ELISA Basics SCST Workshop

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Outline

Goals

History

Definitions

Components

Procedure

Direct or Indirect

Trouble

Goals

1. Introduce and review the basic principles of ELISA.
2. Gain a better understanding of the principles in order to perform better assays.
3. Learn to trouble-shoot on your own.

ELISA Assay History

1798 - First demonstration of vaccination smallpox vaccination (Edward Jenner)

1890 - Demonstration of antibody activity against diphtheria and tetanus toxins. Beginning of humoral theory of immunity. (Emil von Behring) and (Shibasaburo Kitasato)

1900 - Antibody formation theory (Paul Ehrlich)

1938 - Antigen-Antibody binding hypothesis (John Marrack)

1948 - antibody production in plasma B cells

1959-1962 - Discovery of antibody structure

1971 - Peter Perlmann and Eva Engvall at Stockholm University invented ELISA

1975 - Generation of the first monoclonal antibodies (George Kohler) and (Cesar Milstein)

Source: http://en.wikipedia.org/wiki/ELISA

Definitions

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

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.

Epitope

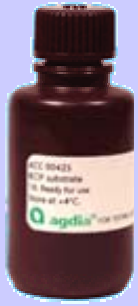
•The region on an antigen to which an antibody binds.

•Sites can be linear, non-epitope (needs digestion), conformational. Denaturation.

ELISA Kits Deconstructed

Solid phase portion of assay. Microtiter plate affinity, etc.

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.



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.

Detergents – removes non-target proteins

Blocking – blocks desorption of target proteins.

Higher concentrations of wash buffer (detergents) – poorly mixed buffers could lead to higher removal of target protein.

ELISA Applications (Agricultural)

- Greenhouse/ornamental growers
  - Detect symptomatic plant material before it is a problem; make management decisions to accept or reject new material.
- Port-of-entry sites
  - Detect plant pathogens being imported or exported.
    – *USDA-APHIS*
- Large commercial plant propagators
  - Check mother stock; screen plants before sending to customers
- Seed producers
  - Used in winter nursery setting Seed/leaf purity testing
  - Determine trait purity percentages
- AP screens (Organics)
  - Screen for the adventitious presence of unintended transgenic events
- Plant breeders
  - Make determinations of trait presence and determine segregation
- Alternate means of testing
  - Confirm other assay results or second-party results.

ELISA Methodology

ELISA methods can be designated according to the way in which analytes are captured and detected.

Capture

Antigen capture ELISA – analyte is captured by the solid phase or microplate

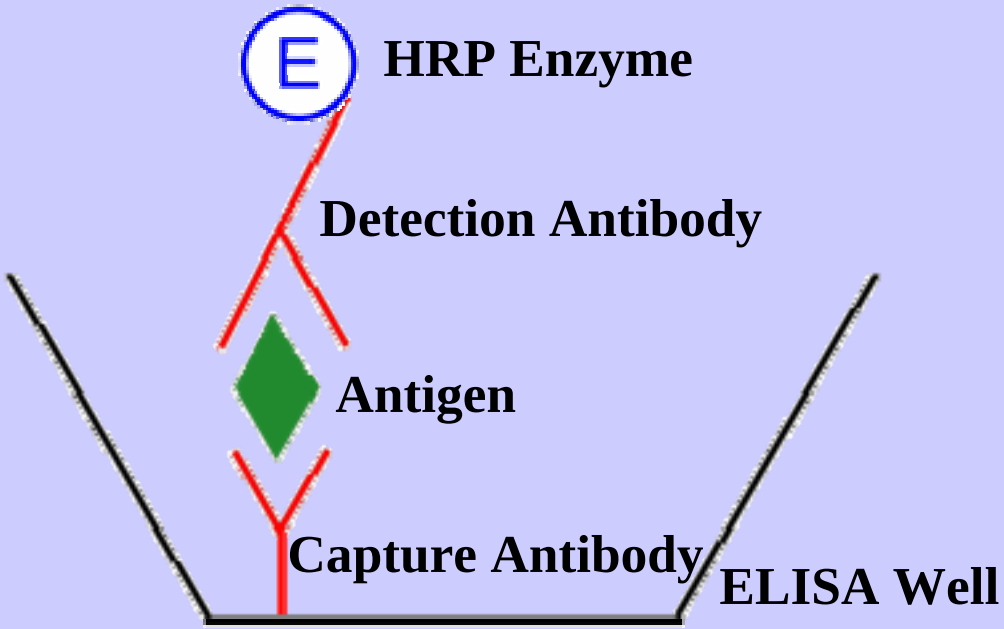
Antibody capture ELISA – an analyte is captured by an antibody attached to the solid phase or microplate

Detection

Direct ELISA (enzyme conjugate binds directly to the antigen)

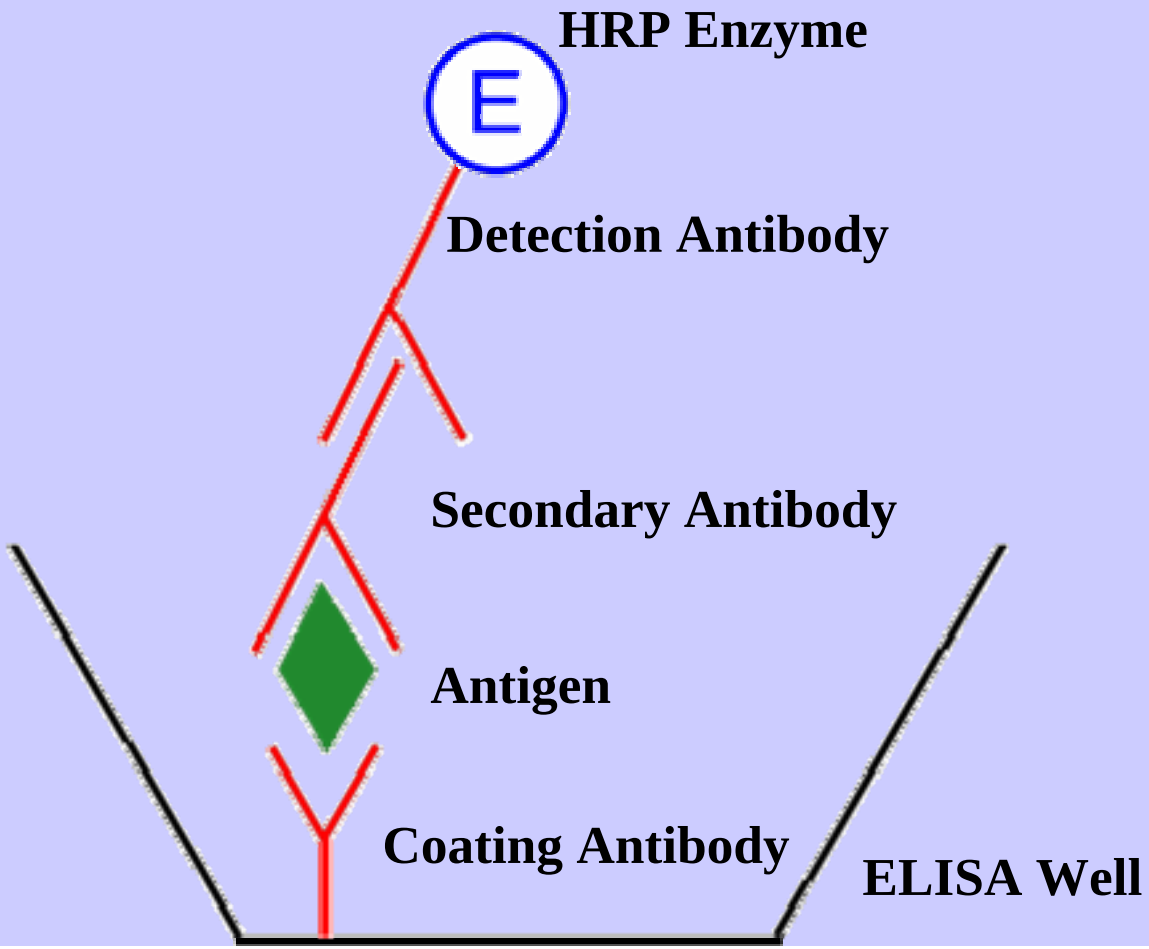
Indirect ELISA (enzyme conjugate binds indirectly to the antigen)

Direct Double Antibody Sandwich (DAS) *Direct antibody capture*

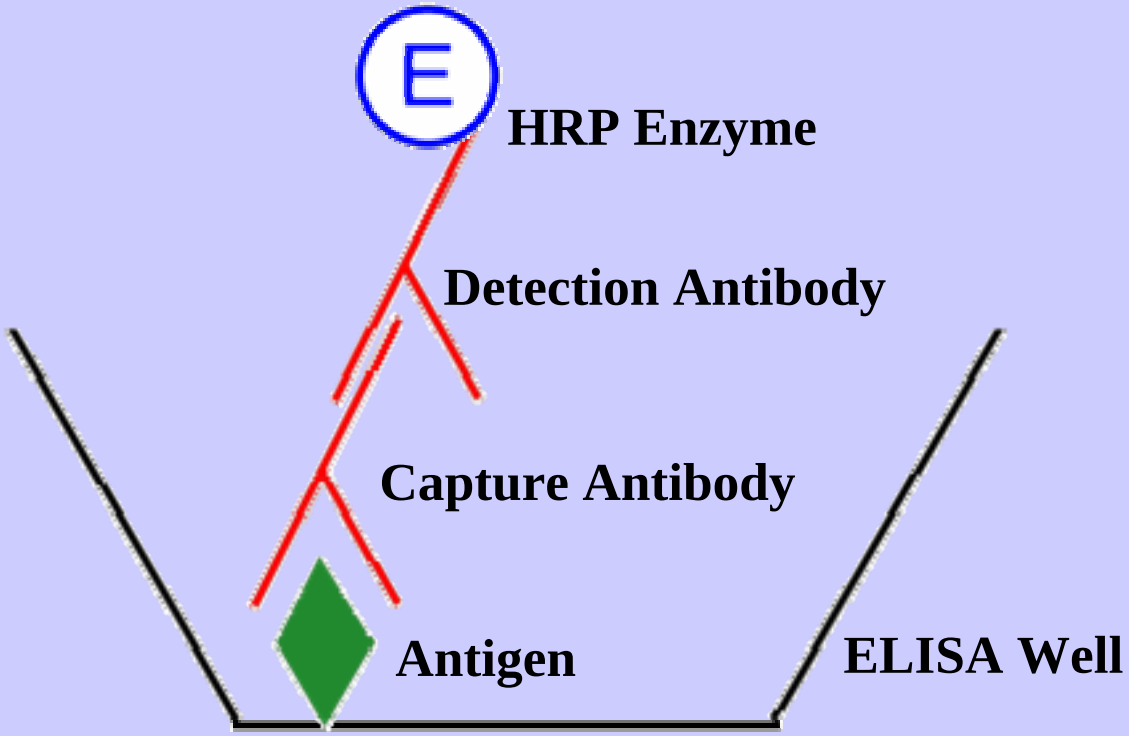


Indirect Triple Antibody Sandwich (TAS)

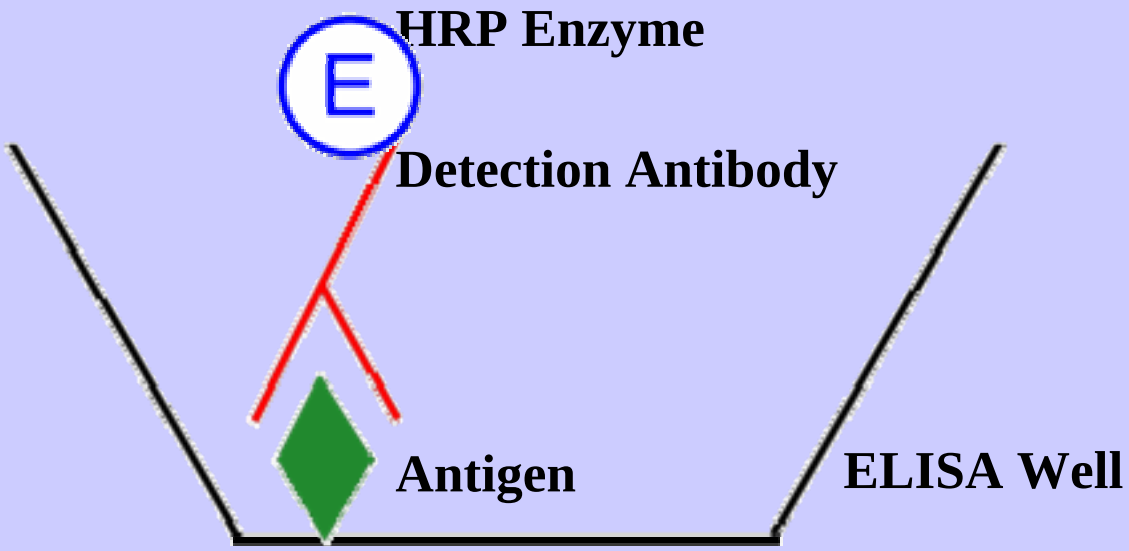
Indirect antibody capture



Indirect Antigen Capture



Direct Antigen Capture



Trouble Shooting

Edge Effects

Edge effects unexpected absorbance value variances from outside to inside. Most typically seen when plates are stacked or incubated at uneven temperatures.

Positive effect – increase in OD values

Negative effects – decrease in OD values

Main causes:

Incubation environment (various inconsistencies)

Cold plates or reagents (outside wells will be warmer)

Color developed in strong light

Edge Effects (Negative)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	0.004
B	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	0.003
C	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	0.003
D	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	1.45
E	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	1.45
F	1.25	1.01	1.3	1.22	1.3	1.5	1.1	0.7	1.20	1.20	1.20	1.20
G	0.98	0.99	0.99	0.97	0.94	0.99	0.97	0.96	0.99	0.91	0.89	0.94
H	0.98	0.99	0.99	0.97	0.94	0.99	0.97	0.96	0.99	0.91	0.89	0.94

Incubated by cold window

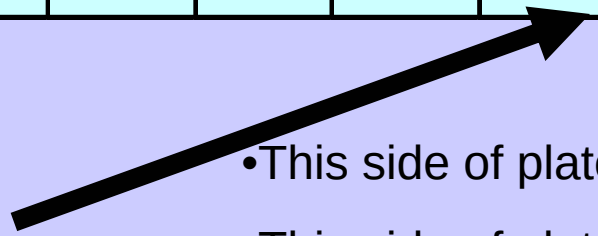
Edge Effects (negative)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	0.0035
B	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	0.0034
C	1.5	1.22	1.19	1.18	1.25	1.23	1.24	1.25	1.25	1.26	1.27	0.0032
D	1.5	1.19	1.18	1.23	1.3	1.11	1.07	1.07	1.05	1.47	1.27	1.45
E	1.5	1.18	1.17	1.23	1.13	1.16	1.15	1.14	1.13	1.12	1.27	1.45
F	1.5	1.22	1.19	1.18	1.25	1.23	1.24	1.25	1.25	1.26	1.27	1.45
G	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	1.45
H	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	1.45

Plates incubated while stacked

Edge Effect (positive)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	0.004
B	1.5	1.49	1.56	1.45	1.48	1.47	1.46	1.46	1.45	1.44	1.43	0.003
C	1.5	1.49	1.56	1.43	1.46	1.45	1.43	1.42	1.4	1.39	1.38	0.003
D	1.45	1.5	1.43	1.44	1.43	1.42	1.41	1.4	1.39	1.38	1.37	1.45
E	1.55	1.54	1.53	1.52	1.51	1.5	1.49	1.49	1.46	1.5	1.44	1.45
F	1.5	1.49	1.48	1.47	1.46	1.45	1.44	1.43	1.42	1.41	1.4	1.39
G	1.5	1.49	1.56	1.45	1.59	1.57	1.55	1.53	1.51	1.49	1.47	1.45
H	1.98	1.99	1.97	1.97	1.97	1.96	1.96	1.95	1.95	1.94	1.94	1.93



- This side of plate exposed to light during substrate development
- This side of plate near heat source during incubation

Preventing Edge Effects

- Plate sealers - prevent evaporation
- Plate warmer
- Incubators (wet recommended)
- Strive for consistent temperature of plates, reagents and incubating environment – allow everything to warm up to the same temperature each time.
- Avoid harsh lighting
- Rotate the plates during incubation
- Avoid stacking plates if possible
- Consistent plate washing

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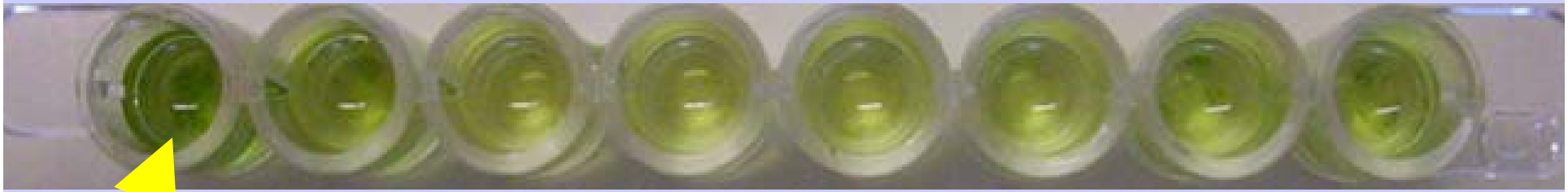
Samples Extracts



This well may require more washes

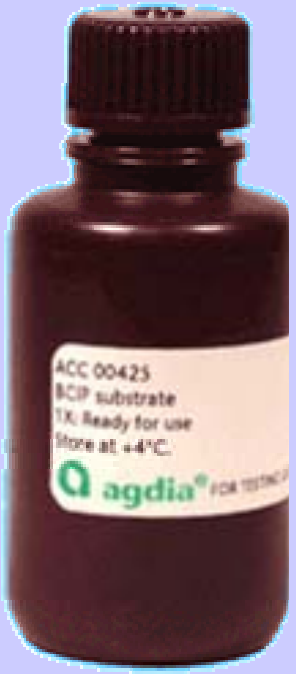
Evaporation

Films and rings can develop from evaporation, which are difficult to wash off. Films can react with substrate creating false positive result.

Substrates

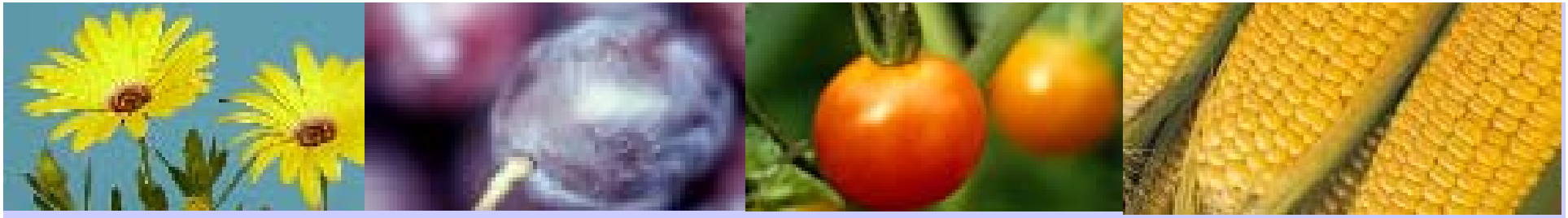
Proper Handling of Substrates

- Do not pipette directly from the substrate bottle.
- Never pour left over substrate back into the substrate bottle.
- Dispense substrate into ultra clean reagent reservoirs, and use immediately. Avoid re-using containers for substrates.
- Avoid intense light.
- Use caution if re-using pipette tips.
- Store at 4 to 5C.



ELISA Control Wells

- Always include a known positive well (+result)
- Always include a known negative well (-result)
- Optional. Can include a buffer + EC minus sample well (-result)
- Optional. Can include a buffer + sample minus EC well (-result)

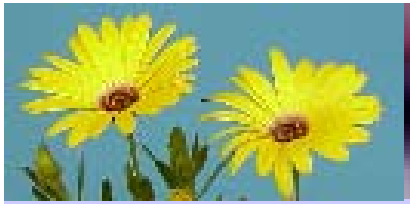
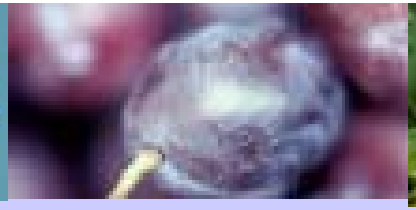


Single Well Multi -Trait ELISA:

- Plates are coated with multiple anti-bodies. (3Bb1 and 1Ab)
- Each anti-body is conjugated to differing enzymes (peroxidase and Alk. Phos.)
- Conjugated enzymes only react to their substrates, allowing for differentiation of signals.

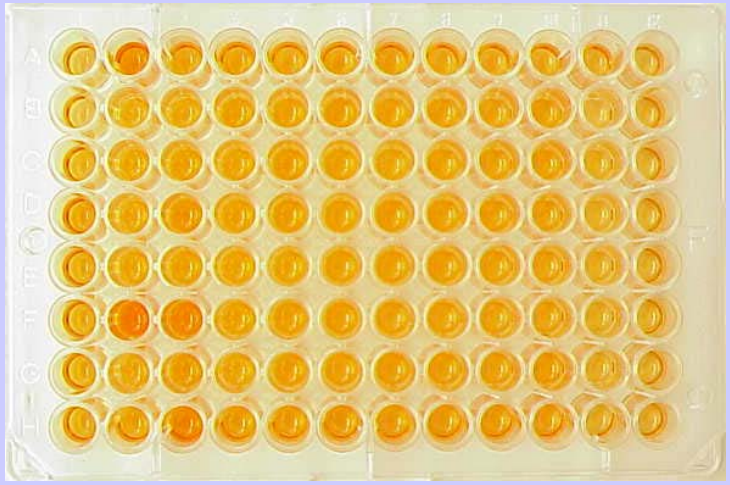
Typical Protocol in Single Well, Multi-analyte ELISA

1. Add 100 uL combo conjugate to well
2. Add 100 uL sample (multiple targets) or controls
3. Incubate at least 1 hour
4. Wash plate
5. Add 100 uL PNP substrate solution
6. Incubate at least 30 minutes, read 405 nm
7. Wash plate
8. Add 100 uL H2O2/TMB substrate solution
9. Incubate 30 minutes, read 650 nm

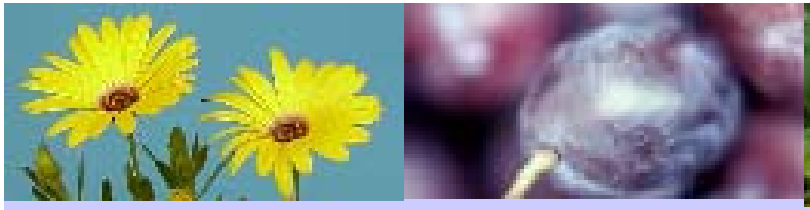

Example of 90 seed kernel check (MON863 stacked with MON810) using Agdia's Multi-Trait ELISA Plates.

- 100ul of Enzyme Conjugate is added to each well.
- MON863/MON810 seed or leaf tissue is extracted and 100ul are added to appropriate wells.



- Sample and Enzyme Conjugate are incubated together for 2 hours.

Enzyme Conjugate is dyed a red color.

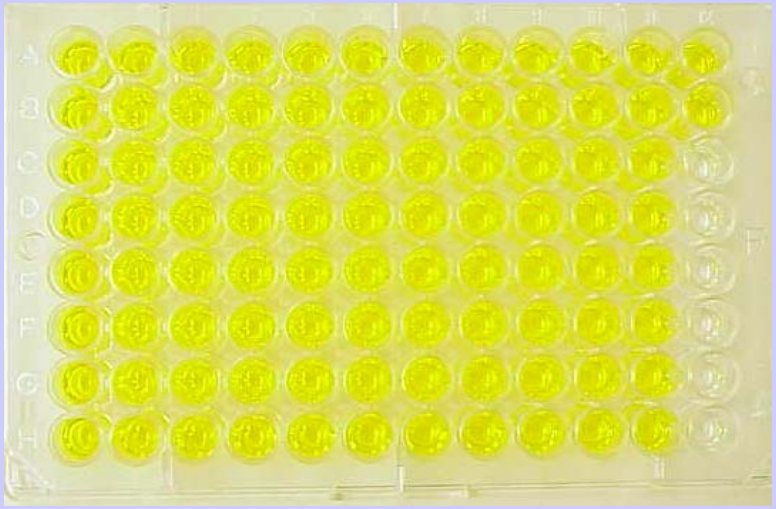


Example of 90 seed kernel check (MON863 stacked with MON810) using Agdia's Multi-Trait ELISA Plates.

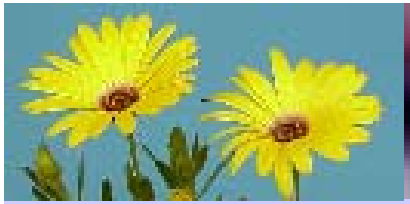
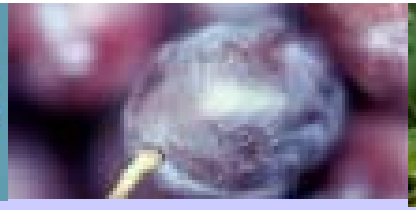
•Plate is washed to remove sample and enzyme conjugate.

•First substrate is added (PNP)

•After 15 minutes, first signal results are observed and recorded.

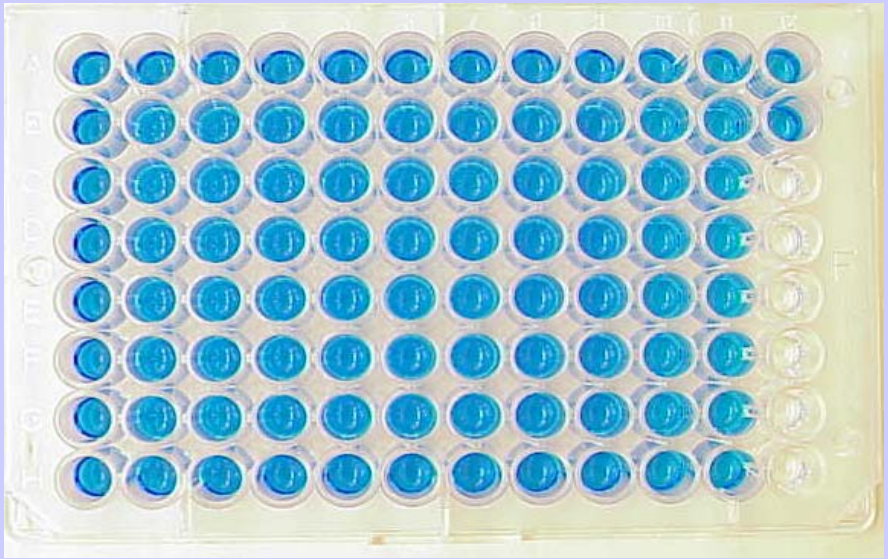


90 seed are positive for BtCry1Ab (MON810)


Example of 90 seed kernel check (MON863 stacked with MON810) using Agdia's Multi-Trait ELISA Plates.

- Plate is washed to remove first developed substrate
- Second substrate is added (TMB)
- After 10 minutes, second signal results are observed and recorded.



90 seed are positive for Bt-Cry3Bb1 (MON863)

Thank You

Questions?