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**Related products**  
UV-Vis Spectrophotometer  
POP

**Technical Note**  
#T250005001

# Microvolume Protein Quantification Using POP UV-Vis and Pierce™ 660 nm 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  $\mu\text{L}$  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  $\mu\text{L}$ , 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.** leads Korea in developing high-precision spectrophotometers with innovative monochromator scanning mechanisms, prioritizing customer satisfaction and continuous improvement.

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## Experimental Conditions

### 1. Instruments

- 1) POP UV-Vis Spectrophotometer (K LAB, Cat.no: UNT0006)
- 2) Microvolume Cell Holder (K LAB, ACC0031)
- 3) Disposable Micro Cuvette (BRAND GMBH, 759200)
- 4) 10 – 1,000 µL pipettors
- 5) 1.5 mL Micro Tube

### 2. Reagents

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

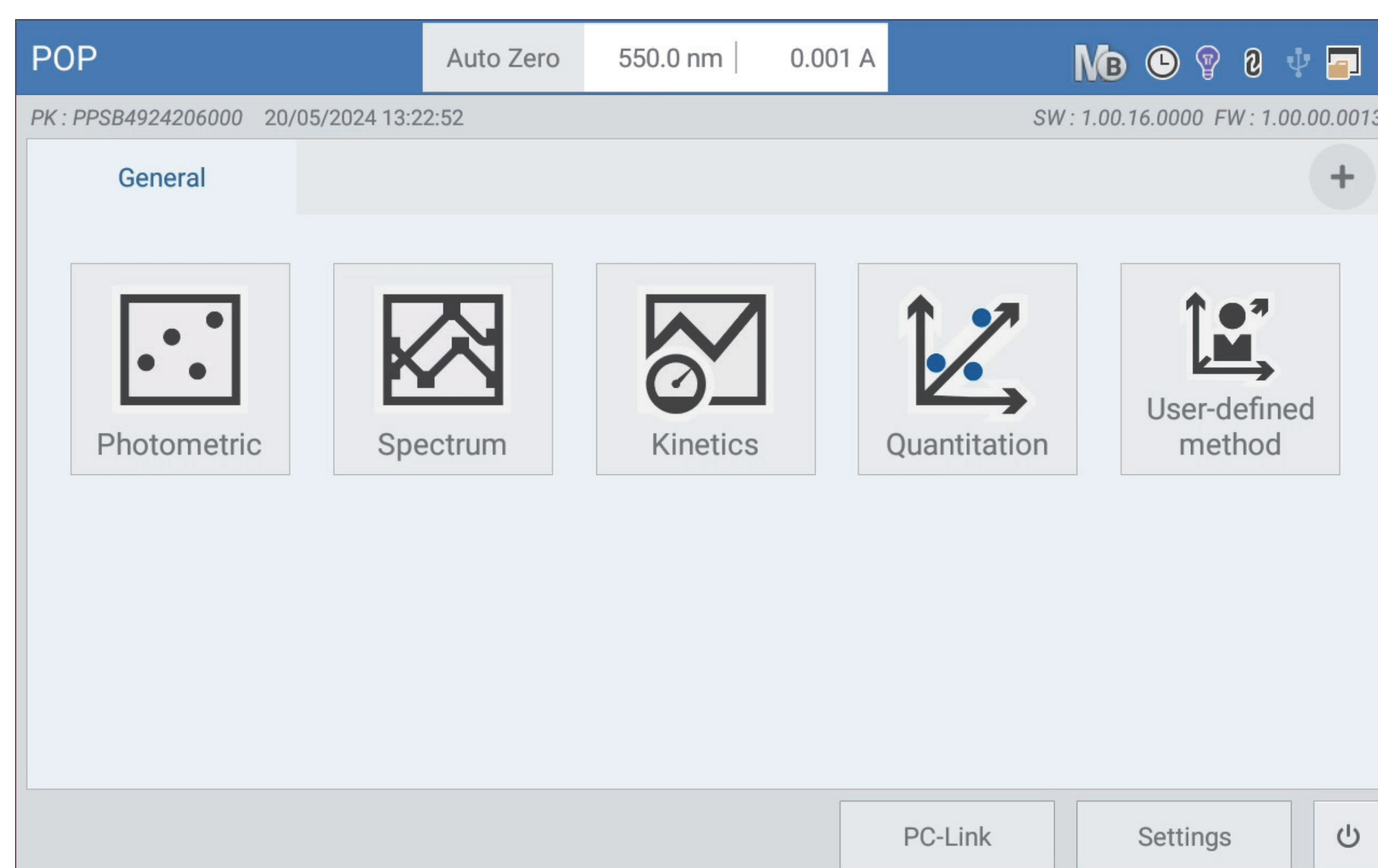
## Experimental Procedure

### 1. 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.

### 2. Preparation of BSA Standard Solutions

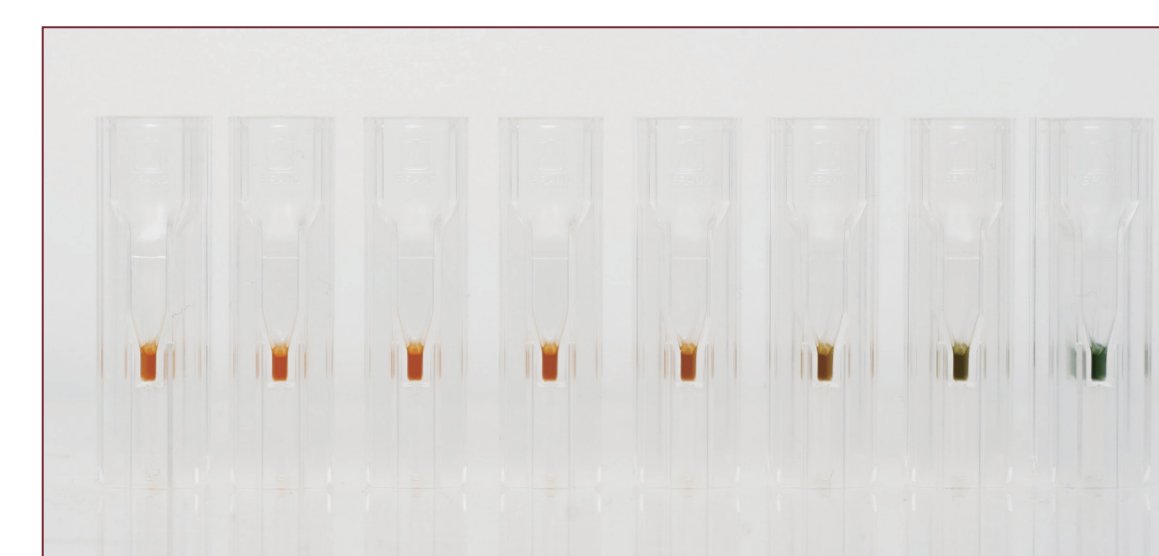
- 1) Dispense 10 µL each of the BSA standard solutions and the blank sample into 1.5 mL microtubes. Add 150 µL of Pierce™ 660 nm Protein Assay Reagent (Thermo Scientific™, Cat.no: 22660) to each tube and mix gently.
- 2) Allow the mixtures to react at room temperature for 5 minutes.
- 3) After the reaction, transfer 70 µL of each mixture into a disposable micro cuvette.
- 4) Select the [Photometric] mode to enter the measurement mode.



- 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.



**[Figure 1] 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.



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

**[Figure 3] Photometric mode selection screen of the POP Instrument.**

## Experimental Results

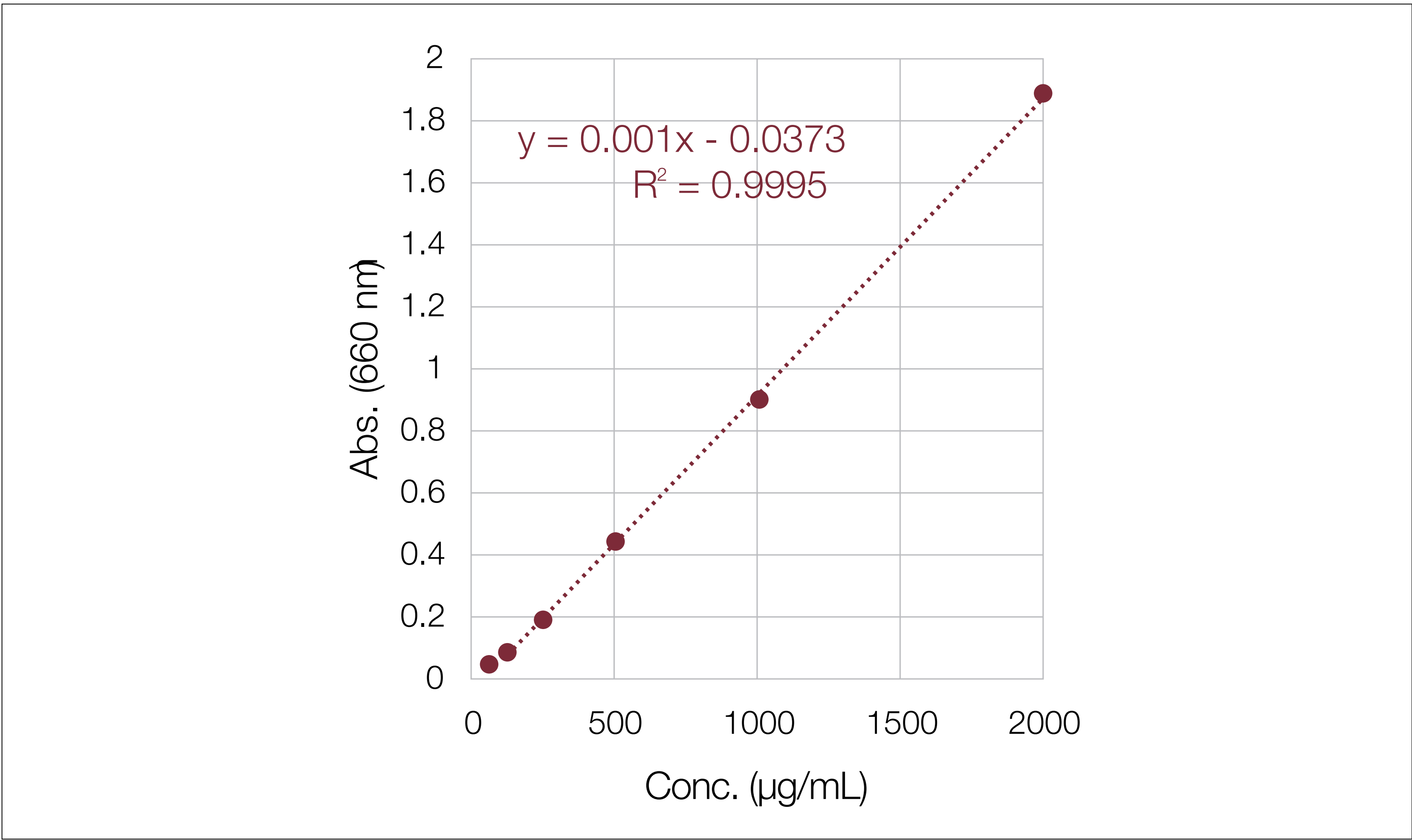
Prepared BSA standard solutions (62.5 - 2,000 µg/mL) using the Pierce™ 660 nm Protein Assay were measured five times each. The results are summarized in [Table 1] and [Figure 5].

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.

Conc. (ug/mL)	Absorbance (660 nm)	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

**[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.



**[Figure 4]** 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.

## 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.

## References

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