bradford assay

stratus plate reader

introduction

Microplate readers are important laboratory tools for optical measurements of biological samples. Common applications include measurements of bacterial growth, cell proliferation, cytotoxicity, and protein concentration, as well as ELISAs and reporter gene assays. The Stratus, a small LED-based plate reader developed by Cerillo, is uniquely versatile in its combination of small footprint, precision, and usability. This application note describes the performance of the Stratus when used for the measurement of the Bradford protein assay.

bradford assay

The Bradford assay is a colorimetric method for total protein quantitation. It measures protein concentration by mixing Coomassie dye reagent with a protein sample. The Coomassie dye binds to specific amino acids, primarily arginine, lysine, and histidine [1]. The binding of Coomassie dye to protein causes the absorbance maximum of the bound dye to shift from 465nm to 595nm. This color change can then be measured by a spectrophotometer or other optical instrument [2].

The ThermoScientific™ Coomassie (Bradford) Protein Assay Kit was used for the measurement of Bovine Serum Albumin (BSA) and Bovine Gamma Globulin (BGG). Serial dilutions of BSA and BGG were made at concentrations ranging from 25 µg/mL to 2000 µg/mL (8 concentrations total). Each concentration was added to a 96-well microplate in triplicate at a volume of 5 µL. For each dilution scheme, one well containing 5µL of diH_2O was tested as the blank control.

After all dilutions and blanks were added to the 96-well plate, 250µl of Coomassie reagent was added. The plate was gently agitated for 30 seconds, then incubated for 10 minutes at room temperature.

After incubation, the plate was measured using Cerillo's microplate reader, the Stratus, at a wavelength of 600nm. The Stratus was controlled by the Cerillo Device Manager software (Figure 1). This software allows for easy data analysis by exporting data directly to a universal format that is compatible with all analysis software packages. To read a single plate, "quick read" is selected on the device menu (Figure 1). The data is then stored under "files" and can be saved to the user's location of choice (Figure 2).

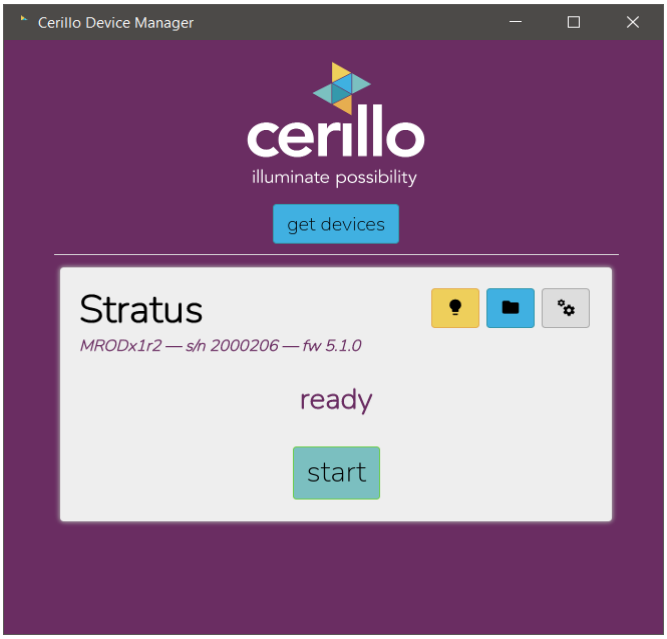

Figure 1. Cerillo Device Manager creates a simplistic interface to manage the Stratus microplate reader.

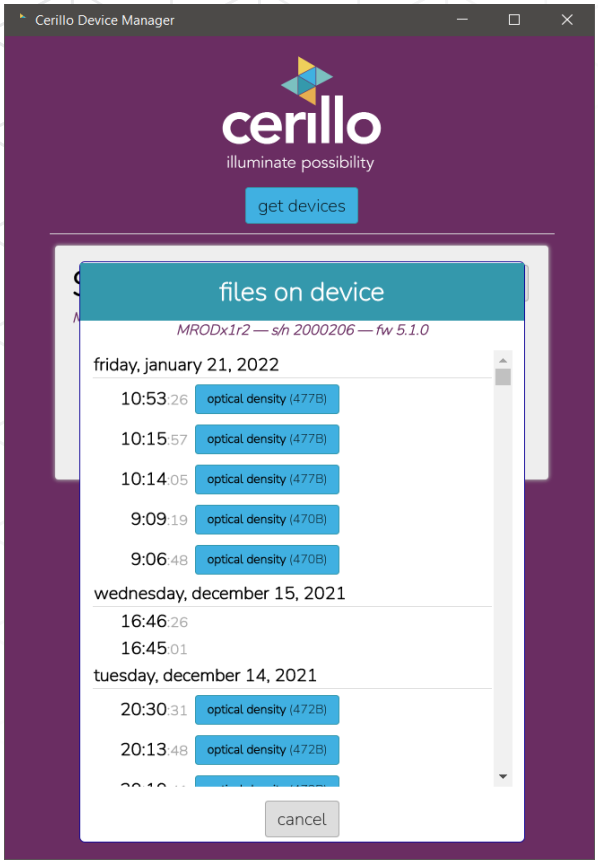

Figure 2. File Storage is easily accessed via the Cerillo Device Manager which allows data to be exported and saved for analysis.

The Bradford assay standard curves were analyzed for quality using a 4-parameter logistic-fit curve. The plotted results show the expected relationship between BSA and BGG (Figure 3). There is low variability between sample replicates and a good curve fit. The ratio between BSA and BGG concentrations is representative of the known offset of 0.56 determined by ThermoScientific™. This indicates that the Stratus produces accurate and highly precise measurements for total protein concentration. A comparison with data from other spectrophotometers on the market revealed equivalent results, confirming the performance of the Stratus is comparable to other absorbance-based microplate readers.

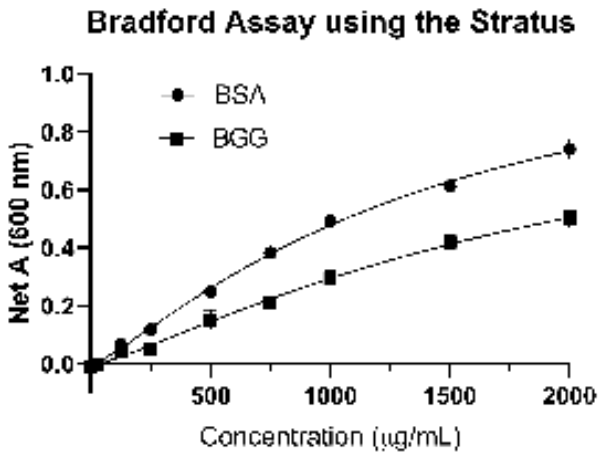

Figure 3. Net Absorbtion measured using the Stratus. The ThermoScientific™ Coomassie (Bradford) Protein Assay Kit was used to measure concentrations of BSA and BGG. A four-parameter logistic curve was fit with an R^2 value of 0.995 for BSA and a value of 0.986 for BGG. Error bars are present but are not visible for most points.

summary

The Bradford assay is widely used to quickly measure protein concentration in liquid samples. Using the Stratus microplate reader BSA and BGG were measured and show accurate and precise results. The Stratus, therefore, provides a versatile alternative to traditional microplate

references

1. Pierce Coomassie (Bradford) Protein Assay Kit. (n.d.). Retrieved October 29, 2019, from https://www.thermofisher.com/order/catalog/product/23200?SID=srch-srp-23200.

2. Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248-54