	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
ELISA **Module Hours: 3.0**	**Effective Date: 03/11/2024** **PRQs: Protein Assay**	**Revision 1.0** **Author: Z. Hazlett-Klein** **Checked by Editor: Alec B.**

Background

The Enzyme-Linked Immunosorbent Assay (ELISA) is a microplate-based technique used to detect and quantify the amount of an antigen (peptide, protein, antibody, or hormone) within a solution.[1] In general, this is achieved by first immobilizing an antigen to a microplate well, then adding an antigen-specific antibody to bind any immobilized antigen, then detecting the presence of the antigen-antibody pair by a chemical reaction catalyzed by an enzyme-linked to either the first (primary, 1º) or a second (secondary, 2º) antibody. This chemical reaction produces a detectable change in the solution usually in the form of a measurable color change. There are different approaches to the ELISA each with unique advantages (Figure 1). The ELISA is a simple method that

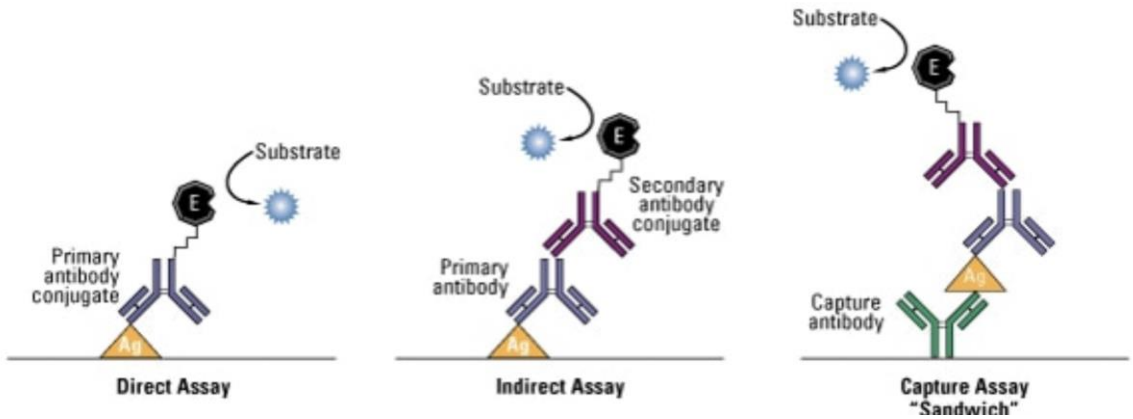


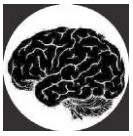
Figure 1. Three common ELISA methods.[1] The direct assay involves detection of an immobilized antigen directly by an enzyme-linked 1º antibody specific to the antigen. The Indirect assay is the same but first requires detection of the antigen by a 1º antibody, then detection of the 1º antibody by a 2º enzyme-linked antibody. The capture, or "Sandwich," assay is the same as the previous, except the antigen is immobilized using a capture antibody as opposed to direct adsorption to the microplate well surface. In each, the antibody conjugated enzyme catalyzes the chemical reaction of a substrate. This reaction provides a quantifiable product for the measurement of the antigen in solution.

allows for the detection of small molecules in a variety of solutions such as cell lysates, blood samples, and more.

The direct ELISA assay is the simplest of the three assays allowing for antigen detection with only a single antibody. The use of only a single antibody also eliminates any non-specific activity associated with a second antibody. With the least number of components, the direct ELISA requires less time for antigen detection than the indirect assay. Where the direct ELISA is relatively simple in execution, it does have drawbacks. With only a single primary antibody, the enzyme required for detection must be fused to the primary antibody. This has the potential of decreasing the antibody's reactivity to the antigen if the linked enzyme interferes at all with the binding reaction. Also, the need for linking enzyme to the primary antibody limits the versatility of the detection setup as an enzyme-linked antibody needs to be produced for every antigen one desires to detect.

The indirect ELISA provides more versatility in assaying for antigens. In this setup, the secondary antibody, conjugated to the detection enzyme, has specificity for the primary antibody as long as it matches the host species in which the primary antibody was produced. This allows for exchange of primary antibodies with specificity toward different antigens. The disadvantages of the indirect ELISA include the increase in time needed for the assay due to having more steps in the procedure as well as the increased potential for non-specific binding of the secondary antibody leading to a false positive signal. Despite these drawbacks, the versatility of this assay compared to the primary ELISA makes this a very useful technique.

As described in Figure 1, there is an alternative approach, called the sandwich ELISA, where the plate is coated first with a capture antibody rather than the antigen. The antigen is then bound and immobilized by the capture antibody. Detection is accomplished with either an enzyme linked 1º antibody as used in the direct ELISA, or the 1º/enzyme-linked 2º antibody pair used in the indirect ELISA. The sandwich ELISA is advantages due to its high degree of sensitivity to low amounts of antigen in a solution. The drawbacks include the cost of potentially needing to purchase pre-capture antibody coated plates or the time needed to make your own. As seen in the indirect approach, there is also the potential for non-specific binding of 1º or 2º antibodies if the specificity of these are not high enough. With the proper preparation, the sandwich

	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
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ELISA is a powerful, as well as common tool for the detection of antigens in biological samples. In this Module, you will perform a sandwich ELISA with biotin-streptavidin detection.

1. Purpose

The purpose of this SOP is to instruct students on the proper procedures involved in the Enzyme-Linked Immunosorbent Assay.

2. Scope

This procedure applies to qualified skills center users.

3. Responsibility

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure.

4. Definitions

4.1 Antigen – a peptide, protein, or hormone that has the potential to produce an immune response in the environment of a foreign host cell.
4.2 Antibody – A protein produced by the immune system with specific binding activity toward an antigen or other antibody.
4.2.1 Primary (1º) Antibody – An antibody with binding specific to an antigen.
4.2.2 Secondary (2º) Antibody – An antibody with binding specific to the 1º antibody.

5. Materials/Equipment[2]

5.1 Test and control samples
- 5.1.1 Test samples: (Apolipoprotein CII unknowns)
- 5.1.2 Control samples: (Apolipoprotein CII Standard 1)

5.2 Detection antibody
- 5.2.1 We will use a monoclonal anti-Apolipoprotein CII antibody conjugated to biotin.

5.3 Streptavidin Peroxidase conjugate.

5.4 Substrate
- 5.4.1 We will use 3,3′,5,5′-tetramethylbenzidine (TMB; a substrate of HRP (Horse Radish Peroxidase))

5.5 Phosphate Buffered Saline (PBS; See recipes below)

5.6 Blocking buffer (See recipes below)

5.7 Wash solution (See recipes below)

5.8 Dilution buffer (See recipes below)

5.9 PBS, 0.1%BSA (See recipes below)

5.10 Stop Solution (See recipes below)

5.11 TMB (tetramethylbenzidine) solution (See recipes below)

5.12 96 Well polystyrene plastic plate(strips), coated with Apolipoprotein CII capture antibodies. (referred to as a microplate)

5.13 Microplate reader

6. Recipes[2]

6.1 Phosphate Buffered Saline (PBS), pH7.4: 0.086 M disodium hydrogen phosphate (Na2HPO4), 0.020 M monopotassium phosphate (KH2PO4), 3.08 M sodium chloride

6.2 Blocking buffer: PBS, 1% BSA, 500 ml PBS, 5 g BSA

6.3 Wash solution (pH 7.4 0.15M PBS): KH2PO4 0.2g, Na2HPO4·12H2O 2.9g, NaCl 8.0g, KCl 0.2g, Tween-20 0.05% 0.5ml, add distilled water to 1000ml.

6.4 Dilution buffer: Bovine serum albumin (BSA) 0.1g, add washing buffer to 100ml or use serum of sheep serum, rabbit serum and other washing liquid to prepare 5-10%.
6.5 PBS, 0.1%BSA: 500 ml PBS, 0.5 g BSA
6.6 Stop Solution: 2 % oxalic Acid
6.7 TMB (tetramethylbenzidine) solution: TMB (10mg/5ml absolute ethanol) 0.5ml, Substrate Buffer (pH 5.5) 10ml, 0.75% H2O2 32µl.

7. Procedures (Adopted from abcam: Sandwich ELISA Protocol)[3]

7.1 Adding Standards and Unknowns
7.1.1 Standard 1 has been created for you. Aliquot 80µL of standard 1 into well 1 of your plate strip.
7.1.2 Add 60µL of 1x Diluent M into wells 2-6.
7.1.3 Create a serial dilution, Diluting 20µl across each well starting from well 1 through well 5, mixing via pipette adequately in each well. After mixing in well 5 dispense of excess 20 µL into a waste beaker.
7.1.4 Aliquot 60 µL of one unknown into wells 7 and 8.
7.1.5 Cover plate strip with a plate cover/seal and uncubate 2 hours at room temperature.
7.2 Plate Washing
7.2.1 Decant the contents of the strip into a sink/paper towel. Hit the back of the plate while decanting into a paper towel to ensure all contents are removed. (make sure not to knock the strip out of the holder.)
7.2.2 Fill each well with approximately 200 µL of 1x wash buffer.
7.2.3 Repeat the previous two steps 4-5 times.
7.3 Incubation with detection and secondary antibody
7.3.1 Add 50 µL of secondary antibody into each well.
7.3.2 Cover and incubate for 1 hour at room temperature.
7.3.3 Repeat the washing steps in 7.2.
7.4 Detection
7.4.1 Add 50 µL of streptavidin-peroxidase conjugate into each well. (possibly labeled SP)
7.4.2 Cover and incubate for 30 minutes at room temperature.

7.4.3 Wash as described in 7.2.
7.4.4 Add 50 µL of chromogen substrate into each well.
7.4.5 After 10-20 minutes, blue color should be visible, add stop solution.
7.4.6 Read the absorbance of the plate at the plate reader at 450 nm.
7.4.7 Analyze results.

8. Troubleshooting

9. References

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10. MODULE METHODS TASK

10.1 Describe the function of each antibody used in this protocol.
10.2 Briefly summarize the purpose of the ELISA.
10.3 Apolipoprotein CII (Apo-CII) is a protein that functions as a cofactor in triglyceride-rich liposome metabolism. Apo-CII deficiency causes the degradation of chylomicrons in the blood to be impaired. Apo-CII overexpression has also been linked to multiple diseases including Parkinson's and Alzheimer's. Describe how an ELISA could be used

to test for Apo-CII in humans but might not work in a separate species. What would be necessary to detect this antigen in other species?
10.4 Run the ELISA protocol, testing for the presence of Apo-CII in two unknown samples. Determine the concentration of detected Apo-CII by establishing a standard curve using your standards. Take a picture, label wells, and write a description of your results and conclusion.