Spectrophotometer: SmartSpec Plus, Bio-Rad

The UV/visible SmartSpec Plus spectrophotomer has a working wavelength range of 200–800 nm. It is the perfect tool for routine applications such as:

- Quantitation of DNA, RNA, and oligonucleotides
- Quantitation of proteins via the Bradford, Lowry, and BCA assay methods
- Monitoring bacterial culture growth
- Simple kinetic assays
- Wavelength scans with peak detection

A simple, menu-driven interface simplifies assays and provides answers to common sample computations at the touch of a button. Conversion factors can be stored and modified. The SmartSpec Plus spectrophotometer is capable of performing calculations and providing results such as:

- A_{260}/A_{280} ratio for nucleic acid purity
- Quantitation that takes dilution factors into account
- Sample concentration in µg/ml (additionally in pmol/µl for oligonucleotides)
- Molar extinction coefficient and molecular weight of oligonucleotides

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1. Power up the instrument. It will go through system status check and display an error message if there are any problems.
2. Press one of the Assay buttons.
   A. DNA/RNA.
      i. Choose type of nucleic acid
         a. dsDNA, ssDNA or RNA: accept or modify the conversion factor
         b. DNA oligo or RNA oligo: input molar extinction coefficient and molecular weight, otherwise choose method for SmartSpec Plus to estimate these values.
      ii. Choose whether to subtract background and, if so, specify background wavelength.
   B. Protein
      i. Choose type of assay.
         a. Bradford. Measure absorbance at 595 nm.
         b. Lowry. Measure absorbance at 750 nm.
         c. BCA. Measure absorbance at 562 nm.
         d. UV. Measure absorbance at 260, 280, and 320 nm.
         e. Other. User specifies the wavelength to read.
   ii. Choose standard curve option.
      a. Create a new standard curve.
      b. Recall a standard curve from memory.
      c. No standard curve. SmartSpec Plus will not be able to convert absorbance to concentration.

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C. Scan

   i. Set upper and lower limits of scan. (200 nm to 800 nm)

   ii. Choose whether to subtract background and, if so, specify background wavelength.

   iii. Choose fast or slow scan.

   iv. For the fast scan, choose number of successive scans.

D. Kinetic

   i. Choose wavelength to read.

   ii. Choose duration of data collection.

   iii. Choose interval between successive readings.

   iv. Choose whether to subtract background and, if so, specify background wavelength.

E. OD600

   i. Accept or modify the conversion factor.

F. λ

   i. Choose number of wavelengths to read.

   ii. Specify the wavelengths to read.

   iii. Choose whether to subtract background and, if so, specify background wavelength.

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3. If your dilution factor is not 1.0, set the dilution factor.
4. Zero the instrument. Place a cuvette containing the blank solution into SmartSpec Plus and press **Read Blank**.
5. Collect absorbance data. Place cuvette containing sample solution into SmartSpec Plus and press **Read Sample**. Continue collecting absorbance data until all samples are read.
6. Absorbance and/or concentration data are displayed automatically as they are collected. If there are absorbance data at other wavelengths, press **Abs** to see them. DNA or RNA oligo concentrations are available in units of 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 Plus and press **Read Sample**.
7. After last sample, press left arrow key to exit assay.
8. For protein assays, save the standard curve (if you made a new one) and print the full report.