APPLICATION NOTE

Protein quantitation with the EMax Plus Microplate Reader

Introduction

Endpoint readers are prolific in the laboratory since absorbance has become the detection of choice for many applications. Examples include ELISAs for quantitation of cytokines and protein concentration determination using the Bradford assay. This application note compares the performance of the Molecular Devices EMax® Plus Microplate Reader to the VMax Reader using a Bradford protein quantitation assay. In addition, a performance comparison is made between the EMax Plus Microplate Reader and the EMax Endpoint Reader using a sandwich ELISA for the quantitation of mouse/rat IL-22.

Bradford assay

The Bradford protein concentration assay is an absorbance assay based upon the addition of an acidic dye to a protein solution[1]. The absorbance maximum for Coomassie Blue dye shifts from 460 nm to 595 nm when binding to protein occurs[2]. Comparing unknown samples to a standard curve provides a relative measurement of protein concentration.

The Bio-Rad Bradford Protein Assay Kit was used for quantitation. A standard curve of bovine serum albumin in the sensitivity range of the assay was prepared in triplicate and pipetted into a 96-well microplate. Coomassie Brilliant Blue G-250 dye was diluted 1:4 per instructions and added to the protein samples. After a 30 minute incubation period, the optical density was read at 585 nm on both the EMax Plus and the VMax readers. SoftMax® Pro Software was used to subtract the plate blank and generate a standard curve (Figure 1). Comparison of data from both instruments showed that the R^2 value for both curves was 0.98. The linear portion of the curve was comparable to the curve in the kit literature.

ELISA assay

Interleukin-22 is a cytokine involved in immune function. It is part of the Interleukin-10 superfamily of cytokines and the family of interferons[3] and it is an important mediator in inflammatory response and tissue repair. IL-22 is expressed on many cells including gut, skin, NK cells and $CD4^+$ Th1 cells[4]. A quantitative mouse/rat IL-22 sandwich ELISA from R&D Systems was used to compare results from EMax Plus and EMax microplate readers. A polyclonal antibody specific to mouse/rat IL-22 was pre-coated onto a 96-well microplate. Standards, controls and blanks were pipetted into the wells and incubated followed by washing with the Molecular Devices MultiWash™+

Benefits

- **8 filters come standard to cover a wide range of applications**
- **Compact footprint**
- **Walk-up usability**
- **Pre-defined protocols with SoftMax Pro Software**

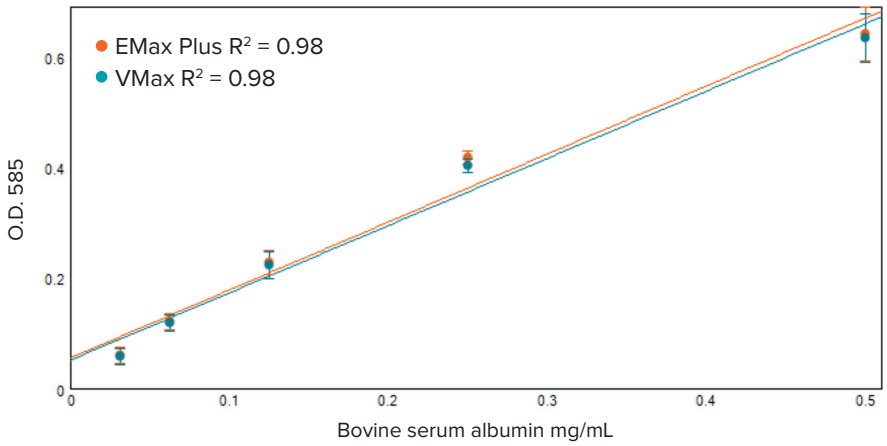


Figure 1: Absorbance reader comparison with Bradford protein assay. The Bio-Rad Bradford Protein Assay Kit was used to determine the concentrations of bovine serum albumin in a standard curve. Signal comparison from both instruments was nearly identical. The assay maintained linearity across five dilutions with R^2 values = 0.98.

Microplate Washer. An enzyme-linked polyclonal antibody specific for mouse/rat IL-22 was added to the wells and incubated followed by an additional wash. Substrate was added followed by stop solution after another short incubation. Absorbance was read on both the EMax Plus and the EMax microplate readers. Color intensity was proportional to the amount of mouse or rat IL-22 bound during the initial incubation. SoftMax Pro Software was used to calculate each standard curve shown in Figure 2. R^2 values for each curve were also very close.

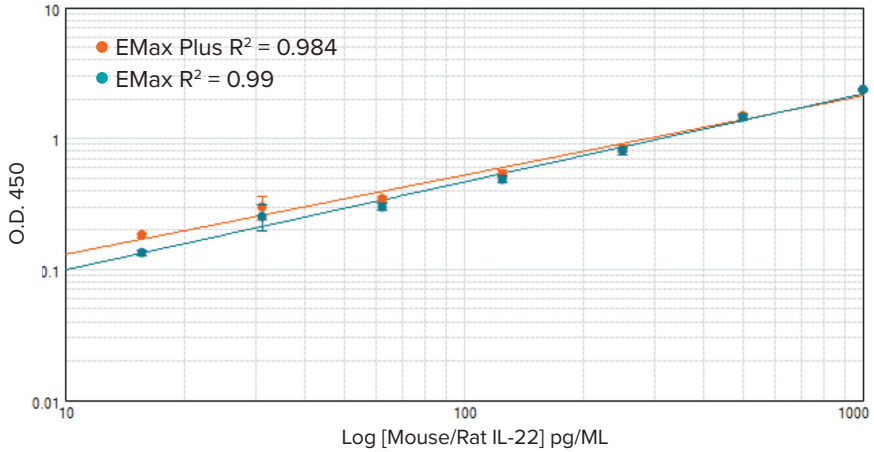


Figure 2: Absorbance reader comparison with ELISA assay. A Mouse/Rat IL-22 Quantikine ELISA from R&D Systems was used to compare performance of the EMax Plus and the EMax endpoint readers in an absorbance assay. An IL-22 standard curve was prepared and a sandwich ELISA performed using the MultiWash+ Plate Washer in strip mode to wash the wells. After reading the ELISA plate on both readers, each standard curve was nearly identical.

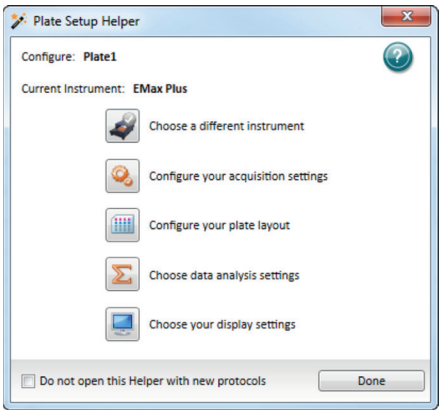
MultiWash+ Microplate Washer

The MultiWash+ Microplate Washer is an automated washer that is configurable for both 96- and 384- well plates. It comes standard with 4 wash/rinse bottles and 1 waste bottle for maximum washing flexibility. The on-board touch panel comes with the option of 20 different wash protocols with up to 8 cycles within each protocol. Washing variations include adjustable speed and volume, adjustable aspiration speed and time and adjustable soak times. Efficient washing is achieved with cross-wise aspiration reducing residual volume within each well. Three modes of shaking are available to mix solutions. The washer has a small footprint and is vacuum and pressure free, with on-board pumps that provide a quiet wash experience.

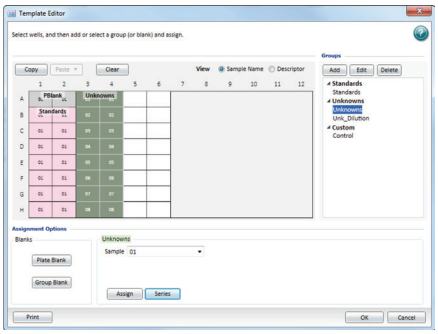
Summary

The EMax Plus Microplate Reader provides a robust solution in an entry level platform. The convenience and power of SoftMax Pro Software makes it possible to easily set up and analyze a broad range of assays. Results from both a protein quantitation assay and an ELISA assay have shown performance equivalent to high end microplate readers.

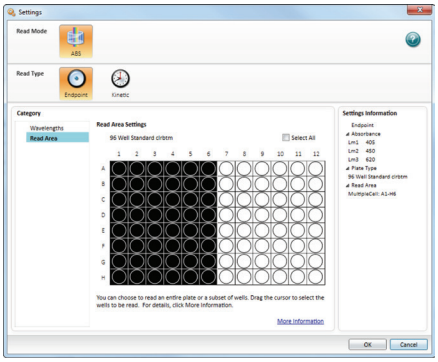
3-step process for setup and data analysis



Step 1. SoftMax Pro assay setup. The Plate Setup Wizard walks the user through the steps to set up the assay. The software automatically recognizes instruments that are connected. It then uses the pre-defined settings for the specified instrument to build a protocol. It this case, the EMax Plus Microplate Reader was the default.



Step 2. Plate layout configuration. User selects appropriate wells for samples and control using plate configuration tool.



Step 3. Data acquisition and analysis. Pre-defined settings for Bradford assays are used to automatically calculate graphs from plate data.

References

1. Bradford, M. (1976) *Anal Biochem.* 72:248
2. Sedmack, J et al. (1977) Anal Biochem. 79:544
3. Pesta, S. et al. (2004) *Annual Rev Immunol.* 22:929-79
4. Liang, S.C. et al. (2005) *J. Exp. Med.* 203:2271.

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