Application Forum

Greiner Bio-One UV-Star® Microplates for UV/VIS of Nucleic Acids and Proteins

UV/VIS of nucleic acids and proteins

The optical density of biomolecules such as DNA, RNA and proteins is influenced by pH, temperature and salt concentration (1). The absorbance of a solution decreases with increasing ionic strength, e.g. DNA has approximately 23% lower absorbance when dissolved in buffered saline rather than water (2). Similarly, exact molar extinction coefficients are dependent upon the nucleotide sequence for nucleic acids or the amino acid type and amino acid sequence in peptides or proteins. Therefore molar extinction coefficients $\alpha\lambda$ are not commonly applied in molecular biology to determine biomolecular concentrations. Instead scientists prefer to use empirically determined standard coefficients. Because these standard coefficients are derived from optical density measurements at 1 cm, they are only valid if the pathlength used for the sample measurement is also 1 cm. For instance, if the optical density of a double stranded DNA sample with 50 µg DNA/ml water is equal to one absorbance unit at 1 cm pathlength, then the standard coefficient of double stranded DNA is 50 µg/ml, also at 1 cm pathlength.

The use of standard coefficients leads to approximations rather than precise determinations of biomolecule concentration within a sample. However standard coefficients have proven to be easy to use with sufficient accuracy for daily laboratory work. To avoid large divergences assay conditions should be considered when determining concentration of nucleic acids or proteins on the basis of standard coefficients.

Calculation of the concentration of an aqueous double stranded DNA sample applying the standard coefficient and the appropriate equation with example

$$\text{Concentration of sample} = OD \cdot sc \cdot f$$

OD Optical density
sc Standard coefficient
f Dilution factor of the sample

Example

An aqueous sample of genomic DNA gave an optical density of 0.8AU. The sample was diluted 1:25 to achieve an OD below 1.0. The measurement was done in a cuvette with a pathlength of 1 cm. With the standard coefficient of double stranded DNA (50 µg/ml) the concentration can be calculated using the equation as shown at left.

$$\begin{aligned} \text{Concentration of sample} &= OD \cdot sc \cdot f \\ &= 0.8 \cdot 50 \mu\text{g/ml} \cdot 25 \\ &= 1000 \mu\text{g/ml} \end{aligned}$$

Commonly used standard coefficients for biomolecules

Biomolecule	λ	Pathlength	Standard coefficient
Double stranded DNA	260 nm	1 cm	50 µg/ml
Single stranded DNA	260 nm	1 cm	33 µg/ml
Single stranded RNA	260 nm	1 cm	40 µg/ml
Oligonucleotides	260 nm	1 cm	20 µg/ml

Microplates for the UV/VIS determination of nucleic acids at 260 nm and proteins at 280 nm

Standard microplates are only partially suitable for transmission in the UV range, as polystyrene absorbs UV light especially in the short-wavelength range below 400 nm. Greiner Bio-One µClear® Microplates with a thin polystyrene based film that display much lower background values and can be used down to 340 nm. The adaptation of the patented µClear® process technology to a new, innovative UV-transparent material enabled the production of microplates that extend the transmission range down to 230 nm.

For the determination of nucleic acid and protein concentrations at 260 nm without background interference, UV-Star® Microplates are the ideal alternative to expensive and fragile quartz glass plates or cuvettes.

UV-Star® Microplates are DMSO resistant, can be stored at -20 °C without issue, and are available in 96 well, 96 well half area, and 384 well formats.

References

- Willfinger W. W.: Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity. *BioTechniques* (1997), Vol. 22, No. 3; 474-481.
- Mc Gown E. L.: UV Absorbance Measurements of DNA in Microplates. *BioTechniques* (2000): Vol. 28, No.1, 60-64.

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Light transmission of UV-Star® and µClear® Microplates compared with a conventional microplate