Total Protein Quantification by Bradford Assay using the Azure Ao Microplate Reader

Introduction

The Bradford assay is a commonly used technique for measuring total protein concentration in solution. Many laboratory applications that evaluate specific proteins in a heterogeneous mixture, such as Western blotting, rely on variable samples to be normalized to the same total protein levels. In the case of Western blotting, loading the same total protein for each sample in the gel is vital for getting semi-quantitative data about specific proteins of interest through immunoblotting.

The Bradford assay is a colorimetric assay where a dye is added that binds directly to proteins in the mixture[1]. Binding to protein in an acidic solution causes a shift in the visible color and absorbance of the dye. The change in absorbance of the solution is indicative of the concentration of proteins and can be used to calculate the concentration by using a standard curve[2].

A convenient method for evaluating a set of samples by Bradford assay is to use a 96-well plate format and an absorbance reader like the Azure Ao Absorbance Microplate Reader. The Ao Microplate Reader has the ability to take measurements and immediately process the raw data to calculate the concentration of the unknowns. The Bradford assay protocol is pre-programmed in the Ao for quick and easy execution and results.

Materials and Methods

The Coomassie (Bradford) Assay kit (Thermo Scientific) was used for this procedure. The kit contains a ready-to-use modified version of the common Bradford Coomassie colorimetric method. When the Coomassie G-250 dye binds protein in acidic medium, the absorption maximum is shifted from 465 nm to 595 nm, with a visible color change from brown to blue.

The unknowns measured for total protein concentration were generated through random dilutions of BSA dissolved in PBS. The standards used in the Bradford assay were prepared by diluting the kit-provided BSA standard in PBS to the concentrations in table 1, with PBS serving as a blank. Once the samples were prepared, they were combined with Bradford reagent in a 96-well microplate. Samples were combined by adding 5 µL of sample or standard to 250 µL of dye, in duplicate wells. Samples were loaded as indicated in Figure 2A, with unknowns in B3/B4 through H3/H4. The plate was shaken for 30 seconds using the shake function of the Ao Microplate Reader. Samples were incubated in the plate for 10 minutes before reading. Absorbance was read at 595 nm using the pre-set Bradford protocol and template on the Ao plate reader.

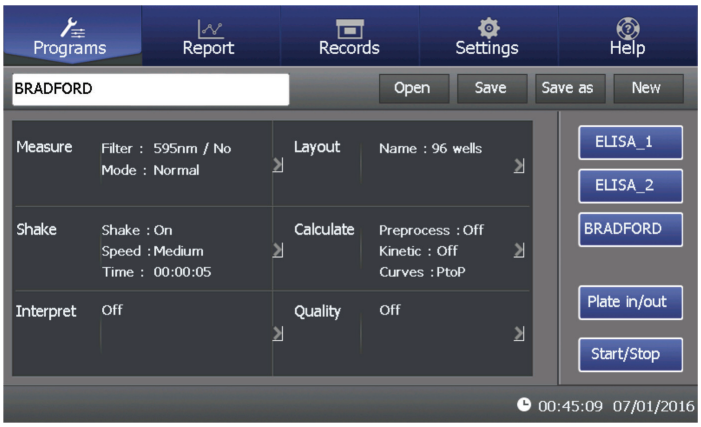

Figure 1. Choosing and setting up the pre-programmed Bradford assay is easy on the Ao Absorbance Reader.

Standards, in µg/µL							
2.00	1.50	1.00	0.75	0.50	0.25	0.125	0.025

Table 1. The above table contains the concentrations of the standards used for this assay.

Once the microplate is loaded, set-up for the Bradford analysis on the Ao Absorbance reader is straightforward and easy using the large touch screen interface. The pre-set Bradford protocol was selected in the Program screen, followed by verification that the plate layout and standard concentrations were correct (Figure 2B). The absorbance of the samples was then measured, and results were viewed, printed and exported from the **Report** tab.

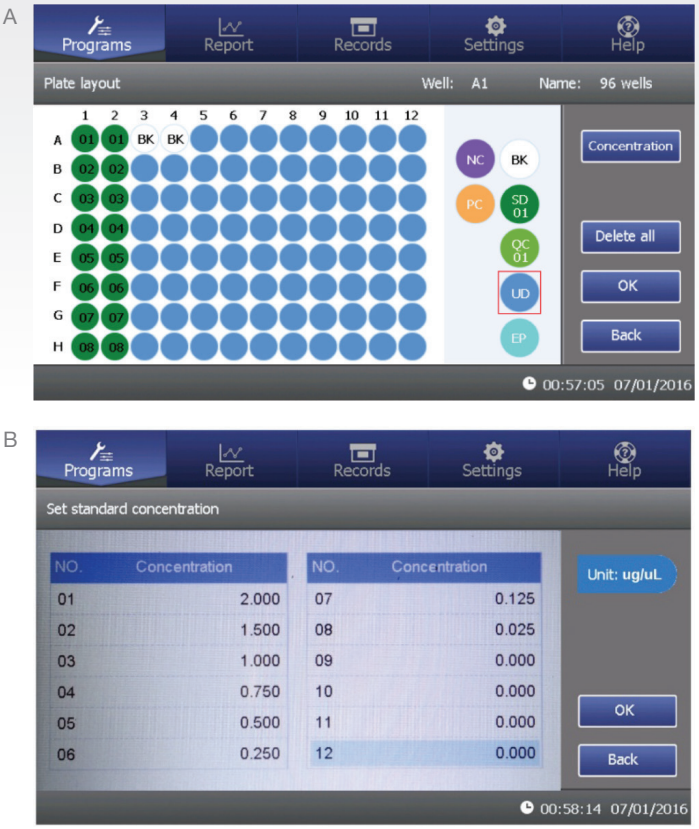

Figure 2. Setting up the plate layout and filling in the standard concentrations using the Ao software. **A.** The plate layout interface allows you to designate where the standards, blank wells, and unknowns are located. Each sample was loaded in duplicate. **B.** Touch **"Concentration"** to enter or change standard concentration values and units.

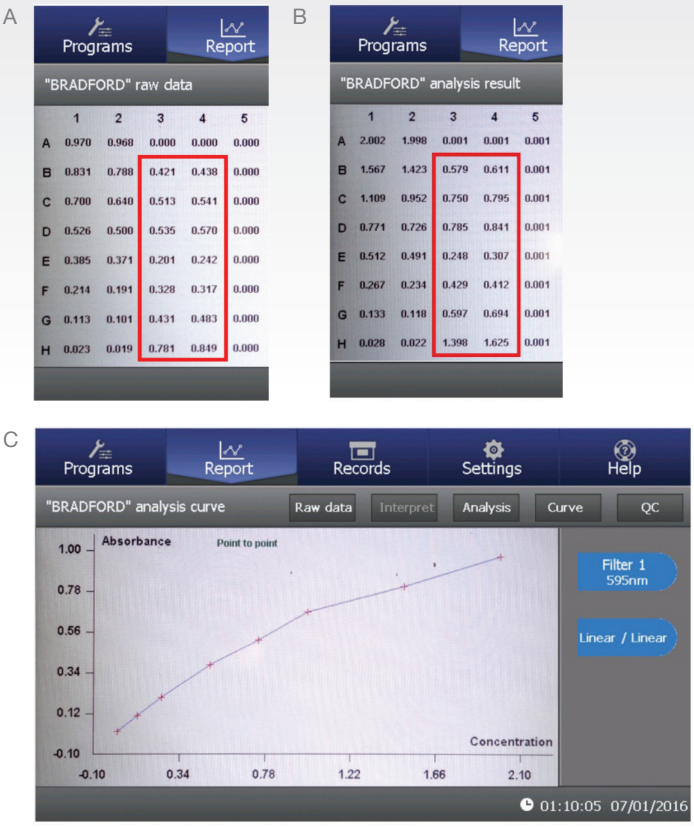

Figure 3. View raw absorbance values, analyzed results and analysis curve easily in the Report tab. **A.** Touch **"Raw data"** to view and export the raw, blank-subtracted OD values. **B.** Touch **"Analysis"** to view and export the unknown concentrations interpolated from the standard curve. In this assay, the unknowns are shown inside the red box. **C.** Touch **"Curve"** to view and export the standard curve generated from the absorption readings of the provided standards.

Results

The purpose of this experiment was to demonstrate the ease and effectiveness of performing a Bradford Assay with the Ao Absorbance Microplate Reader.

Taking measurements and retrieving concentration data is automated with the Ao software. Just prepare the 96-well plate with your samples, load it in the reader, and start the Bradford protocol. After reading the plate, the software opens the **Report** tab with raw data measurements. The standard curve can be viewed by selecting **"Curve"** in the **Report** tab (Figure 3C). A point-to-point curve is the default selection in the pre-set Bradford protocol, but several curve-fit options are available for assays requiring a different fit. The curve-fit and axes can be changed through the **Program** tab or the button beside the curve plot, even after the plate is read.

In this experiment, the protein concentrations of unknown samples were automatically calculated using the standard curve, set to a point-to-point curve-fit, and viewed by selecting **"Analysis"** in the **Report** tab. The concentrations displayed in the **"BRADFORD" analysis result** in Figure 3B were interpolated from the curve.

Conclusions

Performing a Bradford assay using the Azure Ao Microplate Reader is a fast, easy, and reliable method for measuring total protein levels. Data analysis is automatically performed by the Azure Ao plate reader software, and results are easily viewable and exportable using the large touch screen interface, minimizing the need for outside analysis or calculation software.

Quick Protocol: Bradford Assay with the Ao plate reader

Prepare

1. Prepare standards for a standard curve (dilute known concentrations of BSA in PBS).
2. Add appropriate volume standards and unknown samples to duplicate or triplicate wells of a 96-well plate.
3. Add appropriate volume of Bradford reagent to all standard and sample wells.
4. Load plate into microplate reader.
5. Use shake function on plate reader to mix samples. Wait 10 minutes before reading.

Read

6. Select the Bradford protocol from the Programs screen. Set up plate layout by selecting **"Plate Layout"**, and fill in concentrations of standards.
7. Read absorbance at 595 nm using pre-set Bradford protocol.

Analyze

8. Raw data measurements will appear in a Report after measurement.
9. Select **"Curve"** to verify standard curve fit.
10. Select **"Analysis"** to view concentrations of unknowns.
11. Data can be exported through **"Export"** option.

References

1. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976. 72:248-54.
2. Kruger NJ. The Bradford method for protein quantitation. In: The Protein Protocols Handbook. Humana Press. 2009. pp 17-24.

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