Rapid and simple quantification of proteins using the VICTOR Kira multimode plate reader.
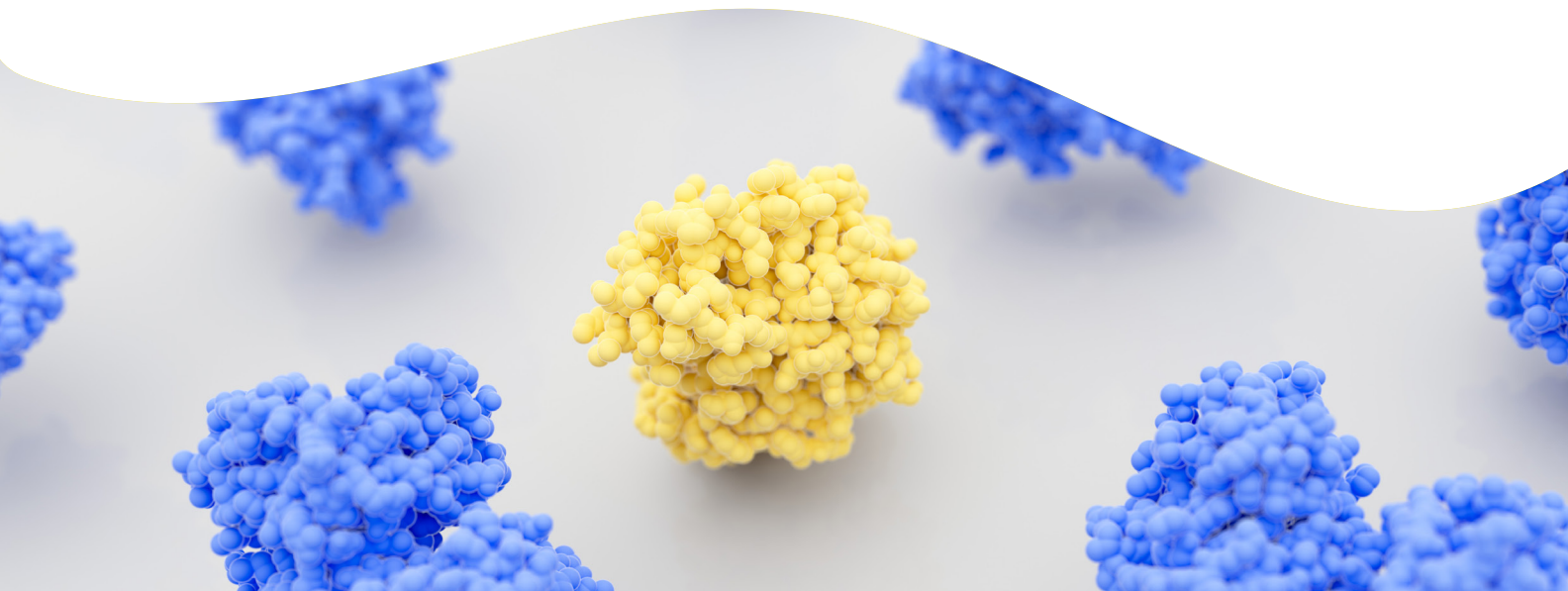
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Introduction

Accurate quantification of proteins is a critical step in many biochemical and molecular biology workflows, influencing downstream applications such as enzyme assays, Western blotting, and cell-based studies. Several colorimetric and fluorometric methods are available for protein quantification, each with distinct advantages and limitations depending on sample type, concentration range, and sensitivity requirements.

In this application note, we present a comparative validation of three widely used protein quantification methods, Bradford, BCA, and Lowry, using VICTOR Kira™, which is a **monochromator-based multimode microplate reader**. This instrument offers flexible wavelength selection, enhanced sensitivity, and reduced background interference, making it ideal for evaluating assay performance across diverse sample conditions. By assessing linearity, sensitivity, reproducibility, and compatibility, we aim to provide practical insights into method selection for routine protein analysis.

- **Bradford assay:** This method relies on the binding of Coomassie Brilliant Blue dye to proteins, primarily through arginine and aromatic residues. The dye undergoes a shift in absorbance from 465 nm to 595 nm upon binding, allowing rapid and simple quantification. It is susceptible but can be affected by detergents and buffer components.

- **BCA assay (bicinchoninic acid):** Based on the biuret reaction, this assay detects the reduction of Cu^{2+} to Cu^+ by proteins in an alkaline medium, followed by the formation of a purple-colored complex with BCA reagent. It offers good compatibility with detergents and reducing agents, with absorbance measured at 562 nm.

- **Lowry assay:** A classical method combining the biuret reaction with the Folin–Ciocalteu reagent, which reacts with tyrosine and tryptophan residues to produce a blue color. Though more time-consuming and sensitive to interfering substances, it provides high accuracy for total protein estimation. In this study, we took two unknown proteins, Slc9c-1 and HPcaf-1.

Material and methods

Protein quantification using Bradford reagent:

A serial dilution of a BSA protein standard (2000ug/ml, #23236, Pierce™ Bradford Plus Protein Assay Kit) was prepared in distilled water and combined 1:30 with the Bradford Reagent. Standard and 2 unknown samples (Slc9c1 & Hpcaf) were made into a clear 96-well microplate (UV-Star® Microplates #655801 (96-well), Greiner Bio-One). Absorbance was detected using VICTOR Kira control software. The assay plate was incubated for 10 min at room temperature inside the reader under shaking conditions before the start of the measurement. OD595 was used for data analysis.

Protein quantification using BCA reagent:

A serial dilution of a BSA protein standard (2000 μg/ml, # 23225 Pierce™ BCA Protein Assay Kit) was prepared. 25 μL of each standard and unknown sample was pipetted in triplicate into a microplate well (working range = 20–2000 μg/mL). (Revvity, SpectraPlate 96-well, Cat. No. 6055620). The working range is limited to 125–2,000 μg/mL. 200 μL of the WR was pipetted to each well, and the plate was mixed thoroughly on a plate shaker for 30 seconds. The assay plate was incubated for 30 min at 37°C inside the reader under shaking conditions before the start of the measurement. OD562 was used for data analysis.

Protein quantification using Lowry reagent:

A serial dilution of a BSA protein standard (2000 μg/mL, #23240 Pierce™ Modified Lowry Protein Assay Kit) was prepared. 40 μL of each standard and unknown sample was pipetted in triplicate into a microplate well (working range = **10–1500 μg/mL**). (Revvity, SpectraPlate 96-well, Cat. No. 6055620). 200 μL of the prepared Modified Lowry Reagent was added to each well, and the plate was mixed thoroughly on a plate shaker for 30 seconds. The assay plate was incubated for **10 minutes at room temperature**. Addition of 20μL of prepared 1X Folin-Ciocalteu Reagent to each well, followed by **30 minutes incubation at 37 °C** inside the reader under shaking conditions before measurement. Absorbance was measured at **750 nm (OD750)** for data analysis.

Results and discussion

The Bradford, Modified Lowry, and BCA protein assays were evaluated using BSA standards, and all three methods demonstrated strong linearity when measured on the VICTOR Kira microplate reader. The Bradford assay showed perfect correlation (R^2 = 1.00), while the Modified Lowry and BCA assays each achieved high linearity (R^2 = 0.99), confirming the accuracy and reliability of these colorimetric methods across their working ranges.

Sample readings from Slc9c1 and HPcaf-1 lysates aligned closely with the respective standard curves in all assays, indicating minimal matrix interference. Overall, the data confirm that all three assays provide robust and reproducible protein quantification on the VICTOR Kira platform, with Bradford offering the highest linearity and BCA providing the widest dynamic range.
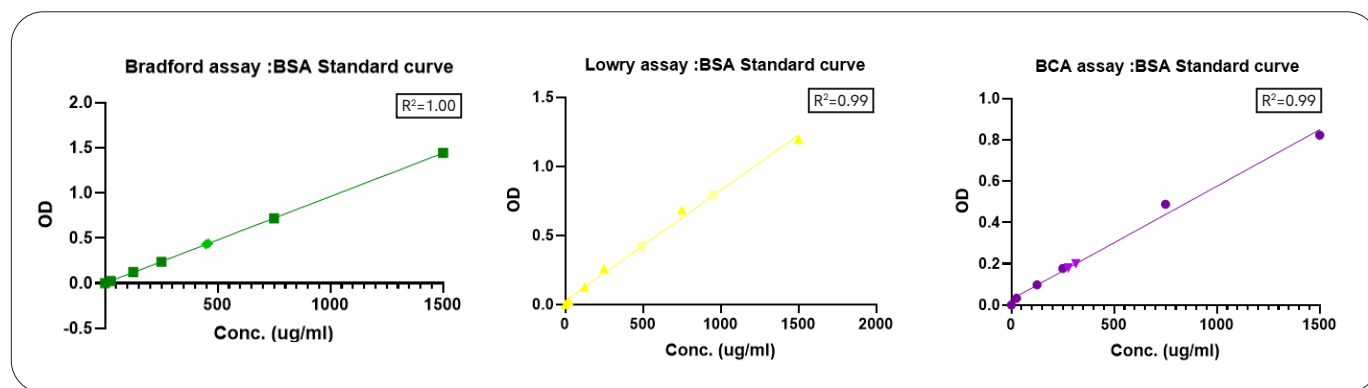

Figure 1: Comparison: BSA standard curve

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