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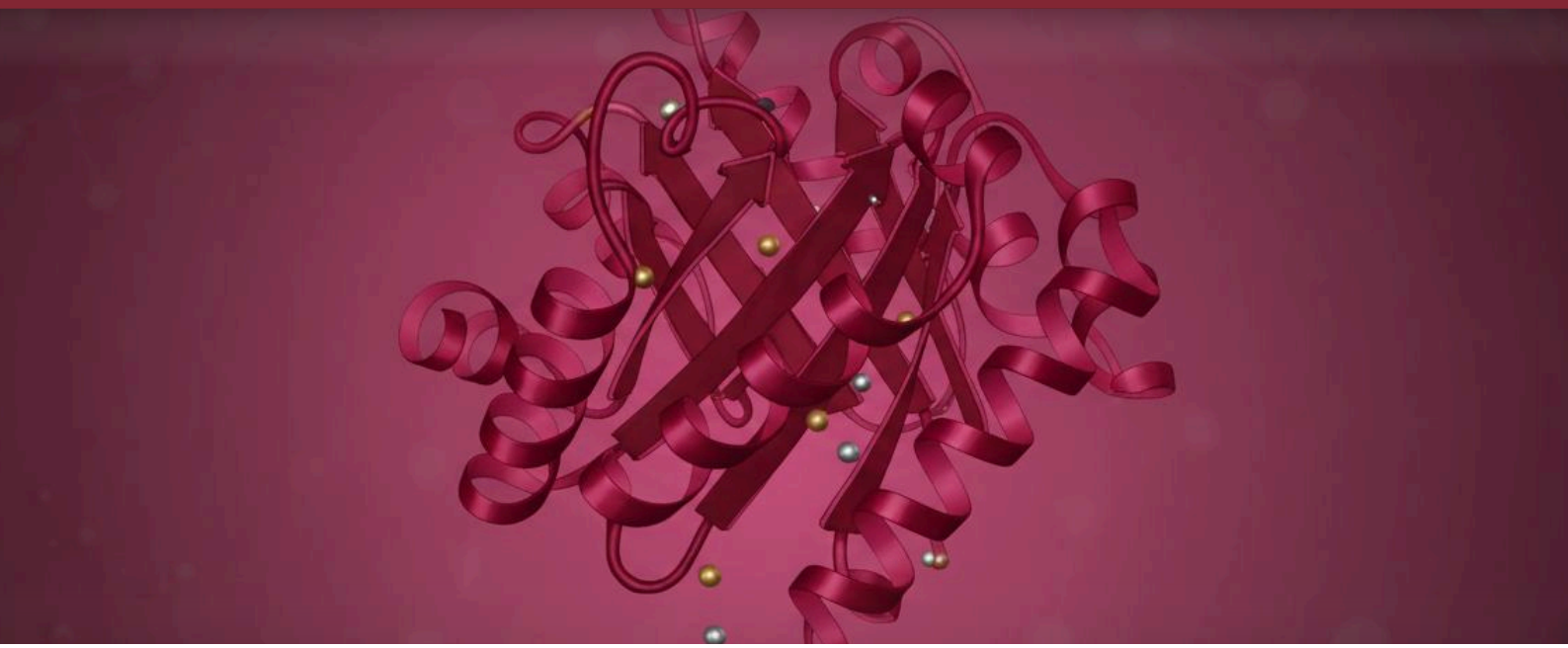
Related products
UV-Vis Spectrophotometer POP
Country of Origin: Korea

Application Notes
#T250005001

Microvolume Protein Quantification Using POP UV-Vis and Pierce™ 660nm Assay



Introduction

This experiment introduces a micro-volume protein quantification solution using the POP UV-Vis Spectrophotometer from K LAB equipped with a Microvolume Cell Holder, enabling the use of as little as 10 µL of protein sample for the Pierce™ 660 nm Protein Assay.

The POP is a standard UV-Vis Spectrophotometer widely used in laboratories and offers flexible cell configurations to suit various analytical purposes, thanks to its compatibility with multiple accessories. When equipped with the Microvolume Cell Holder, it supports the use of disposable micro cuvettes in applications requiring a minimum volume of only 70 µL, making it well-suited for analyzing small-volume samples.

The Pierce™ 660 nm Protein Assay is a colorimetric method that quantifies protein concentration by measuring the absorbance at 660 nm of a blue-colored complex formed through the reaction between the protein and the assay reagent.

K LAB Co., Ltd., a leading company in the domestic analytical instrument industry, is the only specialized research and manufacturing enterprise in Korea that manages the entire process—from R&D to production—under one roof.

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[Figure 1]. POP UV-Vis Spectrophotometer

Experimental Conditions

Instruments

- POP UV-Vis Spectrophotometer (K LAB, Cat. no: UNT0006)
- Microvolume Cell Holder (K LAB, ACC0031)
- Disposable Micro Cuvette (BRAND GMBH, 759200)
- 10 – 1,000 µL pipettors
- 1.5 mL Micro Tube

Reagents

- Protein Standard-analytical standard, 200 mg/mL (BSA) (Sigma, Cat. no: P5369)
- Pierce™ 660 nm Protein Assay Reagent (Thermo Scientific™, Cat. no: 22660)
- Deionized Water (DI Water)

[Figure 2]. Micro Cuvette (BRAND GMBH, Cat. no: 759200) and POP's Microvolume Cell Holder. The POP is equipped with a standard 8-cell multi-cell holder but is also compatible with various accessories, including the Microvolume Cell Holder, allowing for flexible experimental configurations.

Experimental Procedure

Preparation of BSA Standard Solutions

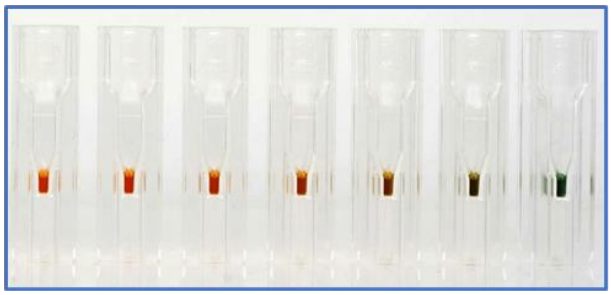
In this experiment, a Protein Standard – analytical standard, 200 mg/mL (Sigma, Cat. no: P5369) was diluted to prepare an initial solution of 2,000 µg/mL. This stock solution was then serially diluted 1:2 to generate a total of six standard solutions with concentrations ranging from 2,000 µg/mL to 62.5 µg/mL.

Preparation of BSA Standard Solutions

① Dispense 10 µL each of the BSA standard solutions and the blank sample into 1.5 mL microtubes. Add 150 µL of PierceTM 660 nm Protein Assay Reagent (Thermo Scientific™, Cat. no: 22660) to each tube and mix gently.

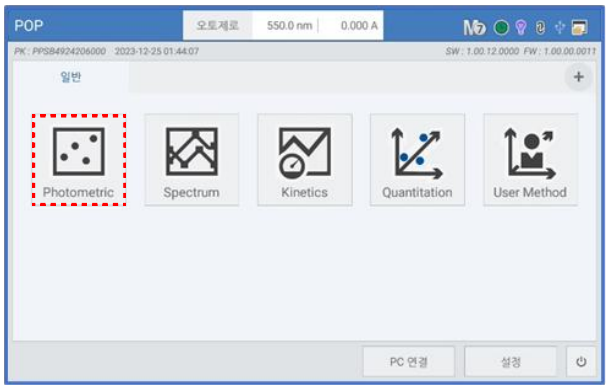
② Allow the mixtures to react at room temperature for 5 minutes.

③ After the reaction, transfer 70 µL of each mixture into a disposable micro cuvette.



[Figure 3]. Loaded blank and BSA Standard solutions (62.5- 2,000 µg/mL) into micro cuvettes after the PierceTM 660 nm assay reaction. After the reaction, the intensity of the blue color varies depending on the protein concentration.

④ Select the [Photometric] mode to enter the measurement mode.



[Figure 4]. Photometric mode selection screen of the POP Instrument.

⑤ Insert the blank sample into the instrument and set the zero (blank) absorbance.

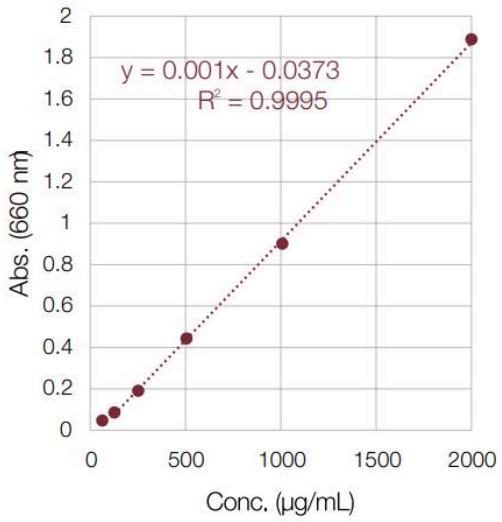
⑥ Then, sequentially insert each BSA standard solution and measure the absorbance at 660 nm.

Results

Prepared BSA standard solutions (62.5 - 2,000 µg/mL) using the Pierce™ 660 nm Protein Assay were measured five times each. The results are summarized in [Table 1] and [Figure 5].

Based on the calculated standard deviation and coefficient of variation (%CV), the reproducibility of the assay was evaluated. At the lowest concentration of 62.5 µg/mL, the %CV was 2.2%, and at all other concentrations, %CV values remained below 2.2%, indicating good precision.

In addition, a standard curve generated from absorbance values at 660 nm showed a coefficient of determination (R^2) of 0.9995, confirming high linearity across the tested concentration range.



[Figure 5]. The standard curve of BSA concentration versus absorbance measured at 660 nm, showing high linearity with a coefficient of determination (R^2) of 0.9995.

[Table 1]. Results of five repeated measurements of BSA standard solutions (62.5 – 2,000 µg/mL). %CV was equal to or below 2.2% across all concentrations.

Conc. (ug/mL)	Conc. (ug/mL)	Standard Deviation	%CV
62.5	0.045	0.001	2.2%
125	0.080	0.001	1.3%
250	0.189	0.000	Below quantifiable variation
500	0.447	0.001	0.2%
1000	0.898	0.000	0.0%
2000	1.891	0.000	Below quantifiable variation

Conclusion

In this study, the performance of the POP UV-Vis Spectrophotometer from K LAB, equipped with a Microvolume Cell Holder, was evaluated for low-volume protein quantification using the Pierce™ 660 nm Protein Assay.

Using 10 µL of protein sample and a 70 µL reaction mixture, repeated measurements showed that the coefficient of variation (%CV) for absorbance was within 2.2%. The standard curve generated from absorbance values measured at 660 nm demonstrated strong linearity, with a coefficient of determination (R^2) of 0.9995.

Based on these results, the POP instrument was experimentally confirmed to be suitable for low-volume protein quantification when used in combination with the Pierce™ 660 nm Protein Assay.

***Reference**

- Pierce™ 660 nm Protein Assay Manual (Thermo Scientific, MAN0016386)