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SmartSpec™ 3000 Spectrophotometer

Instruction Manual

Catalog Number
170-2501

BIO-RAD

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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 3000 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 EN61010-1[*] safety standards. Certified products are safe to use when operated in accordance with the instrument manual. This instrument should no be modified or altered in any way.

Alteration of this instrument will:

Void the manufacturer's warranty
Void the EN61010-1 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 3000 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.

[*] EN61010-1 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 3000 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 3000 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 10.1).
6. Do not place objects on the SmartSpec 3000.
7. The SmartSpec 3000 has no customer-serviceable components, do not open the unit.

Section 2
Product Description

2.1 Packing List

The SmartSpec 3000 Spectrophotometer (catalog number 170-2501) 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 3000 Spectrophotometer	1
Power cable	1
Cuvette spring	1
Thermal paper roll	1

2.2 Identification of System Components



Fig. 1. View of the SmartSpec 3000 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 3000 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 3000 sample compartment.

9. Cuvette chamber	Insert cuvette into the cuvette chamber. Insure 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.

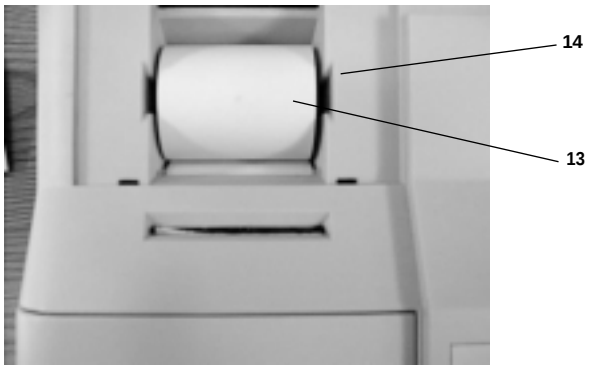


Fig. 4. View of the SmartSpec 3000 integrated thermal printer paper with door removed.

13. Thermal printer paper	Roll of thermal printer paper.
14. Printer paper shaft	

Section 3
Installation

3.1 Environmental Requirements

To insure correct operation and stable performance over an extended period of time, install the SmartSpec 3000 in a location which meets the following conditions:

- Room temperature between 20 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 3000, it will go through a self-diagnostic routine during which it tests the lamp, optical components, diode array, power supply and other components. If a problem is detected, SmartSpec 3000 will alert you to 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

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 3000 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. In the SmartSpec 3000, the xenon flash lamp pulses light only when measuring absorbance of a sample.

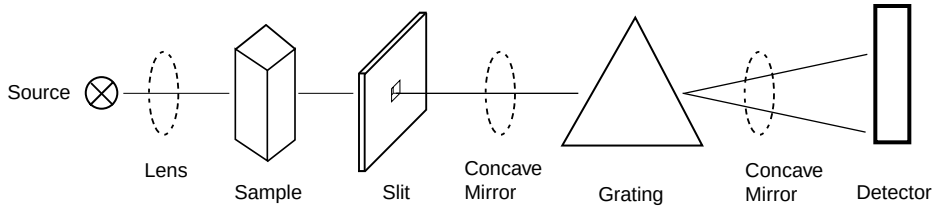


Fig. 5. A symbolic representation of the SmartSpec 3000 optics module.

The path of light in the SmartSpec 3000 is as follows:

Light from the xenon flash lamp
↓
Lens
↓
Sample
↓
Slit
↓
Concave mirror
↓
Grating
↓
Concave mirror
↓
Photodiode array detector

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)

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

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

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

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

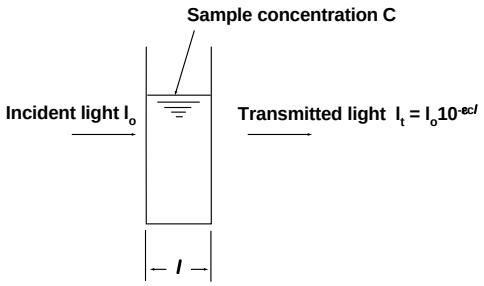


Fig. 6. Lambert-Beer's law.

$$A = \log 1/T$$

$$A = \varepsilon cl$$

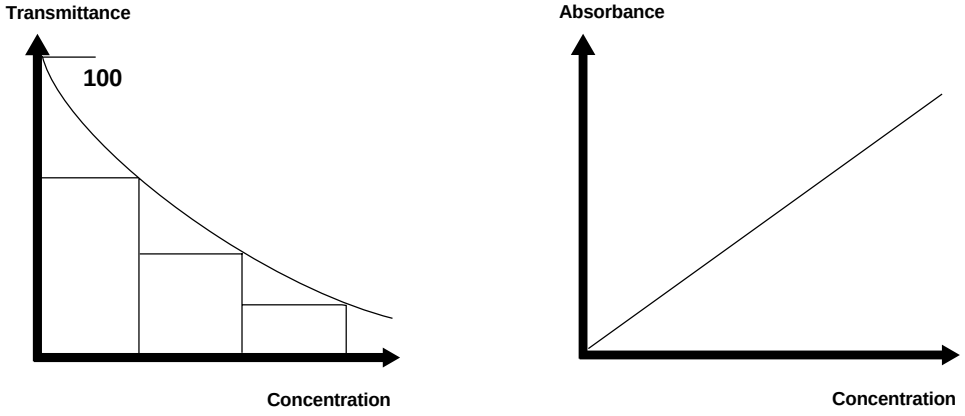


Fig. 7. Relationship between Transmittance and Concentration and Absorbance and Concentration.

4.1 Setup

When the SmartSpec 3000 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 3000 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.

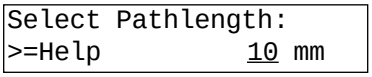
In Setup you can specify the following:

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

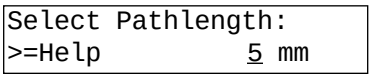
1. **Pathlength**. The pathlength describes the distance that the light travels as it passes through the sample, and is the same as the width of the cuvette. By using a cuvette with a shorter pathlength, you can measure the absorbance of more concentrated solutions. The relationship between absorbance,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

$$A = \varepsilon bc$$

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.



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



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

2. **Background compensation**. When using the DNA/RNA assay, you can choose to have SmartSpec 3000 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 subtract the A320 background. You can toggle background subtraction on and off by pressing **Select**.

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

3. **Default type of Nucleic Acid**. SmartSpec 3000 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
```

4. **Default method to determine oligo molar extinction coefficient and molecular weight**. SmartSpec 3000 uses the molecular weight and molar extinction coefficients of oligonucleotides to convert the absorbance readings into mass (µg/ml) and molar (pmol/µl) concentrations. You can input the molecular weight and molar extinction coefficient directly if you know them, otherwise SmartSpec 3000 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**.

5. **Default number of sample replicates**. SmartSpec 3000 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:_______
```

6. **New Operator**. When SmartSpec 3000 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.

7. **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.

8. **Serial port export.** When this feature is activated, absorbance data and concentration data are sent out through the serial port in all assays. Otherwise only the Scan and Kinetic assay data can be sent out the serial port.

```
 Do you want to send data
 through serial port?  No
```

   - To export data through the serial port, press **Select** to toggle from **No** to **Yes** and press **Enter**. If this feature is active, then absorbance data from the DNA/RNA, Protein, OD600 and λ assays will be exported when you press **Print**. Those data will be printed at the same time.

9. **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.

10. **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.

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

12. **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
```

13. **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.

4.2 Serial Port Functions

The SmartSpec 3000 has the ability to communicate with an external printer or computer via the serial port located at the back of the instrument. The SmartSpec 3000 sends data to these peripherals as ASCII text. When connecting the SmartSpec 3000 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).

DB25F Connector to SmartSpec 3000 3000 (male)	**Signal and Direction**	**DB25M Connector to serial device**
2	TX - - >	3
3	< - - RX	2
4	RTS - - >	5
5	< - - CTS	4
7	Common	7
20	DTR - - >	20

(other pins are open in SmartSpec 3000)

The serial port parameters are: 9600 baud rate, 8 data bits, no parity, 1 stop, and no 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 is sent from the SmartSpec 3000 to a computer or serial printer as an extended hex character. If your computer or printer is not set to accept this character please refer to the instrument's instruction manual for correct settings. (For detailed instructions see Section 12.5.)

Section 5
Keypad Description

Print

SmartSpec 3000 will print data on the current sample, a user-selectable range of samples or all samples in the current assay.

Abs

SmartSpec 3000 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 µg/ml to pmoles/µl. This key has no function for the Scan, Kinetics and λ assays.

A260:A280

SmartSpec 3000 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.

Setup

SmartSpec 3000 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 listed below. Press one of these to begin an assay with SmartSpec 3000. When any assay button is pushed, SmartSpec 3000 resets the dilution factor to 1.0, the sample number to 1, and checks the data buffer for unprinted data.

DNA/RNA

SmartSpec 3000 will automatically collect absorbance data at 260, 280 and 320 nm, and use those readings to calculate nucleic acid concentration. The calculation of concentration depends on the type of nucleic acid specified.

Protein	SmartSpec 3000 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 3000 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 3000.
Scan	SmartSpec 3000 will collect and print the absorbance of a sample over a user-defined range of wavelengths. 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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.
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 3000 calculations. The exp key is used for making entries in exponential notation, and when it is pressed, an **ε** is displayed by SmartSpec 3000. It refers to the base 10 and not to the base of the natural logarithm.

Section 6
Quick Guide

1. Power up the instrument. It will go through system status check and alert you if there are 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 3000 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 3000 will not be able to convert absorbance to concentration.

C. Scan

i. Set upper and lower limits of scan.

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 3000 and press **Read Blank**.
5. Collect absorbance data. Place cuvette containing sample solution into SmartSpec 3000 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 µg/ml and pmole/µl; press **Conc** to toggle between them. If absorbance or concentration data area displayed, press Enter to return the Ready screen, or simply put the next sample cuvette into SmartSpec 3000 and press **Read Sample**.
7. After last sample, press left arrow key to exit assay.
8. Save the standard curve (if you made a new one) and print the full report.

Section 7
Operating Instructions

Note: It is Good Laboratory Practice (GLP) to use the Smart Spec 3000 spectrophotometer to measure absorbances in the range of 0.100 to 1.500 AU.

1. **Choose an assay**. SmartSpec 3000 has six different assay keys in the second row of buttons. When you press one of them, it signals SmartSpec 3000 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 3000 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 3000 will automatically collect absorbance readings at 260, 280 and 320 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.

   Concentration = A260 * Conversion Factor * Dilution Factor

   or

   Concentration = (A260–A320) * Conversion Factor * Dilution Factor

   SmartSpec 3000 is programmed with default conversion factors for dsDNA, ssDNA and RNA. These factors can be modified at any time.

Species	**A260 of 1.000**
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 **Conc**. In order to make these determinations, SmartSpec 3000 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 3000 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 11.2 for a complete explanation of the calculations.

   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 mm) 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 3000 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 3000 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, absorbance readings cannot be converted to concentrations.

The UV Protein assay, developed to find 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 3000 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 3000 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 3000'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 firmware 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 3000 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 3000.

D. **Kinetic**. SmartSpec 3000 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 3000 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 $5x10^8$ cells/ml, then the conversion factor would be $5x10^8$. On the SmartSpec 3000 $5x10^8$ 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 have to have the same number of replicates for each different sample. For each sample replicate group, SmartSpec 3000 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 3000 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 have 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 3000 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 3000 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 3000 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 then SmartSpec 3000 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 3000 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/µl.

```
A260=  x.xxx      Samp #1
Conc=  xxx.xxx  pmoles/µl
```

- 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      µg/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 3000 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 3000 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 3000 will prompt you to save a newly created standard curve 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 8
Description of Assays

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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 µg/ml for an A260 of 1.0). If you do not know either the molar extinction coefficient or the mass/absorbance conversion factor, SmartSpec 3000 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 = _________ µg/ml
```

- Use the number keys to input the conversion factor and press **Enter**. SmartSpec 3000 will now need the molecular weight of the oligonucleotide in order to convert absorbance measurements into concentrations of µg/ml and pmol/µl.

```
  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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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.

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

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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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 3000 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

Concentration = Absorbance*Slope + 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 3000, e represents the base 10, not the base of the natural logarithm. For example, 100,000 would be represented as 1.000 ε 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 ε 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 3000 is now ready to collect absorbance data and to covert 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 3000. 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 3000 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 3000, 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 3000 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 3000 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 3000 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:  µg/ml
```

```
 Select concentration
    units:  ng/ml
```

```
 Select concentration
     units:  none
```

Next SmartSpec 3000 will ask if any of the standards are to be read in replicate.

```
 Select concentration
    units:  µg/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 3000 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 3000 absorbance data are measured to four significant digits.

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

- Place the cuvette with standard #1 in SmartSpec 3000 and press **Read Sample**.

SmartSpec 3000 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 3000 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 3000 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 a 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 3000 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 3000 absorbance data are measured to four significant digits.

Once you have selected the units of concentration, SmartSpec 3000 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 3000 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 3000 and press **Read Sample**.

SmartSpec 3000 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 3000 asks for the concentration of standard #2 and directs you to put the replicates into SmartSpec 3000 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 3000 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 3000 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 3000 will construct the standard curve. Skip ahead to **C**.

b. If there are varying numbers of standard replicates. Each time SmartSpec 3000 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 3000 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 3000 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 3000 absorbance data are measured to four significant digits.

SmartSpec 3000 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 3000 and press **Read Sample**.

SmartSpec 3000 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 3000 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 3000 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 with 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 3000 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 3000 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 replicated 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 3000, press **Select** to toggle from **Yes** to **No** and press **Enter**.

SmartSpec 3000 will now direct you to insert blank (if you chose that option above) and the to insert the specified standard. When the absorbance data have been collected, SmartSpec 3000 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 3000 will complete the calculation of the standard curve.

D. Completing the standard curve. SmartSpec 3000 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

Concentration = Absorbance*Slope + 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 3000, 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 3000 will be ready to collect absorbance data and to covert 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:
```

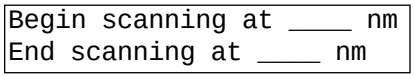
Scan.

SmartSpec 3000 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.

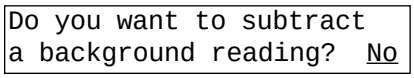
SmartSpec 3000 will identify the local maximum and minimum over the scanned region, or you can specify as many as four different subregions within the scanned area, and SmartSpec 3000 will identify the maximum and minimum in each subregion. This is available through the Options menu.

SmartSpec 3000 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.

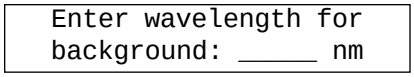
When you press **Scan**, SmartSpec 3000 will prompt for upper and lower limits of the scan.



- 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.

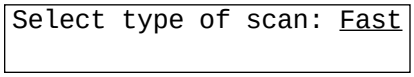


- If you do not want to subtract a background reading, press **Enter** and SmartSpec 3000 is ready to begin the scan. If you do want to subtract a background, press **Select** to toggle from **No** to **Yes** and press **Enter**.



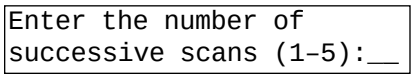
- Use the number keys to input the background wavelength and press **Enter**.

SmartSpec 3000 next lets you choose between the Fast and Slow scans.



- 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.



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

SmartSpec 3000 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 3000 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**.

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 3000 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 3000 is now ready to collect kinetic absorbance data at the specified wavelength. For best results, you should first zero the SmartSpec 3000 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 3000 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.

OD 600.
This assay is useful for monitoring growth of bacterial cultures. When you press **OD600**, SmartSpec 3000 will display the current conversion factor and offer you the choice of accepting or modifying it.

```
A600 1.0=xx.xexx 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.xexx  cell/ml
```

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

SmartSpec 3000 is now ready to collect OD600 readings. For best results, you should first zero the SmartSpec 3000 by inserting a cuvette with media (without cells) and pressing **Read Blank**.

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

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 3000 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 3000 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 3000 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 3000 is now ready to collect absorbance data at the specified wavelengths. For best results, you should first zero the SmartSpec 3000 at specified wavelengths by inserting a blank solution and pressing **Read Blank**.

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

Section 9
Troubleshooting

9.1 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.	2. Check power source.
	Bad power cord.	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.
	Thermal print head/plate damaged.	3. Replace printer. Call Bio-Rad service.
Keyboard buttons not responding	Incomplete system initialization during power up.	1. Power down unit/Restart unit.
	Pressed wrong button for operation.	
RS232 serial port/no response	Incomplete system initialization during power up.	1. Power down unit/Restart unit.
	PC setup incorrect/wrong instruct mode.	2. Check spreadsheet program setup.
	Cable's not fully connected/ wrong adaptor.	3. Check all connections.
Cuvette does not fit in cuvette chamber	Cuvette wrong size.	1. Chamber accommodates cuvettes up to 12.5 mm width.
	Cuvette spring in wrong location.	2. Re-insert spring to correct location.
	Cuvette spring missing.	3. Obtain new cuvette spring.

Problem	Cause	Solution
Negative values/no absorbancy	No sample.	1. Add Sample to solution
	Cuvette light path orientation incorrect.	2. Check cuvette window orientation.
	Wrong wavelength setting.	3. Check assay wavelength requirement.
	May have blanked the instrument using sample instead of blank solution.	4. Reblank the instrument using buffer or water.
	Cuvette window "Z" height incompatible with cuvette chamber. (See Section 12.4.)	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.e.* 0.1–1.5 AU.

Section 10
Maintenance

10.1 Cleaning Up a Fluid Spill

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

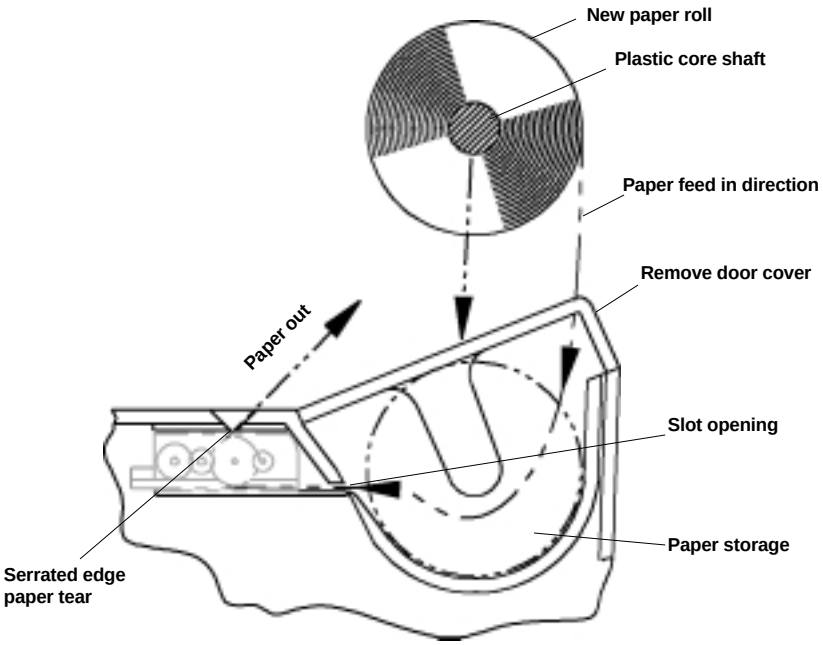
1. Turn the instrument off and unplug the power cord.
2. Use a syringe to clean the cuvette chamber.
3. Use a cotton swab to dry the cuvette chamber.
4. Dampen cloth with water. Use it to clean the instrument from the outside, including the display and the keyboard.

10.2 Cleaning Quartz Cuvettes

Wash cuvettes using a dilute alkaline solution such as 0.1 M NaOH and a dilute acid such as 0.1 M HCl. Rinse cuvettes several times with distilled water. Always use clean cuvettes before taking absorbance measurements.

Rigourous cleaning may be required after using difficult samples. In such cases follow the manufacturer's instructions and use a suitable detergent to clean the cuvettes.

10.3 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.
- 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.

Section 11
Specifications

11.1 Specifications

Product Specifications	SmartSpec 3000 Spectrophotometer
Wavelength range: UV and Visible ranges	200 to 800 nm
Wavelength accuracy	(+/-) 2 nm
Spectral bandwidth	5 nm
Light source	Xenon flash lamp
Detector	Photodiode array
Number of cuvettes	one
Size of cuvettes	100 µl, 700 µl, 1.5 ml, 3.5 ml
Pathlength	1 mm, 2 mm, 5 mm and 10 mm
Display	2 x 24 alphanumeric built-in backlit LCD display
Printer	Built-in alphanumeric/graphic
Interface	RS-232 serial port
Operating temperature	5–40 °C
Photometric range	0–2 AU
Scan speed	200–800 nm in 20 seconds max
Photometric repeatability	± 0.02 AU in a 0.1–2.0 AU range (9 readings) and over a 200–800 nm range.
Dimensions	33 cm (W) x 26 cm (D) x 14 cm (H)
Weight	3.6 k.g.
Power requirement	90–260 VAC, 47–63 Hz
Regulatory	CE, TÜV

11.2 Calculations

I. Molecular Weight (MW):

- Length (L): MW ≈ L * 310 - 61
- Sequence or Composition:

DNA: MW = (#A * 312.2) + (#C * 288.2) + (#G * 328.2) + (#T * 303.2) - 61

RNA: MW = (#A * 312.2) + (#C * 288.2) + (#G * 328.2) + (#U * 289.2) - 61

II. Molar Extinction Coefficient (ε):

- Length: ε ≈ L * 10,000 liter/mole/cm
- Composition:

DNA: ε ≈ (#A * 15480) + (#C * 7340) + (#G * 11760) + (#T * 8850) liter/mole/cm

RNA: ε ≈ (#A * 15480) + (#C * 7340) + (#G * 11760) + (#U * 9000) 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 = $A/(\varepsilon b) * 10^6$ pmole/µl
- Concentration = $A/(\varepsilon b) * MW * 10^3$ µg/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 = $A/\varepsilon * 10^6$ pmole/µl = (µg/ml)* (1000)/MW
- Concentration = $A/\varepsilon * MW * 10^3$ µg/ml = (pmol/µl) * (MW)/1000

IV. dsDNA, RNA, ssDNA concentration:

- A_{260} * Conversion Factor * Dilution Factor = µg/ml concentration

OR

- $(A_{260} - A_{320})$ * Conversion Factor * Dilution Factor = µg/ml concentration

Default conversion factors are:

1.0 O.D. unit = 50 µg/ml dsDNA concentration

1.0 O.D. unit = 40 µg/ml RNA concentration

1.0 O.D. unit = 33 µg/ml ssDNA concentration

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

V. Quantitating UV-proteins:

Protein (mg/ml) = $1.55 * (A_{280} - A_{320}) - 0.76 * (A_{260} - A_{320})$

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

VI. Cells/ml:

Cells/ml = A600 * Conversion Factor * dilution factor

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

Section 12
System, Accessories, and Reagents for the SmartSpec 3000

12.1 Product Information

Catalog Number	Product Description
SmartSpec 3000 Spectrophotometer and Accessories	
170-2501	**SmartSpec 3000 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

12.2 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

12.3 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	75 µl	100 µl	**Sub-microvolume cuvette, quartz**	**10 mm**

12.4 Selecting the Appropriate Cuvette

The SmartSpec 3000 has 1 mm, 2 mm, 5 mm and 10 mm pathlengths. For most (99%) 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 3000 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.

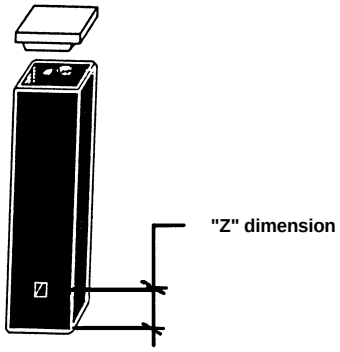
Pathlength	A260 = 0.1 AU	A260 = 2.0 AU**
10 mm	5 µg/ml*	100 µg/ml
5 mm	10 µg/ml	200 µg/ml
2 mm	25 µg/ml	500 µg/ml
1 mm	50 µg/ml	1000 µg/ml

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

** Working range of the SmartSpec 3000 is 0.1–1.5 AU per Good Laboratory Practice.

Cuvettes that can fit the SmartSpec 3000 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 99% of the cases you will use 10 mm pathlength cuvettes.

The SmartSpec 3000 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.



12.5 PC Serial Connection Instructions

1. Connect RS232 9-pin serial cable from SmartSpec 3000 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 ANSI

Tel net terminal ANSI

Terminal Setup

✓ **Underline**

✓ **Blink**

Backscroll buffer line 500

ASCII Setup

✓ **Echo typed characters locally**

✓ **Wrap lines that exceed terminal width**

4. When the SmartSpec 3000 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.

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