Performing an ELISA with the Ao Absorbance Microplate Reader

Introduction

The enzyme-linked immunosorbent assay, commonly called an ELISA, is a widely used biology research method for quantifying proteins or other antigens in an unknown solution. The sensitive and straightforward nature of the assay makes it useful for an array of applications, such as testing blood samples for the presence of infectious disease antibodies, testing the binding between a newly isolated antibody and its target protein, or a variety of other tests that rely on antibody-antigen interactions.

The technique starts with a 96-well plate where the material of the wells allows proteins to adhere to the surface. Immobilization allows the antigen to be bound by its detector-antibody and then washed of any unbound antibodies, followed by measurement of the antigen. An enzyme linked to the detection antibody will interact with a substrate added in the final step to produce a signal proportional to the antigen (Figure 1).

There are four ways that ELISAs can be set up in terms of antibodies and antigens. With a direct ELISA, the unknown sample containing the antigen of interest is adhered directly to the well, followed by detection with a primary antibody. An indirect ELISA begins the same way, but the primary antibody is not linked with the enzyme and a secondary antibody must be used for detection. A sandwich ELISA, depicted in Figure 1, starts with an antibody that is adsorbed to the plate first; then, the antigen-containing sample is added and the antigen is captured by the immobilized antibody and detected similar to the direct or indirect methods. A fourth type is the competitive (or inhibition) ELISA, which can be setup similarly to the other three, but instead of the antibody being labeled, the antigen is labeled; the labeled antigen is added after the unknown samples to see how much unlabeled antigen in the samples competes with the labeled antigen. Unlike the other three types, higher signal from the competitive ELISA means that there is less competition and thus less antigen in the unknown.[1,2]

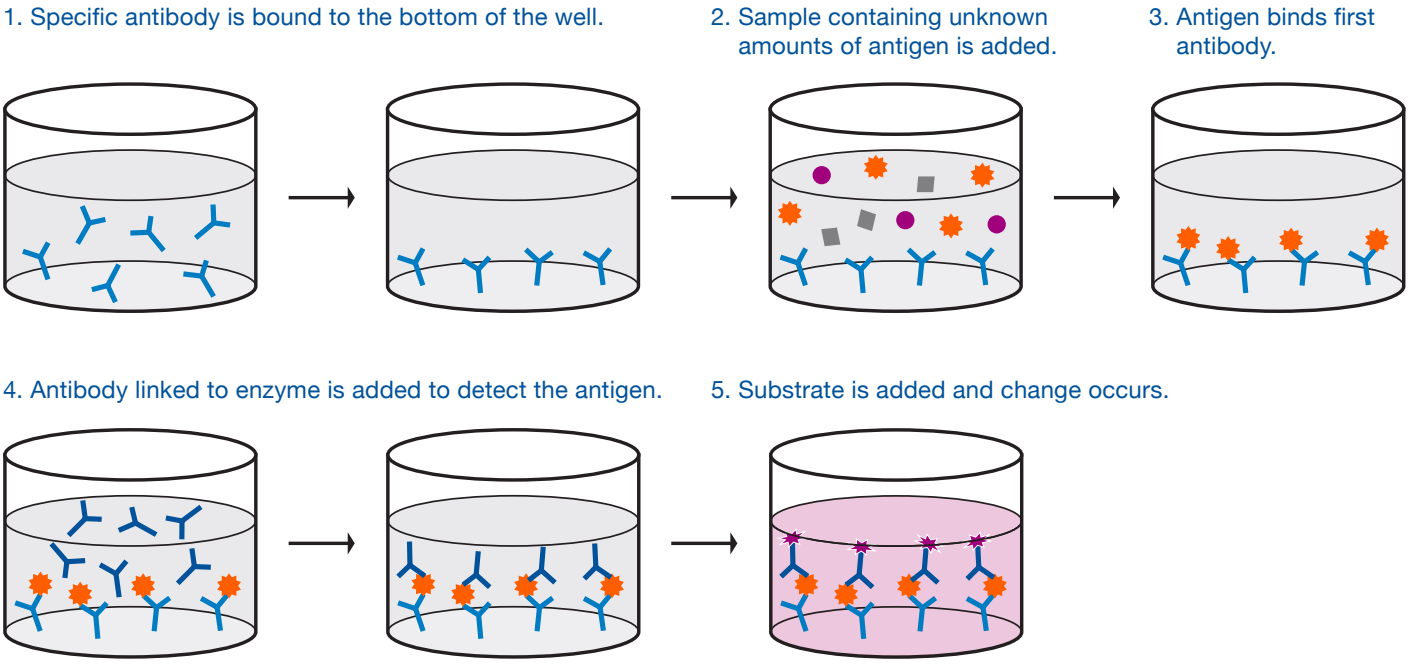

Figure 1. Diagram of an ELISA Assay.

ELISAs are usually performed in 96-well plates, where many conditions and repeat samples can be tested at once, and then measured using a micro plate reader. An absorbance-based plate reader can measure the absorbance of light at chosen wavelengths relevant to whatever is produced from the enzyme reaction, providing quantitative differences between positive and negative samples. In this Application note, we describe use of the Azure Ao Absorbance Microplate Reader for measuring and analyzing results from an ELISA.

Materials and Methods

The Azure Ao Absorbance Microplate Reader was used for all measurement and analysis.

To demonstrate the ELISA protocol, a Histamine EIA Kit (Oxford Biomedical Research) was used to measure histamine levels in biological samples. The kit provided PBS, wash buffer, substrate, histamine-HRP conjugate, histamine standards, and a 96-well plate pre-coated with monoclonal anti-histamine antibody. Unknown samples were prepared by diluting food samples in the kit provided PBS.

The experiment was a competitive indirect ELISA. The plates were pre-coated with anti-histamine antibody and the samples were incubated in the wells to allow binding of the histamine to the antibody. Horseradish peroxidase (HRP) labeled histamine was added with the samples, creating competition between binding of the labeled histamine conjugate and any unlabeled histamine occurring in the samples. After incubation and washing, the substrate was added to the wells to allow the reaction of the HRP enzyme with the substrate to produce a colored substance. The reaction was stopped using 1N Hydrochloric Acid and the change in color produced a change in absorbance that was read at 450nm with a substrate reference read at 630nm using the Azure Ao Microplate Reader.

Results

How to perform ELISAs with the Ao plate reader

The purpose of this experiment was to demonstrate the ease of performing and reading ELISA assays using the Azure Ao Absorbance Microplate Reader. To demonstrate the ELISA protocol, the Histamine EIA Kit (FS35) from Oxford Biomedical Research was used to measure histamine levels in food samples using a competitive indirect ELISA.

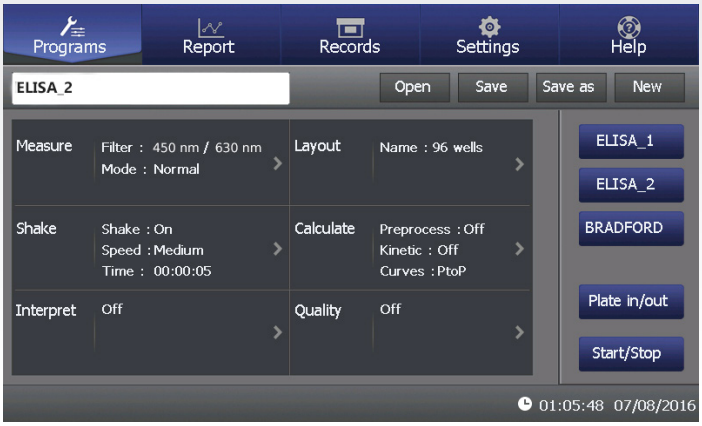

Figure 2. Choosing and setting up an ELISA protocol is easy on the Ao Absorbance Microplate Reader with easy-access pre-programmed protocols.

A

B

Figure 3. Setting up the plate and assigning standard values 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.

Taking measurements and retrieving both raw and analyzed data is simple and intuitive with the Azure Ao Microplate Reader. Just perform the ELISA according to the manufacturer's protocol, adjust the pre-programmed ELISA protocol as needed, load the plate in the microplate reader and start the protocol. After reading the plate, the software opens the **REPORT** tab with raw data measurements. The standard curve can be viewed and printed by selecting "**CURVE**" in the **REPORT** tab (Figure 5). 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.

The antigen concentrations are automatically calculated using the standard curve (set to point-to-point for this assay). Results can be viewed and exported by selecting "**ANALYSIS**" or "**RAW DATA**" in the **REPORT** tab. Figure 4 shows both raw data and analyzed results interpolated from the standard curve.

Conclusion

The Ao is a convenient plate reader for the classic ELISA

ELISAs are valuable in the scientific research community, and the Ao plate reader provides performance and convenience that allows for the best quality research. ELISAs can also use a variety of enzyme and substrate combinations other than horseradish peroxidase, and the Ao plate reader has the flexibility to measure the majority of commonly used colorimetric reagents.

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.

References

1. Goldsby RA, Kindt TJ, Osborne BA, Kuby J. ELISA. In: *Immunology*, Fifth Edition. Freeman, W.H. & Company. 2002. pp 148-149
2. William J. Antigen Measurement Using ELISA. In: *The Protein Protocols Handbook*. Humana Press. 2009. pp 1827-1833

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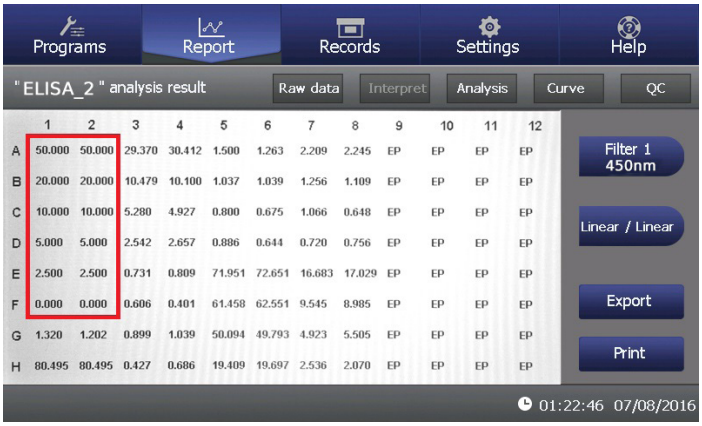

Figure 4. View raw absorbance values, analyzed results and analysis curve easily in the **REPORT** tab. A) Touch "**RAW DATA**" to view and export the raw, blank-subtracted OD values. B) Touch "**ANALYSIS**" to view and export the unknown concentrations interpolated from the standard curve. In this assay, the kit provided standards are shown inside the red box.

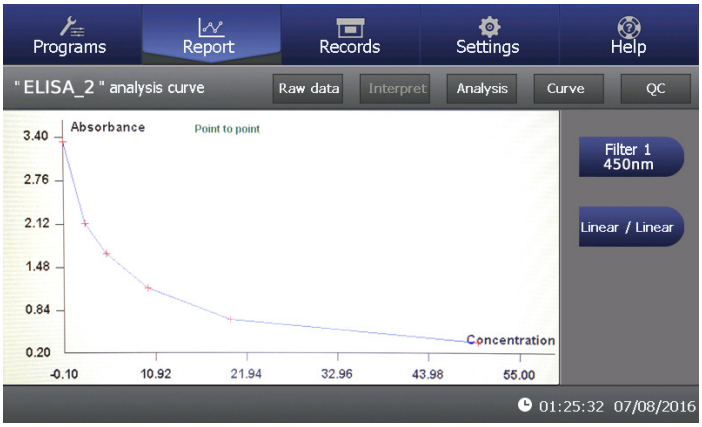

Figure 5. Touch "**CURVE**" to view and print the standard curve generated from the absorption readings of the provided standards.

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