Various Types of ELISA Assays (Biosolutions)

The steps of the general, "indirect," ELISA for determining serum antibody concentrations are:

1. Apply a sample of known antigen of known concentration to a surface, often the well of a microtiter plate. The antigen is fixed to the surface to render it immobile. Simple adsorption of the protein to the plastic surface is usually sufficient. These samples of known antigen concentrations will constitute a standard curve used to calculate antigen concentrations of unknown samples. Note that the antigen itself may be an antibody.
2. A concentrated solution of non-interacting protein, such as bovine serum albumin (BSA) or casein, is added to all plate wells. This step is known as blocking, because the serum proteins block non-specific adsorption of other proteins to the plate.
3. The plate wells or other surface are then coated with serum samples of unknown antigen concentration, diluted into the same buffer used for the antigen standards. Since antigen immobilization in this step is due to non-specific adsorption, it is important for the total protein concentration to be similar to that of the antigen standards.
4. The plate is washed, and a detection antibody specific to the antigen of interest is applied to all plate wells. This antibody will only bind to immobilized antigen on the well surface, not to other serum proteins or the blocking proteins.
5. Secondary antibodies, which will bind to any remaining detection antibodies, are added to the wells. These secondary antibodies are conjugated to the substrate-specific enzyme. This step may be skipped if the detection antibody is conjugated to an enzyme.
6. Wash the plate, so that excess unbound enzyme-antibody conjugates are removed.
7. Apply a substrate which is converted by the enzyme to elicit a chromogenic or fluorogenic or electrochemical signal.
8. View/quantify the result using a spectrophotometer, spectrofluorometer, or other optical/electrochemical device.

Sandwich ELISA

A less-common variant of this technique, called "sandwich" ELISA, is used to detect sample antigen. The steps are as follows:

1. Prepare a surface to which a known quantity of capture antibody is bound.
2. Block any non specific binding sites on the surface.

3. Apply the antigen-containing sample to the plate.
4. Wash the plate, so that unbound antigen is removed.
5. Apply primary antibodies that bind specifically to the antigen.
6. Apply enzyme-linked secondary antibodies which are specific to the primary antibodies.
7. Wash the plate, so that the unbound antibody-enzyme conjugates are removed.
8. Apply a chemical which is converted by the enzyme into a color or fluorescent or electrochemical signal.
9. Measure the absorbance or fluorescence or electrochemical signal (e.g., current) of the plate wells to determine the presence and quantity of antigen.

Competitive ELISA

A third use of ELISA is through competitive binding. The steps for this ELISA are somewhat different than the first two examples:

1. Unlabeled antibody is incubated in the presence of its antigen.
2. These bound antibody/antigen complexes are then added to an antigen coated well.
3. The plate is washed, so that unbound antibody is removed. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence "competition.")
4. The secondary antibody, specific to the primary antibody is added. This second antibody is coupled to the enzyme.
5. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

For competitive ELISA, the higher the original antigen concentration, the weaker the eventual signal.

(Note that some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with your sample antigen (unlabeled). The more antigen in the sample, the less labeled antigen is retained in the well and the weaker the signal).

ELISA Reverse method & device (ELISA-R m&d)

A newer technique uses a solid phase made up of an immunosorbent polystyrene rod with 4-12 protruding ogives (curved shape/feature). The entire device is immersed in a test tube containing the collected sample and the following steps (washing, incubation in

conjugate and incubation in chromogenous) are carried out by dipping the ogives in microwells of standard microplates pre-filled with reagents.

Advantages:

- The ogives can each be sensitized to a different reagent, allowing the simultaneous detection of different antibodies and different antigens for multi-target assays;
- The sample volume can be increased to improve the test sensitivity in clinical (saliva, urine), food (bulk milk, pooled eggs) and environmental (water) samples;
- One ogive is left unsensitized to measure the non-specific reactions of the sample;
- The use of laboratory supplies for dispensing sample aliquots, washing solution and reagents in microwells is not required, facilitating ready-to-use lab-kits and on-site kits.