SHIMADZU

Application News

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Determination of DNA concentration by Nucleic acid analysis mode in UV Biospec series

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Nucleic acid concentration can be determined accurately and simply by spectrophotometric measurement. When a nucleic acid is prepared (e.g. via recombinant DNA techniques) or isolated from a biological sample (e.g. cells, plasmids), its concentration and purity must be determined. Common contaminants include protein, phenol or agarose. DNA has a maximum absorbance peak at 260 nm whilst protein has a peak at 280 nm. The purity of the nucleic acid in a biological sample is determined by the ratio of absorbance at 260 and 280 nm. Pure DNA samples should have A_{260}/A_{280} of 1.8 to 1.9 and the ratio for a pure RNA sample is 1.9 to 2.0. If there is a contamination by proteins, the ratio will be significantly lower.

Shimadzu UV Biospec series offer a DNA quantitation mode, which facilitates the determination of DNA concentration. For non-Biospec series in particular to UV1240-mini, UV-1601 and UV-1700, DNA/Protein Quantitation Program pack is also available as optional. In the DNA/Protein mode of Biospec-1601, absorbance measurements are done in the UV region and the concentration of DNA and Protein is determined using Warburg and Christian's factors. Both the usual wavelength (260/230 nm or 260/280 nm) and optional wavelength can be set for measurement. Optional background correction may be done at 320 nm. In addition, the factors for calculation of concentration can be manually set based on the generally accepted rule of 1 A_{260} is equivalent to 50 µg/ml double stranded DNA. The sample used in this report was a 12 µg/ml of DNA solution prepared, from 1 mg/ml calf thymus DNA Standard obtained from SIGMA. 50 µl.super micro-black cell (optical length 10mm) was used for the DNA analysis in Biospec-1601 and 1.5 ml semi-microcell (optical length 10mm) was used for the analysis in Biospec-mini.

• Spectrum of a pure DNA standard

The DNA sample exhibited an absorbance peak at 260 nm and a valley at 230 nm. An absorbance value of close to baseline was observed at 320 nm. Figure 1 showed the absorbance spectrum of the DNA standard from a wavelength of 200 nm to 400 nm.

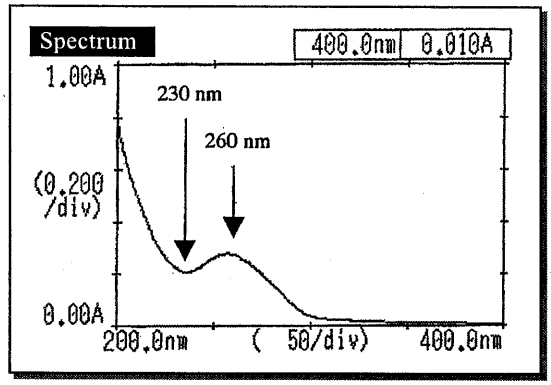

Fig. 1 Absorbance Spectrum of Calf Thymus DNA standard

• DNA/Protein mode in Biospec-1601 (Warburg-Christian factors)

If the DNA sample contains a significant amount of protein as contamination, the default Warburg and Christian's factors based on the wavelength (260nm and 280 nm) can be used.

The ratio of A_{260}/A_{280}, concentrations of DNA and protein were determined. Figure 2 showed the DNA Parameters and the respective measurement results. Similar analysis can be carried out with 260 nm and 230 nm or other manually set wavelengths.

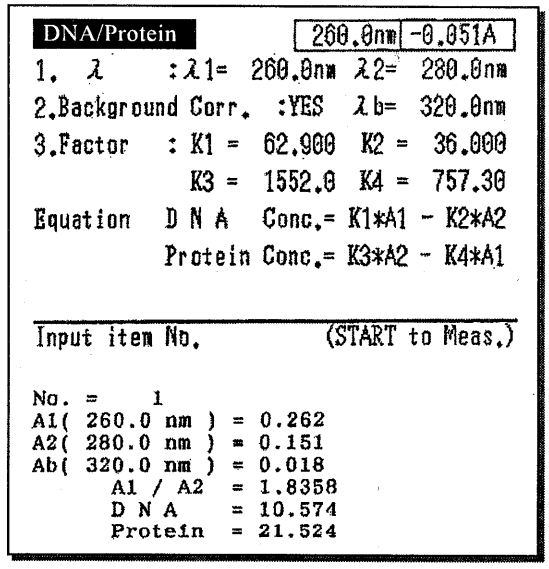

Fig. 2 DNA parameters & respective results

- **DNA/Protein mode in Biospec-1601 (1 Abs = 50 μg/ml dsDNA)**

With today's modern commercially available DNA purification kits, DNA can be isolated at high purity form. The determination of DNA concentration can still be carried out in the DNA/Protein mode, even with negligible amount of protein present in the DNA sample. Based on the generally accepted rule of 1 absorbance value at 260 nm is equivalent to 50 μg/ml of double stranded DNA, the absorption coefficient K1 (for 260 nm) is set to be 50. Figure 3 showed the DNA parameters and the respective measurement results.

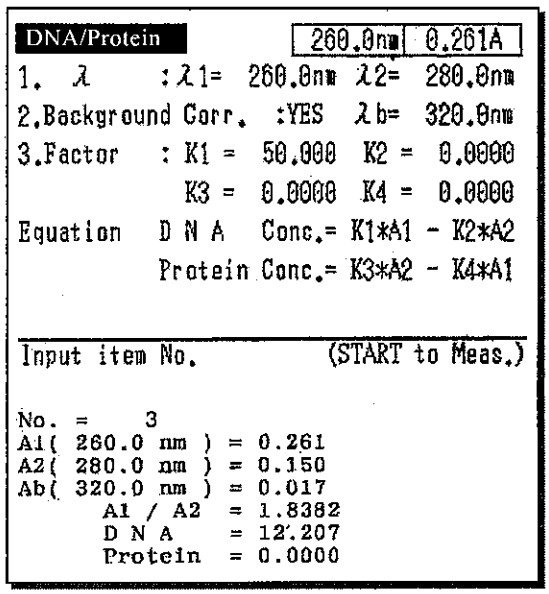

Fig. 3 DNA parameters & respective results

- **DNA/RNA analysis mode in Biospec-mini**

Like Biospec-1601, Biospec-mini has an in-built "DNA/RNA analysis" mode for DNA quantitation. Besides double-stranded DNA, the "DNA/RNA analysis" provides other quantitation modes such as single-stranded DNA, oligoDNA and oligoRNA. For general study of double-stranded DNA concentration, the dsDNA quantitation and Warburg-Christian methods could be adopted. Figure 4 showed dsDNA quantitation parameters and the respective measurement results while figure 5 showed Warburg-Christian parameters and its respective measurement results.

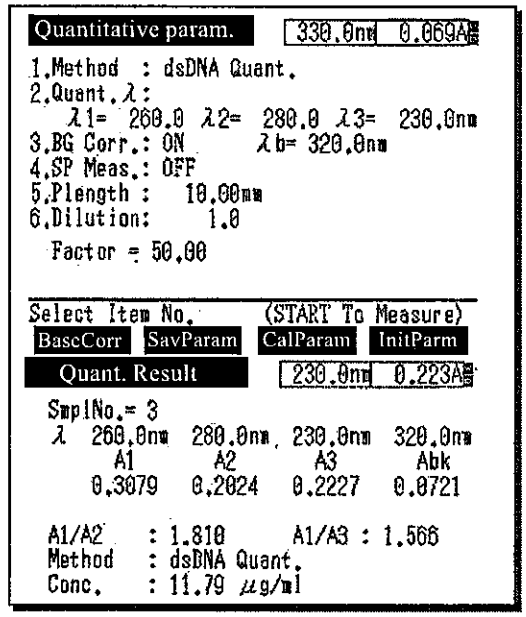

Fig. 4 dsDNA Quantitation parameters & results

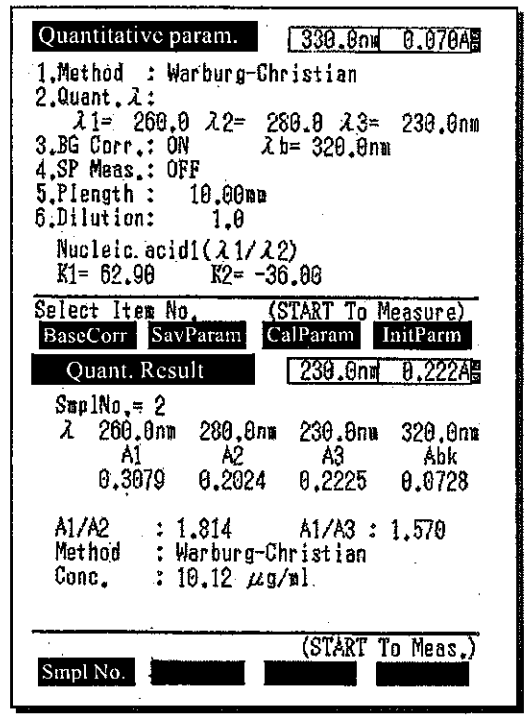

Fig. 5 Warburg-Christian parameters & results

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