Benchmark Plus™ Microplate Reader Scan Well Feature

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The Benchmark Plus microplate reader is a new monochromator-based absorbance reader from Bio-Rad that offers superior convenience, accuracy, and flexibility. The Benchmark Plus is controlled by Microplate Manager® software. A new feature has been designed in Microplate Manager software (version 5.1 and higher) that utilizes the monochromator design of Benchmark Plus and allows the user to prescan a well in order to determine the optimal wavelength prior to running a full plate of samples. In this note, a prescan procedure is described to show how to implement such a function into real-life applications.

In the following procedure, standard protein (bovine IgG) is added to cell culture medium without serum and the Scan Well function in Benchmark Plus is used to determine the best wavelength for protein assay with cell culture medium. Bio-Rad's protein assay kit (catalog # 500-0001) is used to check for measurement inconsistencies in culture medium.

Procedure

1. Serially dilute (1:2) standard bovine IgG protein ranging from 62.5 µg/ml to 1 mg/ml in F-12 Ham's medium without serum.
2. Aliquot 10 µl (in triplicate) of each diluted standard protein into a 96-well clear bottom black microplate; i.e., A1, B1, and C1 for 1 mg/ml protein sample. Aliquot 10 µl (in triplicate) of F-12 Ham's medium into 3 wells (A7, B7, C7) to be used as blanks.
3. Add 200 µl of diluted dye reagent (from Bio-Rad protein assay kit) to each well. Mix thoroughly using a microplate mixer for about 5 sec. Make sure that no bubbles are present. Bubbles may cause inconsistencies in absorbance reading.
4. Incubate 5 min at room temperature.
5. Place microplate into Benchmark Plus microplate reader.
6. Open Microplate Manager. Under File, select Endpoint protocol.
7. Select 96-well format. Under Advanced Options, open Scan Well.
8. Scan well A1 at 340–800 nm using a 5 nm step size. Save data.
9. Scan remaining wells (A2–A7) and save data.
10. For bovine IgG at optimal wavelength, select Endpoint protocol, 96-well format, and single wavelength reading at 590 nm.
11. Highlight the appropriate wells to be read; i.e., A1 to C7 for the concentration range from 62.5 µg/ml to 1 mg/ml and the blank.
12. Read absorbance. Save and analyze data.

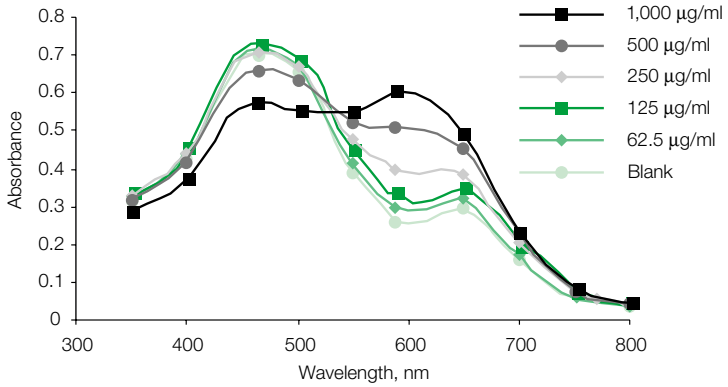

Fig. 1. Absorbance vs. wavelength reading of bovine IgG concentration using Benchmark Plus Scan Well feature.

Results

The most concentrated protein sample shows a higher peak at 590 nm than the least concentrated protein samples (Figure 1). The least concentrated samples and the blank showed higher peaks at 470 nm, whereas the more concentrated protein samples showed similar peak heights at around 470 and 590 nm. From the figure, one can conclude that whereas a peak at 470 nm is apparently caused by the cell culture medium used here, the optimal wavelength for bovine IgG is at 590 nm.

Further reading of bovine IgG concentration at 590 nm on Benchmark Plus yields a linear graph with a correlation coefficient of 0.9993 (Figure 2).

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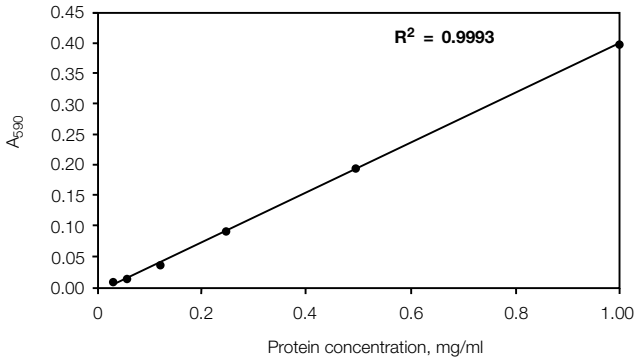

Fig. 2. Linear response of absorbance vs. protein concentration, reading bovine IgG at 590 nm using the Benchmark Plus microplate reader.

Conclusion

The Benchmark Plus Scan Well feature is used to ascertain the best wavelength for reading a desired assay. It allows scanning of a well from 340–800 nm at various wavelength increments. A graph and data of the scan area are provided and can be exported into Microsoft Excel software for further analysis. The Scan Well feature minimizes the problem of finding out at what wavelength samples should be read, thus saving time and effort. At the optimal wavelength for the bovine IgG tested, the correlation coefficient is ≥ 0.99 and linear over the dilution range of 62.5 µg/ml to 1 mg/ml. The dilution in F-12 Ham's medium without serum did not affect the measurement of the protein. The results show that the Benchmark Plus microplate reader is an excellent instrument to use for absorbance studies, as well as an instrument to save time in determining the optimal wavelength to read a sample.

Bibliography

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