



Libra S22

Life Science Modes Operation Manual

English

This section is only a description of the additional Life Science modes now included with every Libra S22 spectrophotometer.

For a complete description of all other modes and the general use of the spectrophotometer, please refer to the main manual included on the CD-ROM.

Life Science mode includes following applications:

- Stored parameters for Nucleic Acid quantification and purity checking
 - DNA
 - RNA
 - Oligonucleotide

Nucleic Modes (3)

Nucleic acids can be quantified at 260 nm because it is well established that a solution of DNA or RNA with an optical density of 1.0 has a concentration of 50 or 40 $\mu\text{g/ml}$, respectively, in a 10mm pathlength cell. Oligonucleotides, as a rule of thumb, have a corresponding factor of 33 $\mu\text{g/ml}$, although this does vary with base composition.

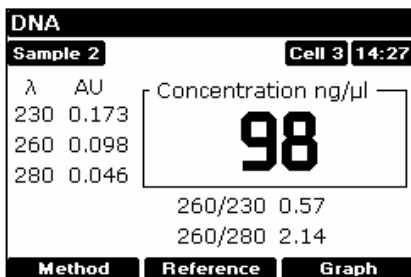
Extracting nucleic acids from cells is accompanied by protein, and extensive purification is required to separate the protein impurity. The 260/280 ratio gives an indication of purity; it is only this, however, and not a definitive assessment. Pure DNA and RNA preparations have expected ratios of ≥ 1.8 and ≥ 2.0 , respectively; deviations from this indicate the presence of protein impurity in the sample, but care must be taken in interpretation of results. An elevated absorbance at 230 nm can indicate the presence of impurities as well; 230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since Tris, EDTA and other buffer salts absorb at this wavelength. When measuring RNA samples, the 260/230 ratio should be > 2.0 ; a ratio lower than this is generally indicative of contamination with Guanidinium Thiocyanate, a reagent commonly used in RNA purification and which absorbs over the 230 - 260 nm range.

Background correction at a wavelength totally separate from the nucleic acid and protein peaks at 260 and 280 nm, respectively, is sometimes used to compensate for the effects of background absorbance. The wavelength used is 320 nm and it can allow for the effects of turbidity, high absorbance buffer solution and the use of reduced aperture cells.

The instrument calculates concentration, displays 260/280 and 260/230 ratios, and compensates for dilution and use of cells that do not have 10mm pathlength. A wavelength scan of a sample can also be obtained for visual inspection of integrity.

The procedure is as follows for DNA (3.1), RNA (3.2) and oligo (3.3):

- Enter pathlength of cell; 10mm (1), 5mm (2), 2mm (3), 1mm (4) or 0.5mm (5)
- Select units; $\mu\text{g/ml}$ (1), $\text{ng}/\mu\text{l}$ (2) or $\mu\text{g}/\mu\text{l}$ (3)
- Select if background correction at 320 nm is required
- Select if sample scan is required (scans 220 to 330 nm, with autoscaling)
- Enter dilution factor
- [Oligo (3.3) only; enter conversion factor. If not known, use 33]
- Insert reference and press green run key
- This reference scan is used for subsequent samples until changed
- Insert samples as required and press \blacklozenge (repeat as necessary)
- To go back and change the parameters press Method (F1)
- Press Graph to view the sample spectrum



Please note that the Libra S22 is not compatible with standard 70 μl UV disposable cuvettes