Enzyme-Linked ImmunoSorbent Assay (ELISA)

Description:

An enzyme-linked immunosorbent assay (ELISA) is used to detect the presence of an antigen in a sample. The antigen is immobilized to the well of a plate by adsorption, or captured with a bound, antigen-specific antibody. A detection antibody is then added forming a complex with the antigen, if present. The detection antibody can be covalently linked to an enzyme, or itself be detected by a secondary, enzyme linked antibody. Enzyme substrate is then added to the wells producing a visible signal that is correlated with the amount of antigen and measured by a spectrophotometer

Materials

Typical ELISA Kit Components

- Antibody-coated 96-well microplate
- Detection antibody (usually biotinylated)
- Standard
- HRP conjugate (antibody or streptavidin)
- Diluent buffers
- Wash buffer
- Chromogenic substrate (usually TMB)
- Stop solution
- Plate covers

Additional Materials Required

- Absorbance-based microplate reader
- Distilled or deionized water
- Squirt wash bottle or an automated 96-well plate washer
- Sample

General Protocol

Run time: *4 hours – 30 minutes hands-on time*
Note: *A standard curve must be run with each assay for quantitation*

1. Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.
2. Add 50-100 µL of prepared standard and sample to wells. Cover plate and incubate at room temperature for 2 hours.

3. Thoroughly aspirate or decant solution from wells and discard the liquid.
4. Wash wells 4 times using a squirt wash bottle or an automated 96-well plate washer.
5. Add 100 µL of diluted detection antibody to wells. Cover plate and incubate at room temperature for 1 hour.
6. Thoroughly aspirate or decant solution from wells and discard the liquid.
7. Wash wells 4 times.
8. Add 100 µL of diluted HRP conjugate to each well. Cover plate and incubate at room temperature for 30 minutes.
9. Thoroughly aspirate or decant solution from wells and discard the liquid.
10. Wash wells 4 times.
11. Add 100 µL of chromogenic substrate to each well.
12. Develop plate at room temperature in the dark for 30 minutes.
13. Add 100 µL of stop solution to each well. The solution in the wells should change from blue to yellow.
14. The plate must be evaluated within 30 minutes of stopping the reaction. Read the absorbance of each well at 450 nm and 550 nm. Subtract 550 nm values from 450 nm values to correct for optical imperfections in the microplate.
15. Use curve-fitting statistical software to plot a four-parameter logistic curve fit to the standards and then calculate results for the test samples.

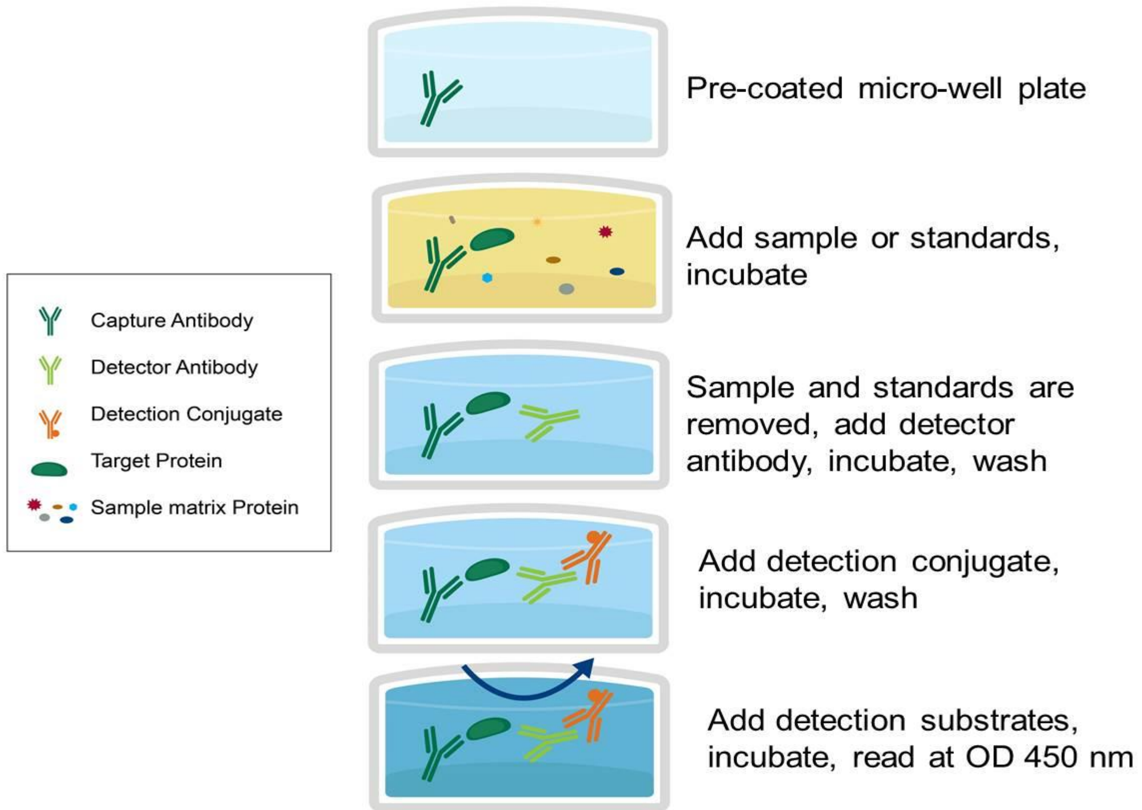
Note: *All amount of reagents can be changed according to the used kit*

Calculation of Results

If samples generate values higher than the highest standard, dilute the samples and repeat the assay. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.)

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

Sandwich ELISA Summary



Four common types of ELISA