Altogen Labs

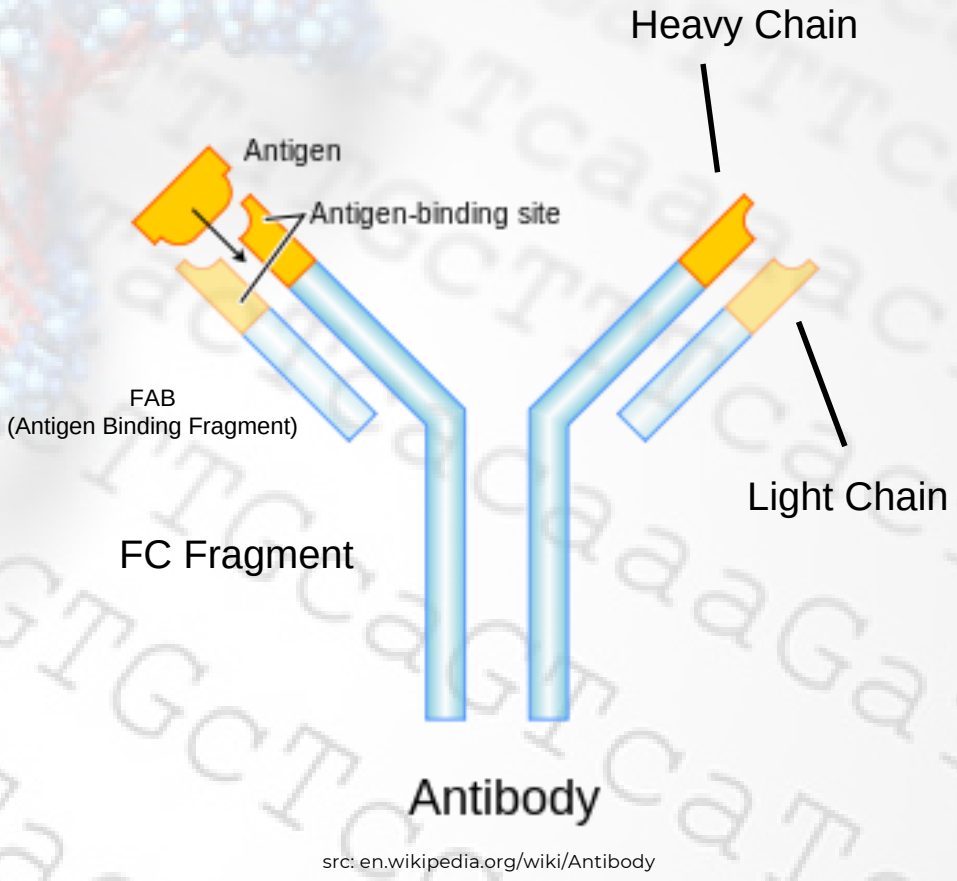
Provider of Preclinical Research Services (GLP/non-GLP) for Drug Discovery
Efficacy and Pharm/Tox IND contract research studies (clients worldwide)
100+ Xenograft Models (validated in-house) and IND-enabling Toxicology studies
100% IP belongs to client, experienced IACUC-regulated barrier facility

ELISA and Cell-based Assay Services

Contact us: **info@altogenlabs.com** | Read more at **AltogenLabs.com**
ALTOGEN® 11200 Menchaca Road 203 • Austin • TX • 78748 • USA 512-433-6177

ELISA (Enzyme-linked Immunosorbent Assay)

- The plate-based assay technique is designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones.
- The substance's binding activity is measured as a linear spectrophotometric response and quantified against a standard curve.

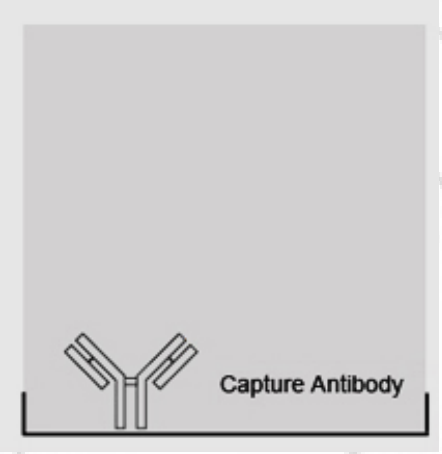


src: en.wikipedia.org/wiki/Antibody

ELISA & Cell-based Assays

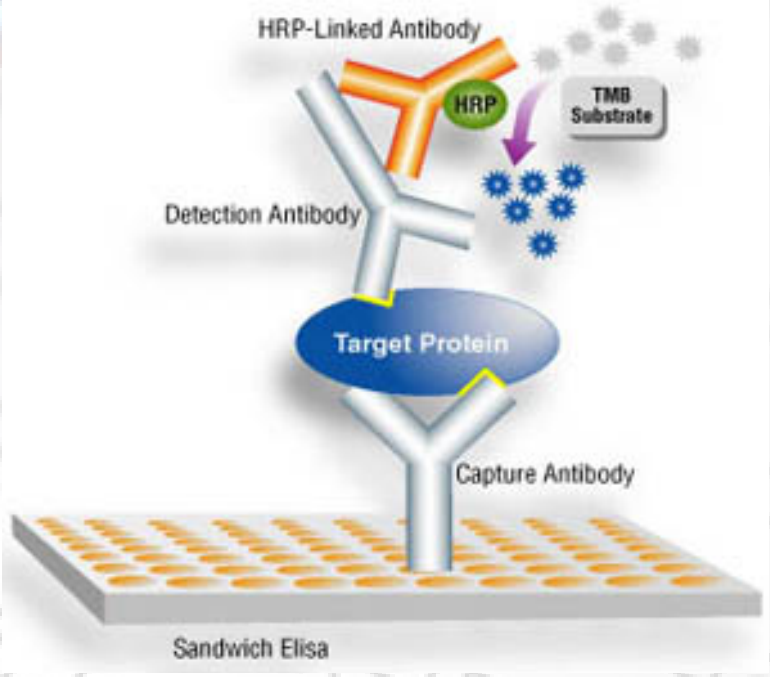
ELISAs (Enzyme Linked Immunosorbent Assays) are typically performed in polystyrene plates which passively bind a known antibody or antigen to the plate surface.

This is known as the *solid phase*.



ELISA & Cell-based Assays

- The plate is loaded with a liquid sample containing the antigen and buffer washes remove any unbound antigen or antibody from the wells. Only target antigen that has bound to the solid phase will remain.
- A buffer containing antibodies and enzyme-conjugated antibody is added to the wells. Bound complexes will be detected by enzymes produced colorimetric substrate.

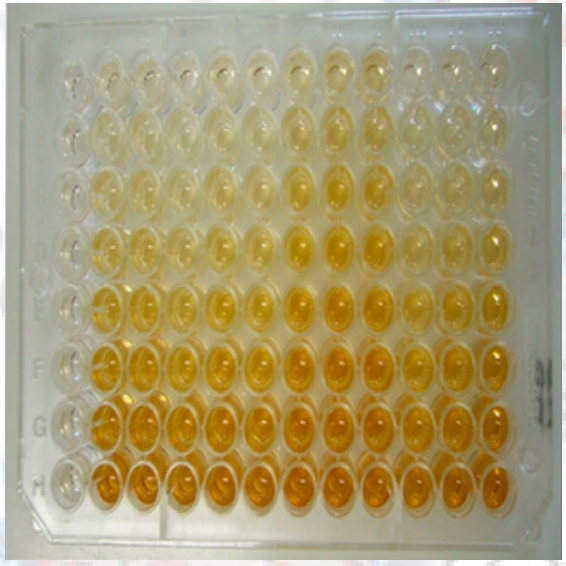


A target antigen bound by 2 capture antibodies detected by HRP activity in a sandwich ELISA

ELISA & Cell-based Assays

Representative ELISA

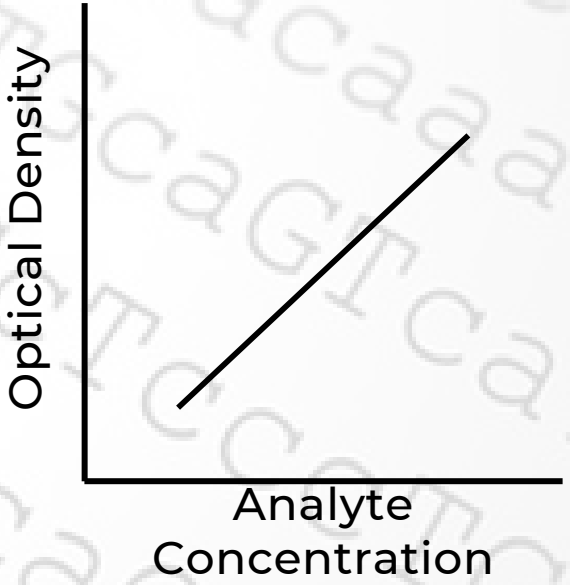
The bottom wells have the most product, hence have the most bound analyte



Analyte Concentration

Optical density is directly proportional to the amount of bound analyte

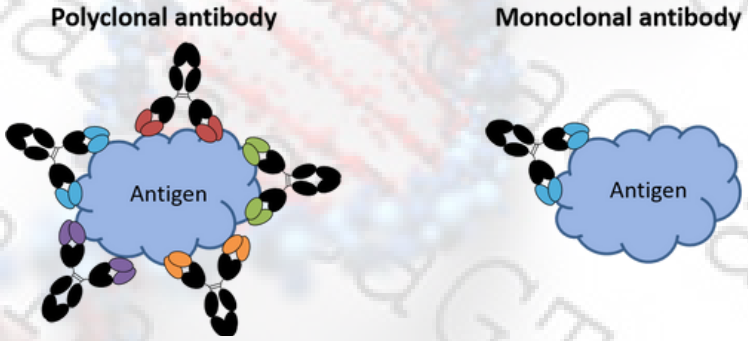
Enzyme production of colorimetric substrate produces an optical signal, which is directly correlated to the amount of bound analyte and correspondently, the concentration of analyte in the original solution.



ELISA

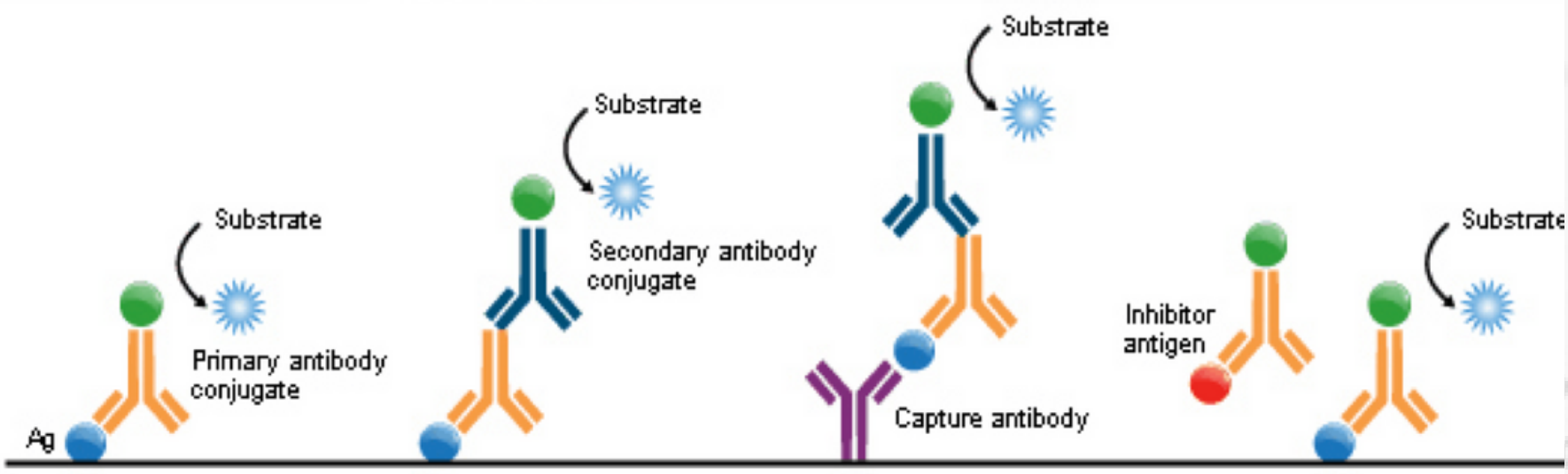
Customizable monoclonal and polyclonal antibodies make the ELISA a very versatile assay able to detect most targets of interest, with high affinity, specificity, and sensitivity. Altogen Labs (www.altogenlabs.com) offers:

- Antibody Testing, Custom labeling, Biotinylation, Purification
- Monoclonal & Polyclonal Antibody Development
- ELISA Assay Development
- ELISA Platform Optimization
- ELISA Assay Format Modification



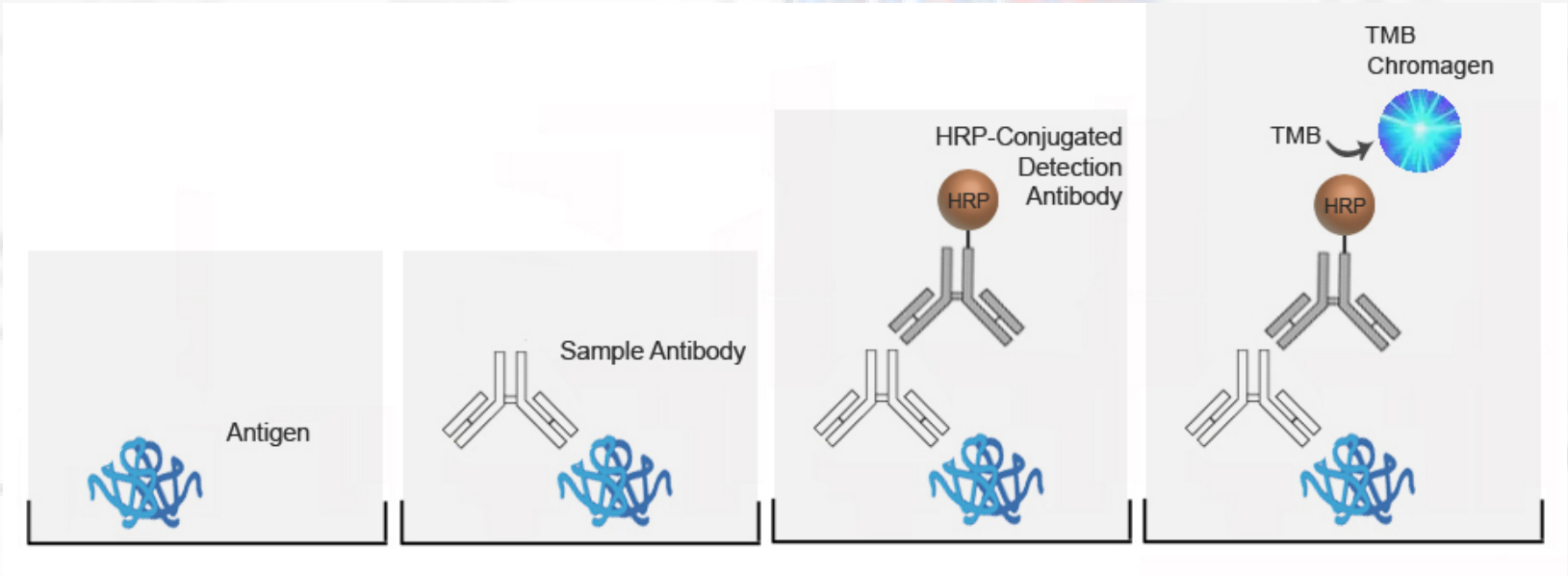
Polyclonal Antibodies can target more than one antigen epitope

ELISA


Substrate
Primary antibody conjugate
Ag
Substrate
Secondary antibody conjugate
Substrate
Capture antibody
Inhibitor antigen
Substrate

Indirect ELISA

1. The antigen that will be tested is diluted with a carbonate buffer (often PBS) and the PVC wells located in an ELISA plate are filled and incubated overnight.
2. The wells are washed and any unbound antigen is removed.
3. A blocking buffer blocks any protein-binding sites in the coated wells to reduce false-positive reactions.
4. Add the primary antibody diluted to the optimal concentration with blocking buffer.
5. Incubate and wash with the carbonate buffer used in step one.
6. Dispense the enzyme-linked secondary antibody into each well and incubate.
7. Add a stop solution to stop the detection substrate's reaction and read the optical density (OD) of each well.
8. The concentration of each well is calculated against the standard curve.



Antigen adheres to well and unbound antigen is removed

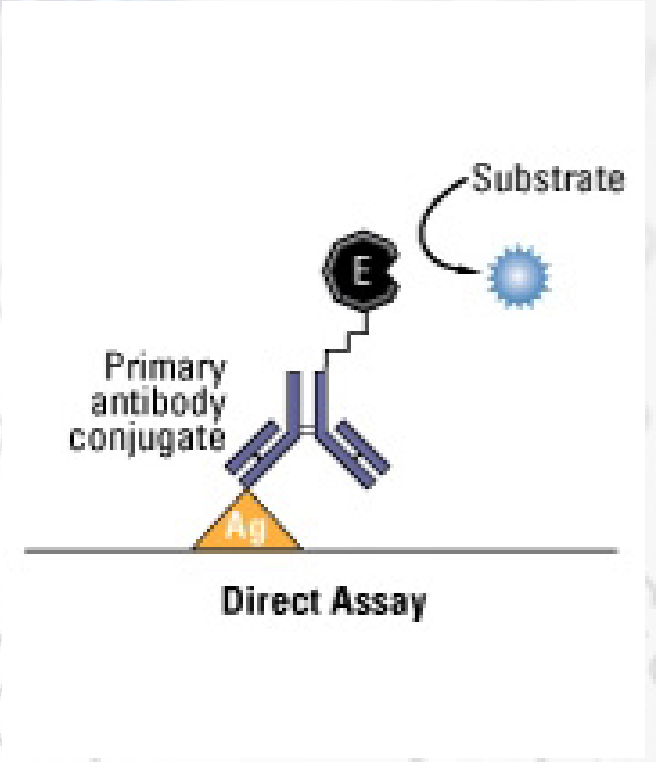
Antibody is added and binds to any antigen present

Detection antibody is added and binds to the primary antibody

Enzyme present turns over substrate producing a color change

Direct ELISA

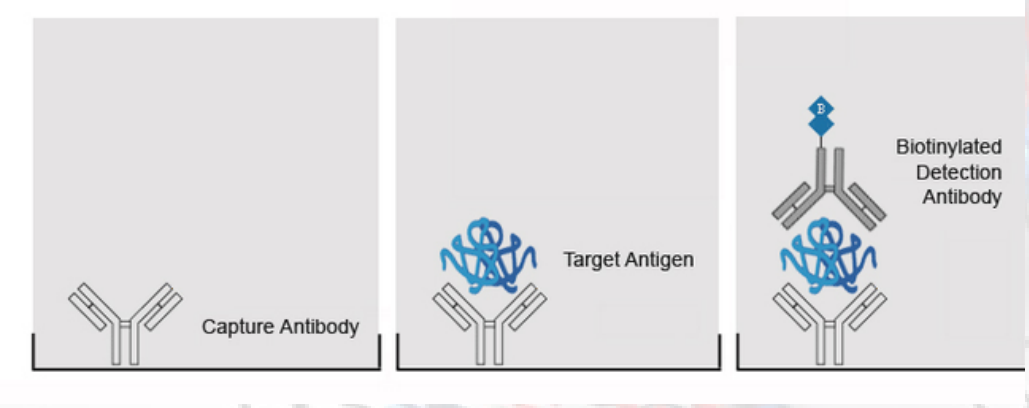
- The antigen is incubated overnight in a microtiter plate as the solid phase of the experiment. Unbound antigen is removed by buffer wash steps.
- The enzyme-conjugated primary antibody is added to each well. Subsequent buffer wash steps removes unbound antibody.
- Upon the addition of substrate, conjugated enzyme produces an optical signal via colorimetric product. This signal is directly proportional to the number of bound primary antibodies and accordingly the amount of antigen present, and the concentration of antigen present in the original solution.



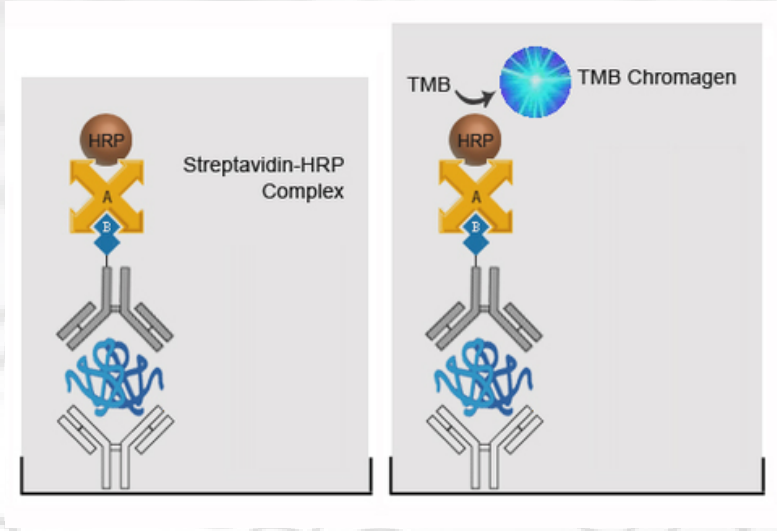
Sandwich ELISA

1. A known concentration of capture antibody is incubated overnight in a microtiter plate.
2. Any unbound antibody is removed through buffer wash steps and non-specific surface binding sites are blocked.
3. Antigen containing solution is added to the plate.
4. Any unbound antigen is removed through buffer wash steps.
5. A primary antibody is added, and the wells are washed again to remove non-binders.
6. A secondary, enzyme-conjugated antibody targeting the Fc region of the primary is added. Wash steps are repeated to remove any non binders.
7. The substrate for the conjugated-enzyme is added and the colorimetric reaction is allowed to develop for a finite time period.
8. A stop solution is added and the optical density (OD) of the wells is measured.
9. Test solution concentrations of antigen can be calculated from linear regression of a standard curve.

Biotin Conjugation



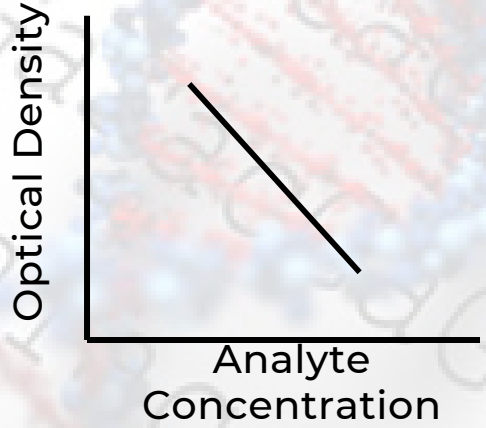
The biotinylated primary antibody binds to an epitope on the antigen's surface producing the antibody "sandwich".



A streptavidin-conjugated enzyme is added, which specifically targets the biotin moiety of the primary antibody, linking enzyme produced colorimetric product to bound antibody and bound antigen.

Competitive ELISA

1. A microtiter plate is coated with antigen overnight. Non-bound antigen is removed by wash steps.
2. Unlabeled antibody is incubated with antigen-containing solutions.
3. Antibody-antigen complexes are added to antigen-coated wells. Non-bound enzyme is removed through buffer wash steps.
4. An enzyme-conjugated secondary antibody, targeting the Fc region of the primary antibody, is added and wash steps are repeated to remove any non-binders.
5. The substrate is added and allowed to develop before reading OD.



The more antigen is present in the test solution, the less free antibody will be available to be captured by surface-bound antigen, hence a higher concentration of analyte in solution, the lower the optical signal.

ELISA & Cell-based Assays

Advantages

1. ELISAs are generally more accurate, sensitive, and specific compared to other assays.
2. ELISAs are safer as they don't need radioisotopes.
3. They can detect down to nanogram levels of target antigen.
4. The use of antibody-antigen binding in ELISAs makes it a highly specific test, binding only to target epitopes relevant to the study.

Disadvantages

1. ELISAs are time sensitive assays and require appropriate incubation for accurate results; incubating too long or too short can distort data.
2. Every step of the assay is critical, therefore washes must be thorough in order to avoid skewed data.
3. For optimal results, antibodies may need to be produced and engineered, which can be expensive and time-consuming.

Altogen Labs Custom ELISA Services

- Antibody Labeling, Biotinylation, Purification
- Monoclonal Antibody Development
- ELISA: Assay Development
- ELISA: Platform Optimization
- ELISA: Assay Format Modification

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Altogen Labs

Enzyme-Linked Immunosorbent Assay

Standard ELISA services include testing activity of the compound of interest on a panel of 15 intracellular Ser/Thr kinases & 17 Tyr kinases to detect substrate-specific phosphorylation activity induced by kinase activation.

Altogen Labs' ELISA service is multifaceted and thorough

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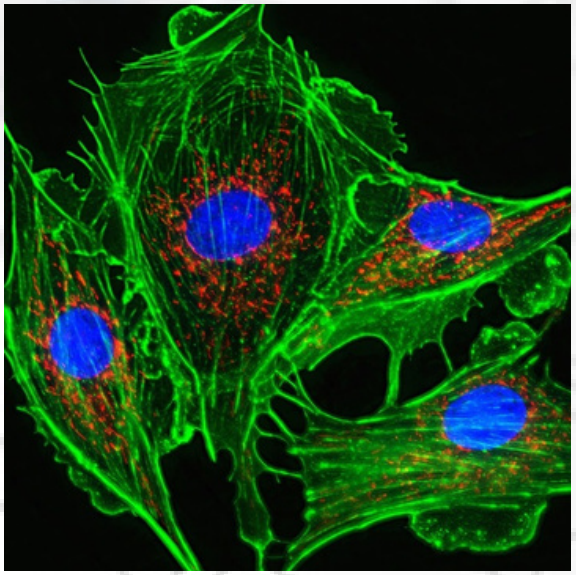
Benefits of Altogen Labs Custom ELISA Services

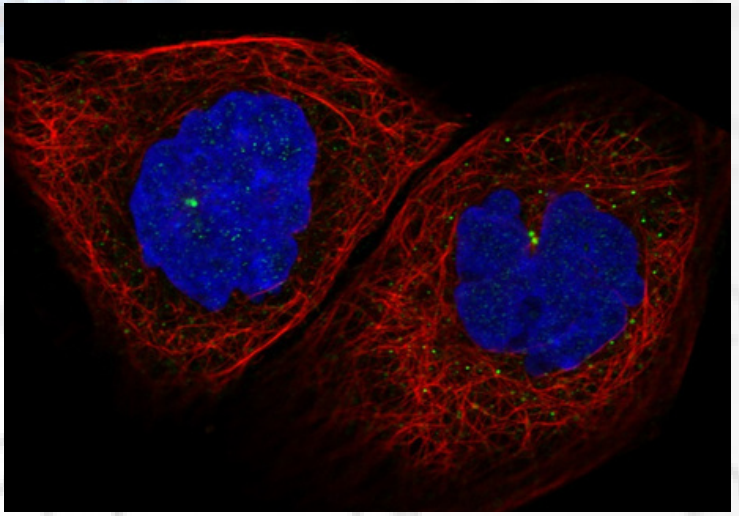
- Allows the study of cellular processes
- Leveraged to get an accurate assessment of the system functioning as a whole, not just the “sum of the cellular parts”
- Can be utilized to investigate receptor activation, receptor binding, cell signaling, and ligand internalization



Altogen Labs offers custom cell-based assay development and testing
http://www.ddw-online.com

Types of Cell-based Assays

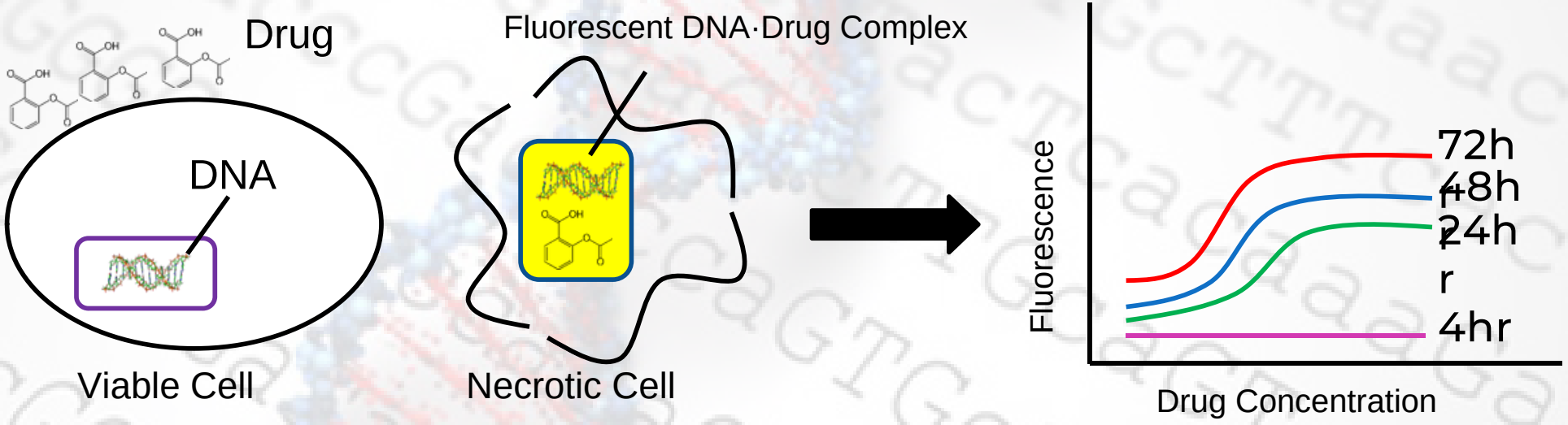
- Cytotoxic Assays
- Cell Senescence Assays
- Enzyme-Linked Immunosorbent Assay (ELISA)
- Cell Viability Assays
- LC-MS
- ICP-MS



Cell-based assays have a wide range of applications
http://www.proteinatlas.org

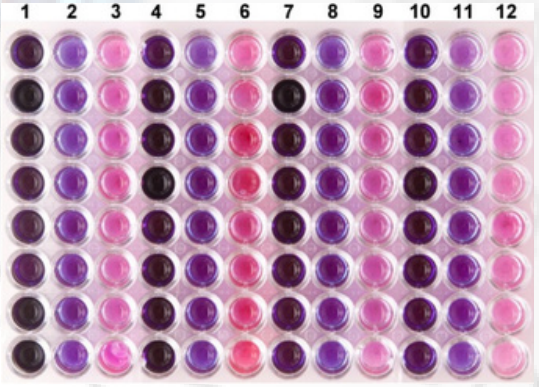
Cytotoxic Assay

- Cytotoxic assays are used to assess the cytotoxicity (toxicity to living cells) of a compound of interest.
- The drug of interest is administered to cells.
- Reagent is added to cells per time interval post-drug treatment
- Necrotic cells have compromised membranes, allowing reagent access to DNA resulting in a visible signal


Drug
DNA
Viable Cell
Fluorescent DNA·Drug Complex
Necrotic Cell
Fluorescence
72h
48h
24h
4hr
Drug Concentration

Contact Us

- Altogen Labs has a staff of scientists experienced with ELISA development, optimization, and modification.
- Altogen Labs' standard ELISA service includes screening against a panel of 15 intracellular Ser/Thr kinases and 17 Tyr kinases for substrate phosphorylation activity.



Altogen Labs can tailor cell-based assays to clients' needs

Hudman DA, Sargentini NJ. Resazurin-based assay for screening bacteria for radiation sensitivity. *SpringerPlus*. 2013;2:55.

Contact us to discuss details, timeline estimates, and price!

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