| Negative values/no absorbancy | No sample. Cuvette light path orientation incorrect. Wrong wavelength setting. May have blanked the instrument using sample instead of blank solution. Cuvette window "Z" height incompatible with cuvette chamber. (See Section 11.4.) | 1. Add Sample to solution 2. Check cuvette window orientation. 3. Check assay wavelength requirement. 4. Reblank the instrument using buffer or water. 5. Use only 8.5 mm "Z" dimension cuvettes. |
| Fluctuations | Interference in light path. | 1. Check that there are no scratches on the cuvette. 2. Check that there are no particles floating in the cuvette. 3. Wipe the cuvette walls with lint-free tissue. 4. Make sure the sample is in the recommended working range <i>i.e.</i> 0.1–1.5 AU. 5. Clean cuvette chamber as described in Section 9.1). |

Section 9 Maintenance

9.1 Cleaning Up a Fluid Spill

In case of a spill in the cuvette chamber, clean up the spill using the following procedure:

1. Turn the instrument off and unplug the power cord.
2. Use a damp cotton swab to clean the cuvette chamber.
3. Use a cotton swab to dry the cuvette chamber.

4. Dampen cloth with water. Use it to clean the instrument from the outside, including the display and the keyboard.

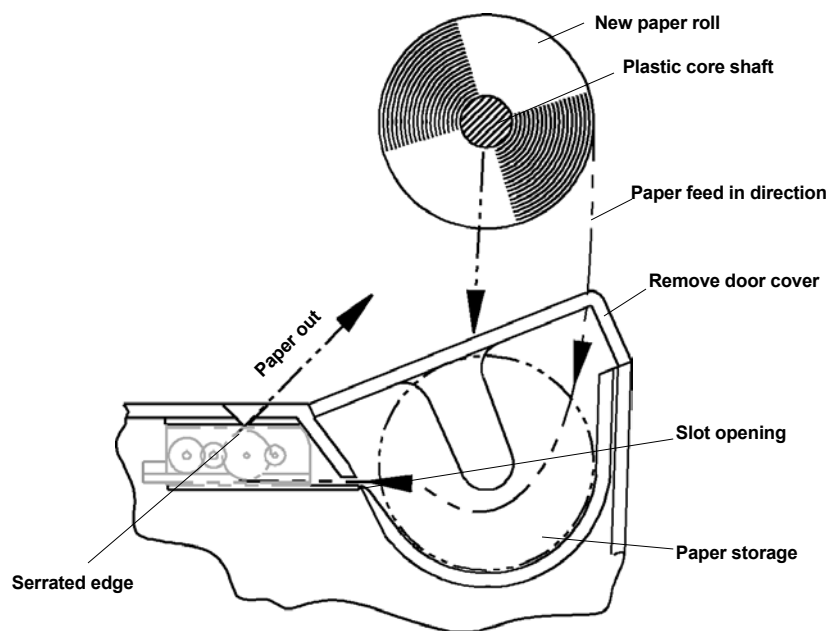
9.2 Cleaning Quartz Cuvettes

It is important to maintain the cleanliness of quartz cuvettes so that residues from prior analyses do not contribute errors to future measurements. For most biological samples, the following cleaning procedure will help keep these cuvettes working optimally for your spectrophotometry system.

1. Wash cuvette with warm water.
2. Clean the cuvette with a mild acidic solution (0.1M HCl).
3. Rinse the cuvette with copious amounts of water.

Be sure not to use abrasive materials to clean the cuvettes as they may scratch the optical surfaces and shorten the life of the cells. Similarly, care must be taken not to use highly acidic (HF) or highly basic ($>pH 9$) to clean the cells as the optical surfaces could be damaged.

9.3 Changing Thermal Printer Paper



Installing New Thermal Printer Paper

Note: Use only Bio-Rad recommended thermal paper catalog number 170-2506.

- Remove printer paper door cover by pushing down on the center of the door and sliding the door back away from the unit.
- Remove plastic core shaft from old paper roll and insert into center hole in new paper roll.
- Un-roll enough paper to allow for enough space to feed leading edge of paper into slot opening in paper storage area as shown in above figure.

Note: Feed paper from bottom of roll as shown. Do not fold or crease leading edge of paper.

- Trim leading edge of paper roll with scissors if uneven or torn.
- Feed paper into slot opening until it stops. Press the "Paper Feed" key located on the keypad and guide the paper into the printer until it grabs and self feeds.
- Continue to press "Paper Feed" button until paper comes out of printer outlet located on top of case as shown.
- Wind any excess paper back on roll and drop new paper roll with shaft into grooves in paper storage area as shown.
- Re-install printer door cover.

Tearing Paper

- When tearing off print outs, always tear paper forward against serrated edge of printer outlet as shown on previous page.

Section 10 Specifications

| Characteristic | SmartSpec Plus Specification |
|--------------------------------------|--|
| Light Source | Xenon lamp |
| Detector | Photodiode array |
| Display | 2 x 24 alphanumeric backlit LCD |
| Printer | Built-in alphanumeric printer |
| Interface | RS-232 serial port |
| Wavelength Range | 200 to 800 nm |
| Wavelength Accuracy (18–27°C) | < ±1.0 nm from 200–250, 300–800 nm < ±0.5 nm from 250 to 300 nm |
| Spectral Bandwidth | <5 nm |
| Absorbption Accuracy | ±0.01 @ 0.5 AU ±0.02 @ 1.0 AU |
| Absorbption Repeatability | ±0.005 @ 0.5 AU |
| Stray Light | <0.2% @ 230 nm using NaI Standard |
| Operating Temperature | 15 to 35°C |
| Beam Height (Z-dimension) | 8.5 mm |
| Dimensions | 33 x 26 x 14 cm (W x D x H) |
| Weight | ~3.9 kg |
| Power Requirement | 90–260 VAC, 47–63 Hz |
| Regulatory | CE, TÜV |

Section 11

System, Accessories, and Reagents for the SmartSpec Plus

11.1 Product Information

| Catalog Number | Product Description |
|---|--|
| SmartSpec Plus Spectrophotometer and Accessories | |
| 170-2525 | SmartSpec Plus Spectrophotometer |
| 170-2502 | Standard 1–3.5 ml Cuvette , quartz |
| 170-2503 | Semi-microvolume 1.4 ml Cuvette , quartz |
| 170-2504 | Microvolume 700 µl Cuvette , quartz |
| 170-2505 | Sub-microvolume 100 µl Cuvette , quartz |
| 170-2415 | Standard Cuvette, 1–3.5 ml , polystyrene disposable cuvettes, 100 |
| 223-9950 | Standard 3.5 ml Disposable Polystyrene Cuvettes , 100 |
| 223-9955 | Semi-microvolume 1.5 ml Disposable Polystyrene Cuvettes , 100 |
| 170-2506 | Printer Paper , 5 pk |
| 170-2507 | Spring , cuvette holder |
| 170-2509 | Performance Validation Package , includes NIST traceable cuvette, certificate of analysis |

11.2 Bio-Rad Protein Assays

| Catalog Number | Product Description |
|----------------|---|
| 500-0001 | Bio-Rad Protein Assay Kit I , 450 standard assays, bovine gamma globulin standard, based on the method of Bradford |
| 500-0002 | Bio-Rad Protein Assay Kit II , 450 standard assays, bovine serum albumin standard, based on the method of Bradford |
| 500-0006 | Bio-Rad Protein Assay Dye Reagent Concentrate , 450 ml |
| 500-0005 | Protein Standard I , bovine gamma globulin |
| 500-0007 | Protein Standard II , bovine serum albumin |
| 500-0111 | Bio-Rad DC Protein Assay Kit I , 2,500 standard assays, bovine gamma globulin |
| 500-0112 | Bio-Rad DC Protein Assay Kit II , 2,500 standard assays, bovine serum albumin |
| 500-0116 | Bio-Rad DC Protein Assay Reagents Package , contains 250 ml alkaline copper tartrate solution, 2,000 ml dilute Folin reagent, 5 ml surfactant solution |

11.3 Cuvette Selection Guide

| Catalog Number | Minimum Volume | Maximum Volume | Cuvette Type | Pathlength |
|----------------|----------------|----------------|----------------------------------|------------|
| 170-2502 | 1,000 µl | 3,500 µl | Standard cuvette, quartz | 10 mm |
| 170-2503 | 500 µl | 1,400 µl | Semi-microvolume cuvette, quartz | 10 mm |
| 170-2504 | 200 µl | 700 µl | Microvolume cuvette, quartz | 10 mm |
| 170-2505 | 80 µl | 100 µl | Sub-microvolume cuvette, quartz | 10 mm |

11.4 Selecting the Appropriate Cuvette

The SmartSpec Plus has 1 mm, 2 mm, 5 mm and 10 mm pathlengths. For most absorbance measurements you will be using 10 mm pathlength cuvettes. Cuvettes of shorter pathlength allow the measurement of concentrated samples without dilution. If you put a solution in a 10 mm pathlength cuvette and get a reading of 2.0 AU, you can use a 5 mm pathlength cuvette to get an absorbance of 1.0 AU for the same solution. SmartSpec Plus corrects for pathlengths shorter than 10 mm when converting absorbances into concentration.

The following table gives you the absorbance at A260 and corresponding concentration for the different pathlength cuvettes.

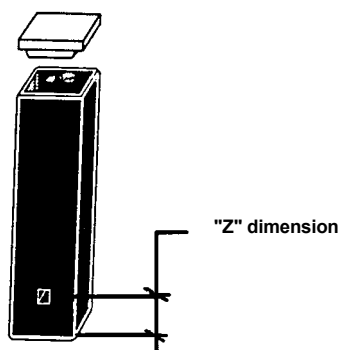
| Pathlength | A260 = 0.1 AU | A260 = 1.5 AU** |
|------------|---------------|-----------------|
| 10 mm | 5 µg/ml* | 75 µg/ml |
| 5 mm | 10 µg/ml | 150 µg/ml |
| 2 mm | 25 µg/ml | 375 µg/ml |
| 1 mm | 50 µg/ml | 750 µg/ml |

* DNA concentration based on 1 O.D. = 50 µg/ml for dsDNA

** Working range of the SmartSpec Plus is 0.1–1.5 AU per Good Laboratory Practice.

Cuvettes that can fit the SmartSpec Plus should have 8.5 mm 'Z' dimension and either 1, 2, 5 or 10 mm pathlength and 12.5 mm width. In general there are two types of 'Z' dimensions: 15 mm and 8.5 mm. This is only applicable for sub microvolume cuvettes *i.e.*, for volumes 100 µl or lower. In most cases you will use 10 mm pathlength cuvettes.

The SmartSpec Plus has 8.5 mm 'Z' dimension. This is 8.5 mm from the bottom of the cuvette and it is the distance required for the light path to hit the sample. See Figure below.





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