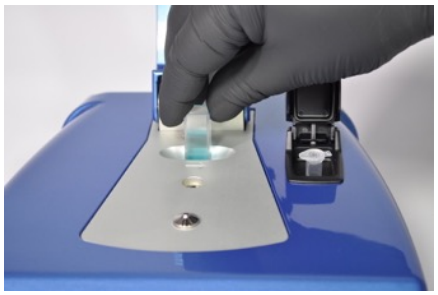
DeNovix

Spectrophotometer / Fluorometer

Absorbance and Fluorescence Quantification

Introduction

DeNovix DS-11 FX Spectrophotometer / Fluorometer Series instruments enable precise absorbance and fluorescence quantification across a wide dynamic range. The dual mode spectrophotometer incorporates SmartPath® Technology, which facilitates accurate and reproducible measurements for both cuvette and 1 µL modes. The patent-pending optical core of the fluorescence component utilizes LED sources and highly sensitive photodiodes capable of detecting minute amounts of fluorescence across four wavelength ranges.



Basics of Absorbance Measurements

UV-Vis absorbance measurements have long been a standard method for purified biomolecule quantification in the life science laboratory. This method allows for the rapid detection of molecules based on their absorbance profiles at specific wavelengths. Absorbance also provides an indication of sample contamination, as the shape of the absorbance spectrum will change based on the presence of other molecules that absorb at or near the same wavelengths as the molecule of interest.

Basics of Fluorescence Quantification

Fluorophores are molecules that absorb light at one wavelength (excitation wavelength) and then emit light at another (emission wavelength). Certain fluorophores' structures can be manipulated to fluoresce only when bound to a specific molecule (ie: double-stranded DNA). Fluorescence assays use this binding specificity to establish a direct correlation between the amount of fluorescence emitted by a sample and the concentration of the biomolecule of interest in solution. By mixing a fluorophore with a sample of known concentration and measuring the Relative Fluorescent Units (RFU), a relationship between concentration and measured RFU can be plotted and used as a standard curve. The emission of the same fluorophore, bound to unknown samples, can then be plotted against this standard curve to determine the sample concentration.

Absorbance vs. Fluorescence

The advantages of absorbance measurements are:

- Reagents are not required. The measured absorbance is a direct result of the molecule of interest absorbing light at a known wavelength.
- The amount of light absorbed corresponds directly to the concentration of the molecule of interest.

Alternatively, fluorescence is an indirect measurement. Advantages Include:

- High Sensitivity: Due to the high extinction coefficient of the fluorophore, fluorescence assays are extremely sensitive, allowing for the detection of molecules at concentrations hundreds of times lower than what is detectable by traditional absorbance.
- Specificity: The binding properties of the fluorophore make these methods highly selective for specific molecules. These assays are ideal for samples that may contain contaminants that would interfere with an absorbance measurement.

Summary

Absorbance and fluorescence are unique but complementary methods of quantitation. Quantitation via absorbance using the microvolume or cuvette based capabilities of the DS-11 FX Series is ideal for the rapid and accurate measurement of purified samples including nucleic acids and proteins.

Fluorescence quantitation utilizing a secondary reporter fluorophore is ideal for samples that fall below the detectable threshold for UV-Vis absorbance. In some cases, fluorescence quantitation methods can also be used to detect samples in the presence of contaminants or buffer elements that would interfere with UV-Vis measurements. DS-11 FX Series instruments offer both UV-Vis and fluorescence capability in a small bench top footprint. Both methods share the same EasyApps® software and sample export features, making data analysis fast, easy, and intuitive.

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