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Application News

UV-Vis Spectrophotometer UV-1900i Plus

Using the Spectral Evaluation Function for Quantitative Analysis of Nucleotides and a Simultaneous Pass/Fail Judgment on Nucleotide Sample Purity

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User Benefits

- Use the Spectral Evaluation Function to set threshold criteria and provide a pass/fail judgment based on any chosen wavelength or photometric value.
- Use the Spectral Evaluation Function to perform quantitative analysis of nucleotides and simultaneously provide a pass/fail judgment on nucleotide sample purity.

■ Introduction

UV-Vis spectrophotometers are used for quantitative analysis of solution, and in accordance with the Beer-Lambert law, the amount of light absorbed (measured as absorbance) in a cell of fixed length is proportional to the concentration of solution. Nucleic acids are quantified by measuring absorbance at 260 nm because light absorption by nucleic acid bases is greatest at this wavelength. However, the amount of light absorbed by nucleic acids varies by base sequence, sequence length, solvent used, and other factors. To account for this variability and obtain accurate quantitative results, an extinction coefficient is typically calculated for each oligonucleotide. The amount of light absorbed by nucleic acids is also affected by the presence of proteins and other contaminating substances that absorb light at a nearby wavelength (proteins absorb light at 280 nm). This Application News uses the UV-1900i Plus UV-Vis spectrophotometer and LabSolutions™ UV-Vis control software to measure the absorption spectra of nucleic acids and then uses the Spectral Evaluation Function to perform a quantitative analysis using a calibration curve based on absorbances measured at the wavelengths of maximum absorption. The Spectral Evaluation Function was also used to simultaneously check for contaminating substances that affect the quantitative analysis by providing a pass/fail judgment on nucleic acid sample purity based on the ratio of absorbance at 260 nm and 280 nm (OD_{260}/OD_{280}).

■ Quantitative Analysis Using LabSolutions UV-Vis

An important step in quantitative analysis is to prepare a calibration curve using a parameter that varies in proportion with concentration. Parameters used for this purpose include absorbance at a specific wavelength, or absorbance value or area value at the wavelength of maximum absorption within a specific wavelength range.

LabSolutions UV-Vis is the control software for Shimadzu UV-Vis spectrophotometers and is equipped with applications that perform measurements to meet a variety of analyst needs. These applications are selected from the LabSolutions UV-Vis launcher (Fig. 1).

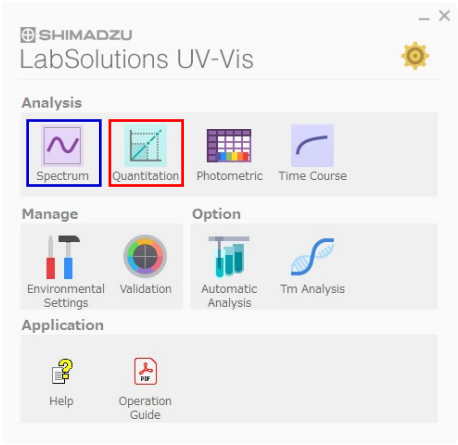

Fig. 1 LabSolutions UV-Vis Launcher

The “Quantitation” application (inside red square in Fig. 1) can perform quantitative analyses based on a calibration curve prepared from absorbance value at a specific fixed wavelength. The “Spectrum” application (inside blue square in Fig. 1) and the quantitative processing options in the Spectral Evaluation Function can use spectra recorded in a given wavelength range to prepare calibration curves based on user-defined criteria, which are then used to perform quantitative analysis of the analyte.

■ Quantitative Processing Options of the Spectral Evaluation Function

Spectral Evaluation Function

The Spectral Evaluation Function can automatically perform a preconfigured analysis of a recorded spectrum and use the result of that analysis to provide a pass/fail judgment on the sample. To use the Spectral Evaluation Function, select the "Spectrum" application in the LabSolutions UV-Vis launcher (Fig. 1) and configure "Evaluation" - "Table Settings" - "Evaluation Items." Fig. 2 shows an advanced settings window in the Spectral Evaluation Function.

The parameters that can be chosen for evaluation include point pick, maximum value, minimum value, peak, valley, area, statistics, and cutoff. These can be used individually or in combination and support a variety of different types of spectrum-based evaluations.

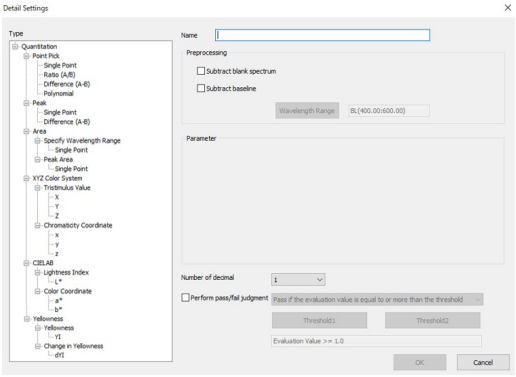

Fig. 2 Advanced Settings Window in the Spectral Evaluation Function

Quantitative Processing Options of the Spectral Evaluation Function

Select and configure which quantitative parameters to process under "Evaluation Items" - "Quantitation" in the Spectral Evaluation Function. Fig. 3 shows a quantitative processing options settings window. A total of 19 different parameters can be selected and combined in quantitative processing, including point pick, peak, and area, as well as color systems such as tristimulus values (XYZ color system)* and yellowness*.

The results of quantitative processing can be combined with the OD_{260}/OD_{280} absorbance ratio and other evaluation items to perform a variety of different quantitative evaluations.

*Requires the separate optional software product LabSolutions UV-Vis Color

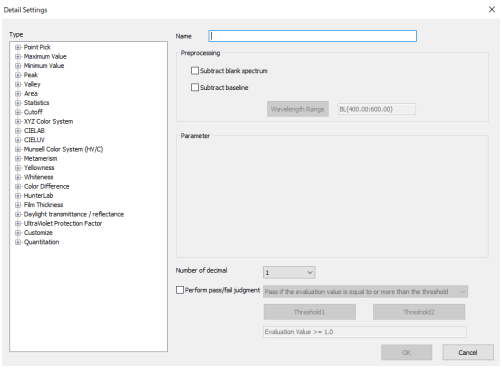

Fig. 3 Quantitative Processing Options Settings Window

■ Performing Various Quantitative Analyses with Nucleic Acid Absorption Spectra

Sample solutions of an M13-F25mer oligonucleotide were prepared at oligonucleotide concentrations of 0.2 to 10.0 ng/µL by diluting from 10-fold to 500-fold with a buffer (66.7 mM phosphate buffer).

A Super-micro cell holder was attached to the UV-1900i Plus UV-Vis spectrophotometer, and a 10 mm path length micro black cell was used to measure the spectra of the oligonucleotide samples. A cell path length of 10 mm can be used to obtain the spectra at low nucleotide concentrations of 1.0 ng/µL and below.

Fig. 4 UV-1900i Plus System

The detailed analysis conditions used to measure the absorption spectra are shown in Table 1, and the absorption spectra of the oligonucleotide at each concentration are shown in Fig. 5.

Table 1 Measurement Conditions

Instrument:	UV-1900i Plus Super-Micro Cell Holder Micro black cell
Wavelength Range:	220 to 400 nm
Data Interval:	0.5 nm
Scan Speed:	Medium speed
Slit Width:	1 nm

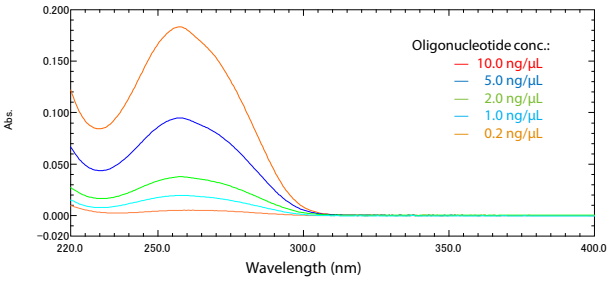

Fig. 5 Absorption Spectra of Oligonucleotide at Various Concentrations

The wavelength of maximum absorption of each of the absorption spectra recorded in Fig. 5 is shown in Table 2.

Table 2 Oligonucleotide Wavelengths of Maximum Absorption

Oligonucleotide conc. (ng/µL)	Wavelength of Maximum Absorption (nm)
10.0	257.0
5.0	257.5
2.0	258.5
1.0	257.5
0.2	259.0

Table 2 shows that the position of the wavelength of maximum absorption varies with dilution factor. Next, two calibration curves were prepared: one using absorbance measured at a fixed wavelength and one using absorbance measured at the wavelength of maximum absorption. For quantitative analysis based on absorbance at a fixed wavelength, a wavelength of 260 nm was entered in "Point Pick" under "Evaluation Item" - "Quantitation" in the Spectral Evaluation Function. For quantitative analysis based on absorbance at the wavelength of maximum absorption, "Peak" was selected under "Evaluation Item" - "Quantitation" and wavelength ranges were set for the wavelength of maximum absorption and the baseline.

The calibration curves prepared under each of the above conditions are shown in Figs. 6 and 7.

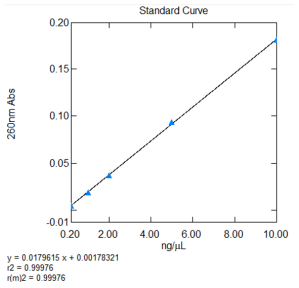

Fig. 6 Calibration Curve Prepared Using a Fixed Wavelength

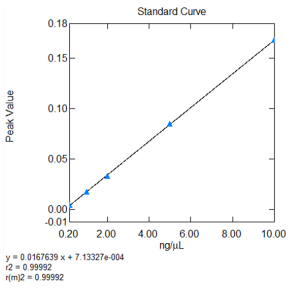

Fig. 7 Calibration Curve Prepared Using the Wavelength of Maximum Absorption

The squares of the correlation coefficient (r^2) in Figs. 6 and 7 were 0.99969 and 0.99992, respectively.

As shown above, when the wavelength of maximum absorption varies with sample concentration, more accurate results can be obtained by performing a quantitative analysis based on the wavelength of maximum absorption rather than a fixed wavelength.

■ Pass/Fail Judgment on Purity of Nucleotide Sample Adulterated with a Contaminating Substance

The calibration curve in Fig. 7 was used to perform a quantitative analysis of an unknown sample and, simultaneously, the Spectral Evaluation Function was used to provide a pass/fail judgment on the nucleic acid purity of the sample based on the 260 nm/280 nm absorbance ratio (OD_{260}/OD_{280}). Nucleic acid samples with a 260/280 absorbance ratio of 1.8 or higher are typically considered to be of high purity and are recommended for analysis with a next-generation sequencer. Accordingly, the pass/fail criterion for purity was set at 1.8 and above[1)].

A mock sample adulterated with contaminating material was prepared by adding bovine serum albumin to M13-M25mer oligonucleotide to a final oligonucleotide concentration of 0.2 ng/µL. Fig. 8 shows the absorption spectra obtained from a 1.0 ng/µL M13-M25mer oligonucleotide solution and the adulterated 0.2 ng/µL M13-M25mer oligonucleotide/bovine serum albumin solution measured under the conditions shown in Table 1. The nucleic acid purity of each sample determined by the Spectral Evaluation Function in terms of a pass/fail judgment is shown in Table 3. The oligonucleotide concentration of the mock solution was measured at 0.4 ng/µL and its 260/280 absorbance ratio was 1.3, showing that the contaminating material caused the sample to fail to meet the criterion for nucleic acid purity.

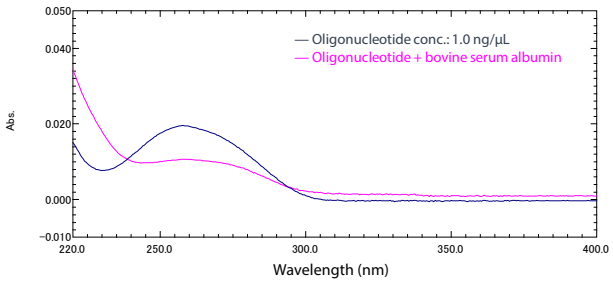

Fig. 8 Absorption Spectra of Oligonucleotide Samples

Table 3 Pass/Fail Judgments on Nucleic Acid Purity of Samples Using the Spectral Evaluation Function

File Name	General Judgment	Value	Judgme	Value	Judgme
UNK_Contaminant_OligoDNA_Protein.vspd	FAIL(1)	0.4	N/A	1.34	FAIL

■ Conclusion

The quantitative processing options in the Spectral Evaluation Function were used to perform a quantitative analysis of nucleic acid samples and simultaneously provide a pass/fail judgment on the purity of the nucleic acid samples. The Spectral Evaluation Function allows users to measure levels of nucleic acids in a sample while simultaneously passing judgment on the purity of the sample. The Spectral Evaluation Function is also useful when an analysis requires accurate nucleic acid concentrations, such as in thermal stability (Tm) analysis or thermodynamic parameter analysis of nucleic acids.

<References>

1. L. Braglia, S. Giani, D. Breviario, F. Gavazzi : Anal. Bioanal. Chem., 408, 8299 (2016).

<Related Application News Articles>

1. Evaluating Thermal Stability (Tm) Analysis and Thermodynamic Properties of Nucleic Acid Drugs
Application News No. 01-00322-EN

01-00873-EN First Edition: Feb. 2025

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