



ELISA BASICS

IVDW

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**Courtesy of:
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Agdia, Inc.**





ELISA

Enzyme Linked Immuno-Sorbent Assay

ELISA – an immunological test, using an enzyme as a label to determine presence of target protein.

•The enzyme linkage or labeling allows you to follow your target protein and if present (qualify) and at what amounts (quantify).

•An enzyme conjugate is an enzyme bound or joined with an antibody which binds with your target protein. This enzyme labeling is a safe and effective way to track your antibody.

Components

Antigen

- **Any substance that stimulates an immune response.**
- **The antigen is your target protein which comes from your sample extract. Example: Bt protein in corn sample. The antigen binds to the antibody.**





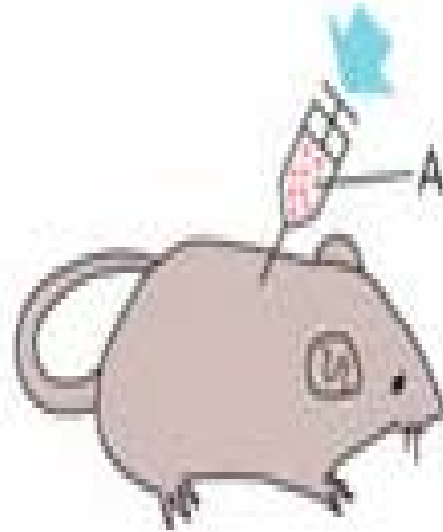
Components

Antibody

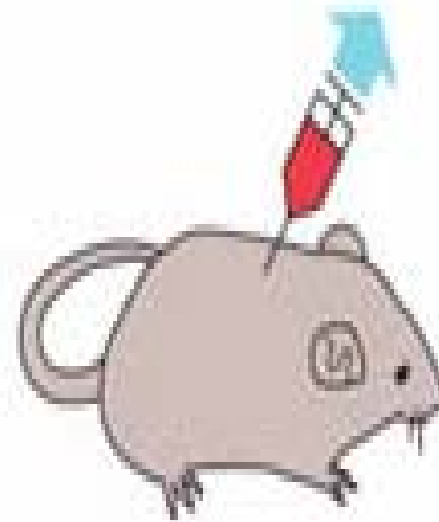
- **An antibody is a protein made in response to an antigen.**
- **Each antibody binds only to its antigen.**



Producing Antibodies



Animals are injected over a period of weeks with purified antigen (ex. Bt protein)



Serum is drawn and antibodies are separated and purified

Components

Enzyme Conjugate

An enzyme conjugate (EC) is an antibody joined with an enzyme. Enzyme labeling allows the researcher to follow the antibody. This joining of the enzyme to antibody is often called conjugation.





Enzyme Function

Enzymes are proteins that speed up the rate of a chemical reaction without being used up and usually react only to particular substrates. The rate of this reaction is proportional to the amount of enzyme present. In the case of non-competitive ELISA, the more binding you have of the enzyme conjugate to the antigen, the stronger your color development will be.



Substrate Function

A substrate is a compound or substance that undergoes change. Substrates bind to active sites on the surface of enzymes and are converted or changed. In ELISA the specific substrate used changes color.



ELISA Formats

(Sandwich)

Direct sandwich ELISA – antibodies (Ab) are coated to micro wells. Antigen (Ag) is added and binds with antibody. Excess antigen is washed away. Enzyme conjugate (Ab-E) is added and binds with antigen to form the double antibody sandwich. Wells are washed to remove any excess (Ab-E). Substrate is added and color development is observed. The enzyme conjugate binds ‘directly’ to the antigen.

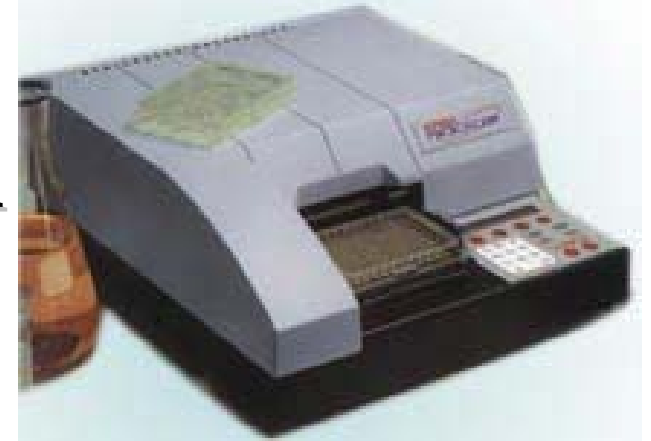
Ab + Ag + Ab-E

Double Antibody Sandwich (DAS)

1. Plates are typically coated with antibody by vendor.
2. Sample extract is added and if present, target proteins bind to antibodies. Incubate 1 to 2 hours.
3. Plate is washed. Any unbound material is washed away.
4. Enzyme conjugate* is added. Incubate 1 to 2 hours.
5. Plate is washed. Any unbound material is washed away.
6. Substrate is added.
7. Stop solution (optional) is added.
8. Plate is read visually or by plate reader.



PLATE READER



- Machine which measures color density in plate well.
- A specific wave-length of light is chosen (depending upon the color you expect to capture).
- Light is cast from the bottom of the well through through the sample.
- No color change wells absorb very little light; wells with color change absorb more light; this is the optical density or OD.



Reading plates

- Select proper wave-length on machine.
- Carefully wipe bottom of plate to remove excess moisture. Plates that are wet may diffuse your light source, giving inaccurate readings.
- Mix the plate. This will distribute color evenly. Some machines have a 'mix' setting.

OD Values – good results

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.02	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.3	
B	0.02	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	
C	0.04	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.5	
D	0.02	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.3	4 control wells
E	0.01	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	0.02	Buffer + Known pos no EC
F	0.02	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.03	Buffer + EC
G	0.03	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.3	Known positive
H	0.02	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.04	Known negative

A1 – H1 are expected negatives

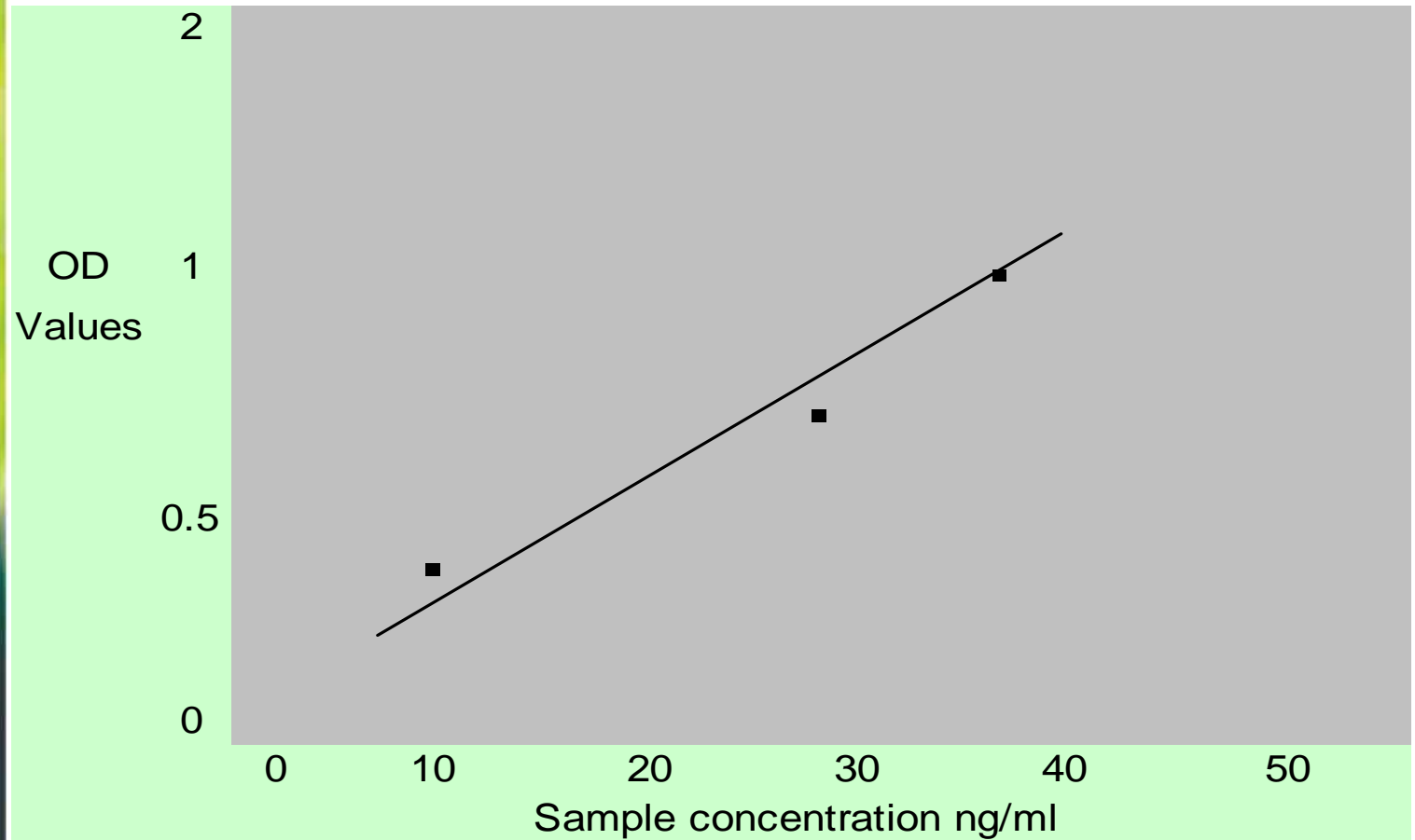


OD Values – unclear results

	1	2	3	4	5	6	7	8	9	10	11	12	
A	1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.3	
B	0.9	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	
C	0.9	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.5	
D	2.4	2	1.5	2	1.5	1.5	0	1.5	1.5	1.5	1.5	1.3	4 control wells
E	0.01	2	1.6	1.6	0	1.6	1.6	1.6	1.6	1.6	1.6	2	Buffer + Known pos no EC
F	2	1.2	1.2	1.2	1.2	1.2	0	1.2	1.2	1.2	1.2	0.9	Buffer + EC
G	1.2	0	1.2	1.2	1.2	2	1.2	1.2	1.2	1.2	1.2	1.3	Known positive
H	2.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1	Known negative

A1 – H1 are expected negatives

Standard Curve Example



Curve set using dilutions: 40ng/ml, 30ng/ml, 10ng/ml





Questions?



Thank You

