Application Note

PROTEIN QUANTIFICATION USING THE APOLLO LB 917 ABSORBANCE READER

Abstract

Some of the most popular methods for protein quantification are BCA, Coomassie Plus (Bradford), and Lowry, and they are most often measured using a microplate absorbance reader. The suitability of the Apollo LB 917 to perform those assays was assessed and confirmed. Furthermore, the Apollo is easy to program and performing the assays is simple and straightforward.

Introduction

The quantification of the protein content of a sample is necessary for many applications, such as enzymatic activity measurement, western blotting, development of immunoassays, and more.

There are many protein quantification methods available, and the choice of the most suitable method depends on a range of factors: from the protein to be quantified (protein mixture or specific proteins), the presence of detergents in the buffer, the amount of sample available, and others. Some of the most popular methods are BCA, Coomassie Plus (Bradford), and Lowry [1]. All three are colorimetric methods which are quantified using absorbance in the visible range and are most often performed in microplates.

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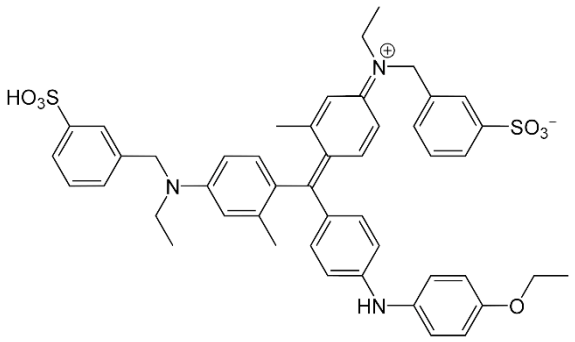

***Figure 1**: Coomassie brilliant blue G-250, the binding dye for the Bradford assay.*

The BCA assay combines the reduction of Cu^{+2} to Cu^{+1} by the peptide bonds in protein in an alkaline medium with the colorimetric detection of the cuprous cation (Cu^{+1}) using a reagent containing bicinchoninic acid (BCA) and is measured at 562 nm.

The Bradford/Coomassie Plus assay uses Coomassie dye. When it binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm, with a concomitant colour change from brown to blue.

The Lowry assay is based on two chemical reactions: the biuret reaction, in which the alkaline cupric tartrate reagent complexes with the peptide bonds of the protein, and reduction of the Folin & Ciocalteu's phenol reagent, which yields a purple colour. Absorbance of the coloured solution is read at a suitable wavelength between 500 nm and 800 nm.

This application note demonstrates the suitability of the Apollo absorbance reader for protein quantification.

Apollo Absorbance Reader

Reliable ELISA & absorbance analysis in just 6 seconds

The Apollo Absorbance Reader is an intuitive and reliable filter-based microplate reader that can be used for a wide variety of research and routine applications. The system has been designed to help you accelerate your research combining fast measurement of 96-well plates in just 6 seconds with intuitive 7-inch colour touchscreen stand-alone operation.

Apollo benefits at a glance:

- Wide variety of applications: With its wavelength range from 340 to 750 nm it is ideal for ELISA, cytotoxicity assays, protein colorimetric assays, endotoxin assays and more.
- Fast measurement: Read your plate in just 6 seconds (fast mode).
- Trust your data: Accuracy of ±0.005 OD or ±1% (whichever is greater) at 0-3 OD.
- Intuitive operation: 7‘‘ colour touchscreen and preprogrammed protocols simplify operation of the system.
- Large dynamic range: The system´s dynamic range of 4.0 OD is large enough to cover any assay´s requirements.

Materials

- Apollo LB 917 absorbance reader from Berthold Technologies (73664-10) equipped with 560, 595 and 650 nm filters.
- Pierce™ BCA Protein Assay from ThermoFisher Scientific™ (23227).
- Total Protein Kit, Micro Lowry, Peterson's modification from Sigma-Aldrich® (TP0300).
- Pierce™ Coomassie Plus (Bradford) Assay Kit from ThermoFisher Scientific™ (23236).
- Pierce™ Bovine Serum Albumin Standard Ampules, 2 mg/mL (23209).
- 96-well clear microplates from ThermoFisher Scientific™ Nunc (269620).
- Deionized water (to prepare BSA standards).
- Pipettes and pipette tips (various models and volumes).

Methods

All reagents were prepared following the manufacturer's instructions. A standard curve of BSA was prepared with the following concentrations: 2000 (Standard 1), 1500 (Standard 2), 1000 (Standard 3), 750 (standard 4), 500 (Standard 5), 250 (Standard 6), 125 (Standard 7) and 25 (Standard 8) µg/mL, and deionized water was used as blank. In addition, 1 unknown sample was measured: it was prepared by mixing equal volumes of the 750 and 1000 µg/mL standards and had hence a theoretical concentration of 875 µg/mL. All measurements were performed in duplicate.

Each assay was performed following the manufacturer's instructions (see below). The BCA assay was measured at 560 nm, as a 562 nm filter was not available.

BCA protocol

1. Pipette 25 µL of each standard or sample to each well
2. Add 200 µL of working reagent to each well
3. Shake 30 seconds
4. Cover plate and incubate 30 minutes at 37° C
5. Measure absorbance at 560 nm

Micro Lowry protocol

1. Pipette 50 µL sample or standard to each well
2. Pipette 50 µL Lowry reagent to each well
3. Incubate 20 minutes at RT
4. Pipette 25 µL Folin & Ciocalteu's phenol reagent to each well and mix immediately
5. Incubate 30 minutes at RT
6. Measure absorbance at 650 nm

Coomassie Plus (Bradford) protocol

1. Pipette 10 µL of standard or sample to each well
2. Pipette 300 µL Coomassie Plus reagent to each well
3. Shake 30 seconds
4. Incubate 10 minutes at RT
5. Measure absorbance at 595 nm

The positions of standards, blank and samples were entered in the Apollo built-in software. The plate layout used is displayed in Figure 2:

***Figure 2**. Plate layout programmed in the Apollo software. Wells in green are the standards (Standard 1 to Standard 8), wells in white are the blanks, and the rest of the plate is left for samples. The unknown sample was pipetted in duplicate in wells C3 and D3.*

To perform the calculations, 4 Parameter Logistic was selected as curve fitting algorithm (labelled "Logistic" in the software). Calculated protein concentrations were directly obtained from the built-in software. Data were exported to CSV format using a USB drive for further processing (reporting and publication of results). Curve fitting graphics were exported to BMP format using a USB drive.

Instrument settings

Common settings:

- Mode: Normal
- Plate: 96 wells (see Figure 2)
- Shake: Off
- Preprocess: Off
- Kinetic: Off
- Curves: On
- Interpret: Off
- Quality: Off

BCA settings:

- Filter: 650/Off

Coomassie Plus settings:

- Filter: 595/Off

Micro Lowry settings:

- Filter: 560/Off

Results

As expected, the standards produced curves of slightly different shapes for each assay, with shapes very similar to the examples displayed in the user manual of each assay. In all cases, correlation was excellent (R^2 ≥0.997). All values were within the dynamic range of the Apollo reader (0.0-4.0 OD). Obtained standard curves are displayed in Figure 3.

The calculated concentrations of the unknown sample were quite close to the expected concentration: within a 5% difference for the Coomassie Plus and Lowry assays, and a 6.84% lower for the BCA assay. Numerical results are summarized in Table 1.

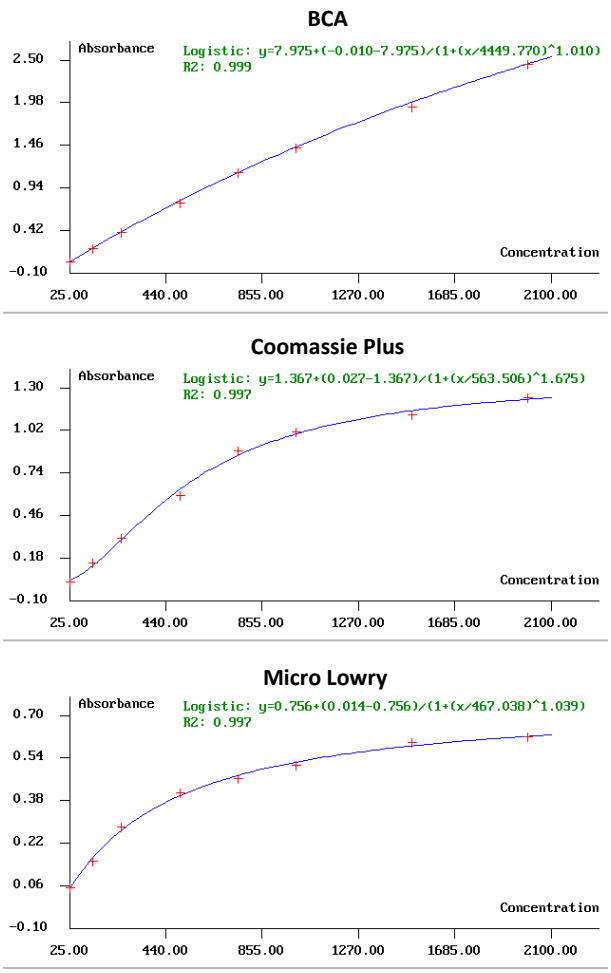

***Figure 3**. Standard curves generated by the Apollo built-in software using the Logistic function (4PL). Graphs were exported as BMP files.*

Standards			
µg/mL	OD		
BSA Standard	BCA	Coomassie Plus	Lowry
2000	2.447	1.232	0.620
1500	1.931	1.122	0.599
1000	1.421	1.012	0.513
750	1.124	0.888	0.463
500	0.751	0.590	0.409
250	0.392	0.307	0.281
125	0.197	0.146	0.151
25	0.031	0.022	0.052
0	0.000	0.000	0.000
Unknown sample (875 ug/mL)			
	BCA	Coomassie Plus	Lowry
OD	1.208	0.955	0.510
Calculated concentr. (µg/mL)	815.1	915.1	918.8
Difference from expected (%)	-6.84	4.58	5.00

***Table 1.** Numerical values obtained for the standards (top) and unknown sample (bottom). All values are the average of duplicate measurements.*

As the BCA assay should be measured at 562 nm but only a 560 nm filter was available, the impact of this difference in the measurement wavelength was assessed. To test this, the same plate was measured with a monochromator-based microplate reader (Tristar 5), both at 562 nm and at 560 nm. Measurements at 560 nm were a 0.86% lower as average than the measurements at 562 nm (data not shown).

Conclusions

Using the Apollo LB 917 to measure the BCA, Coomassie Plus/Bradford and Lowry assays was simple and straightforward thanks to the intuitive built-in software, which allowed to easily set measurement settings, standard positions and concentrations, and curve fitting functions. The standard curves produced were similar to the examples provided with the kit's instructions and had a high correlation coefficient. Although a 562 nm filter was not available to measure BCA, the impact of measuring it at 560 nm instead was negligible. The results obtained for the unknown sample were very close to the expected values, with differences which can be explained mostly by pipetting error.

Concerning the comparison of the different assays tested, the closest concentration to the expected one was obtained with Coomassie Plus (only 4.58% higher than expected) and the most different with BCA (6.84% lower than expected), but it's difficult to assess at what extent these differences are due to the performance of the assay or to pipetting error.

The obtained results confirm that the Apollo LB 917 is a suitable reader for convenient and reliable measurement of protein concentrations.

References

1. Goldring, L.P.D. Protein Quantification Methods to Determine Protein Concentration Prior to Electrophoresis. Methods Mol Biol (2012), 869: 29-35.

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