



## Protein Detection with Antibodies

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### DESCRIPTION

#### Principle of immunoassay

Immunoassays take advantage of the specificity of antibody-antigen binding that occurs naturally in the immune system. Antibodies produced by the body's adaptive immune response are highly specific for specific antigens. This is why, for example, they are vaccinated to help initiate an antibody repertoire response to some of the antigens before the immune system encounters a more pathogenic condition. Immunoassays use these highly specific antibodies to look for the molecule of interest when mixed with other molecules.

#### Applications of immunoassay

Immunoassays can be applied in situations where you want to detect or separate molecules in a mixture. This assay can be used to identify the presence of pathogens in clinical samples or to measure the amount of target biomolecules. A reporter system is required when measuring target doses using an immunoassay. The following describes different types of detection / detection systems. If the purpose of the immunoassay is to separate a specific molecule, a separate system is needed. When separation is achieved by magnetic separation with magnetic particles, it is called a magnetically actuated immunoassay. The most commonly used particles in these assays consist of a magnetite core that is coated with a biocompatible material and chemically modified by antibody attachment. However, before designing magnetic particles for an immunoassay, it is necessary to determine which type of immunoassay is best suited to the experimental goal. Steps and parameters for developing and running an immunoassay.

1. Consider sensitivity, throughput requirements, and cost to determine the best immunoassay technique for your experiment. The types of immunoassays are described below. It is also the place to consider the type of surface or environment on which the immunoassay is performed.

2. Determine antibody / antigen pairs to test and determine their commercial availability or prepare reagents in the laboratory.
3. It binds an antibody or antigen to the surface. Plates or beads, depending on the target you need to observe. Common techniques are sandwich assays, competitive assays, or antigen-preferred binding of antibody probing.
4. Optimize the blocking and cleaning steps to minimize non-specific binding.
5. Incubate with a secondary molecule or secondary antibody for detection.
6. Analyze the data depending on the type of immunoassay used.
7. Validate the immunoassay procedure. There are many published guidelines on validation designed to ensure consistency and accountability of protocols and methods.

As biotechnology advances and the understanding of nanotechnology grows, we can expect more immunoassay options available. For now, we can focus on the following five types of immunoassays

- Radioimmunoassay (RIA)
- Counting Immunoassay (CIA)
- Enzyme-linked immunosorbent assay (EIA) or enzyme-linked immunosorbent assay (ELISA)
- Fluoroimmunoassay (FIA)
- Chemiluminescence Immunoassay (CLIA)

It is also worth noting that there is currently a move towards the development of label-free immunoassays. These clever techniques are based on physical principles such as constructive and destructive interference of light, resonance conditions, and how changes in the effective index of refraction change these conditions. Label-free assays can detect antigen-antibody bonds without the use of additional luminescent labels. This increases the sensitivity of the assay and reduces working hours.

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### Radioimmunoassay

Radioimmunoassays are probably the oldest type of immunoassay. Here, the radioisotope binds to the antigen of interest and to its complementary antibody. Next, add a sample containing the antigen to be measured. It competes with the radioactive antigen and knocks it out of the binding site to replace it. After flushing the unbound antigen, measure the radioactivity of the sample. The amount of radioactive signal is inversely proportional to the amount of target antigen. The health hazards of using radioactive materials have led to a move towards safer methods.

### Counting immunoassay

In immunoassays count, polystyrene beads are coated with a variety of antibodies that are complementary to the target antigen. During incubation, the beads bind to multiple antigens and aggregate into large clumps. Some pearls remain unbound. The entire solution passes through the cell counter and only unbound beads are counted. The number of unbound beads is inversely proportional to the amount of antigen.

### Enzyme linked immunosorbent assay

In ELISA, the antibody binds to the enzyme. After incubation with the antigen, unbound antibodies are washed away. Observe the antibody enzyme bound to the target antigen by adding the

substrate to the solution. This enzyme catalyzes the chemical reaction of the substrate to produce a quantifiable color change. A practical example is the Magneto ELISA system for detecting CD4 + cells for the diagnosis of AIDS.

### Fluoroimmunoassay

In the fluoroimmunoassay, the antibody is labeled with a fluorescent probe. After incubation with the antigen, the antibody-antigen complex is isolated and the fluorescence intensity is measured.

### Chemiluminescent immunoassay

The principle of chemiluminescent immunoassay is the same as for ELISA or fluoroimmunoassay, but the reporter is different. Emission is the emission of light when an electron is promoted to a higher energy state and the emission of a photon when the electron relaxes. This is the same principle as for fluorescence. The difference lies in the mechanism that gives electrons higher energy in the first place. With fluorescence, this is achieved at a specific light frequency. In chemiluminescence, this is achieved by a chemical reaction. These reactions require an emitter and reactants. A magnetically activated chemiluminescent assay was developed to detect the presence of Zika virus in patient samples.