Section11:Immunoassay Formats

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Immunoassay Formats

An enzyme-linked immunoassay (ELISA) is one of several methods used in the laboratory to detect and quantify specific molecules. ELISA's rely on the inherent ability of an antibody to bind to the specific structure of a molecule. In order to optimize an ELISA and obtain the sensitivity and dynamic range required for the particular assay being developed, all the various components of the assay must be evaluated. The components will vary depending on the immunoassay format selected.

Following is a description of the various types of ELISA formats as well as reagents that needed to be optimized in order to obtain a robust assay.

Types of ELISA Formats

Three frequently used types of ELISA are: sandwich assays, competitive assays and antigen down assays. The format selected depends on the reagents that are available and the dynamic range required for the particular assay. Sandwich assays tend to be more sensitive and robust and therefore tend to be the most commonly used.

Sandwich Immunoassay (ELISA)

A Sandwich Immunoassay is a method using two antibodies, which bind to different sites on the antigen or ligand. The primary antibody, which is highly specific for the antigen, is attached to a solid surface. The antigen is then added followed by addition of a second antibody referred to as the detection antibody. The detection antibody binds the antigen to a different epitope than the primary antibody. As a result the antigen is 'sandwiched' between the two antibodies. The antibody binding affinity for the antigen is usually the main determinant of immunoassay sensitivity. As the antigen concentration increases the amount of detection antibody increases leading to a higher measured response. The standard curve of a sandwich-binding assay has a positive slope. To quantify the extent of binding different reporters can be used. Typically an enzyme is attached to the secondary antibody which must be generated in a different species than primary antibodies (i.e. if the primary antibody is a rabbit antibody than the secondary antibody would be an anti-rabbit from goat, chicken, etc., but not rabbit). The substrate for the enzyme is added to the reaction that forms a colorimetric readout as the detection signal. The signal generated is proportional to the amount of target antigen present in the sample.

The antibody linked reporter used to measure the binding event determines the detection mode. For an ELISA, where the detection is colorimetric, a spectrophotometric plate reader is used. Several types of reporters have been recently developed in order to increase sensitivity in an immunoassay. For example, chemiluminescent substrates have been developed which further amplify the signal and can be read on a luminescent plate reader. Also, a fluorescent readout where the enzyme step of the assay is replaced with a fluorophor tagged antibody is becoming quite popular. This readout is then measured using a fluorescent plate reader.

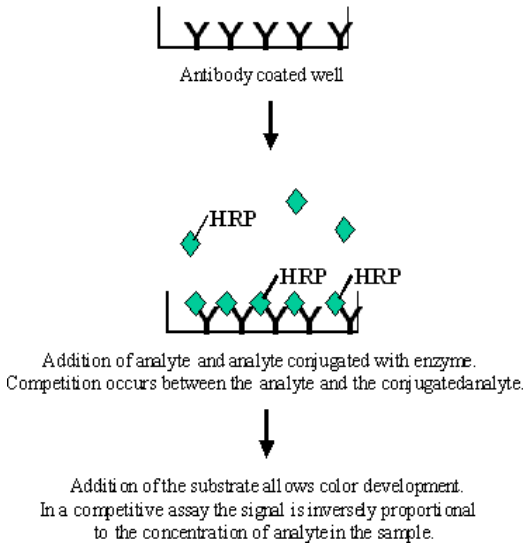

Figure 1

Competitive Binding Assay

A competitive binding assay is based upon the competition of labeled and unlabeled ligand for a limited number of antibody binding sites. Competitive inhibition assays are often used to measure small analytes. These assays are also used when a matched pair of antibodies to the analyte does not exist. Only one antibody is used in a competitive binding ELISA. This is due to the steric hindrance that occurs if two antibodies would attempt to bind to a very small molecule. A fixed amount of labeled ligand (tracer) and a variable amount of unlabeled ligand are incubated with the antibody. According to law of mass action the amount of labeled ligand is a function of the total concentration of labeled and unlabeled ligand. As the concentration of unlabeled ligand is increased, less labeled ligand can bind to the antibody and the measured response decreases. Thus the lower the signal, the more unlabeled analyte there is in the sample. The standard curve of a competitive binding assay has a negative slope.

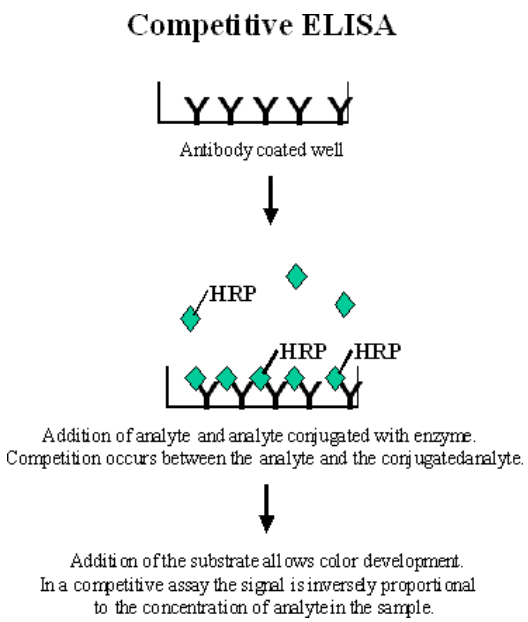

Figure 2

Antigen-Down Immunoassay or Immunometric Assay

Signal is directly proportional to the amount of antibody present in the sample, the more antibodies there are in the sample the higher the signal.

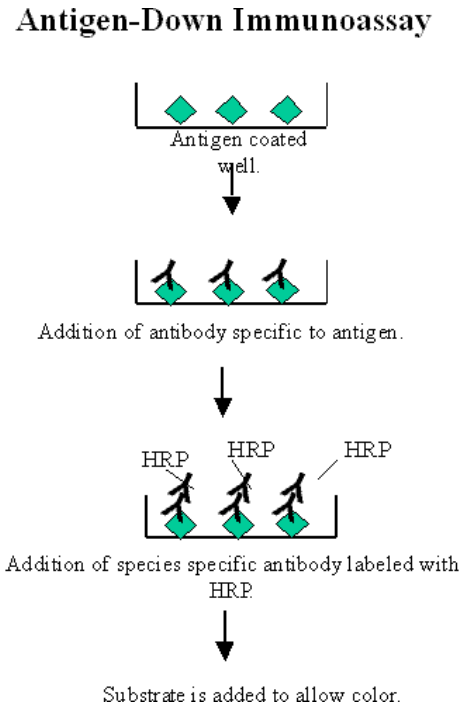

Figure 3

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