
Biotechnology
CAPTIVATING SCIENCE EDUCATION
Explorer™
BIO-RAD
Professional Development

Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

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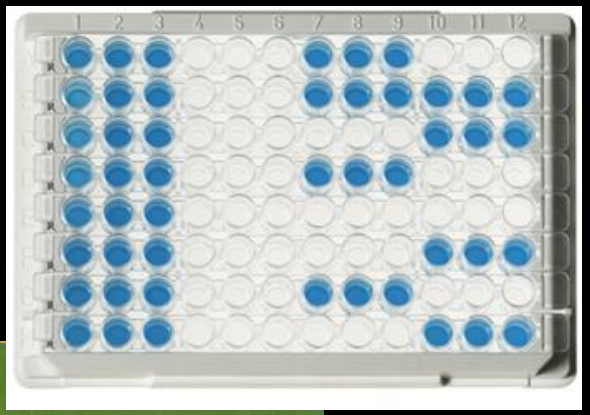
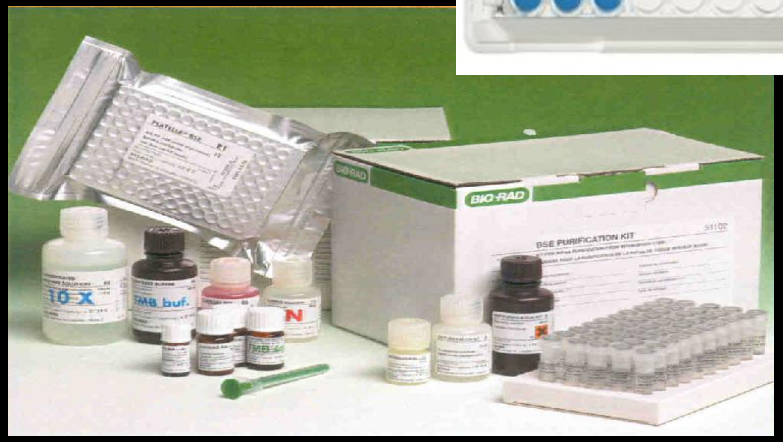
ELISA Immuno Exlorer™: Antibodies in Agriculture From Mad Cow to GMOs





Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

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ELISA Immuno Explorer™

Instructors

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Why Teach ELISA?

- **Hands-on Immunology**
- **Tangible results**
- **Laboratory extensions**
- **Real-world connections**
- **Link to careers and industry**
- **Standards-based:**
  One lesson integrates multiple standards
  - **Health sciences**
  - **Immunology**
  - **Biodefense**
  - **Immune response –** antibody/antigen interactions
  - **Disease –** infection, detection, transmission

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Scientific Inquiry

- Tapping nature's tool kit to solve human problems
- Use of immunodetection to hunt for proteins
- Use of positive and negative experimental controls
- Interpretation of experimental results

Chemistry of Life

- Enzyme-substrate interactions
- Protein structure and function
- Properties of antigens and antibodies

Genetics

- DNA > RNA > protein — antibodies
- Antibody structure and function
- Antibody production via genetic recombination

Evolution

- Animal immune systems response
- HIV mutation and evolution
- Viral drug resistance
- Biowarfare in nature

Cell and Molecular Biology

- Immune response
- Manufacturing antibodies
- Virology and immunology

Environmental and Health Science

- HIV, mad cow disease, and bird flu testing
- Epidemiology and biodefense
- Drug, pregnancy, and GMOs testing
- Soil, water, air testing

ELISA Immuno Explorer Kit Advantages

- **Lab completed in a 45 min period**
- **Supplies for 48 students** (12 workstations)
- **Comprehensive and flexible curriculum**
- **Compelling real-world links**
- **Striking results**
- **Cost effective**
- **Classroom Safe**

Workshop Time Line

- **Introduction**
- **Antigen Detection by ELISA**
- **Ways the ELISA-Immuno Explorer Kit can be used**
- **Real-World Examples**

ELISA

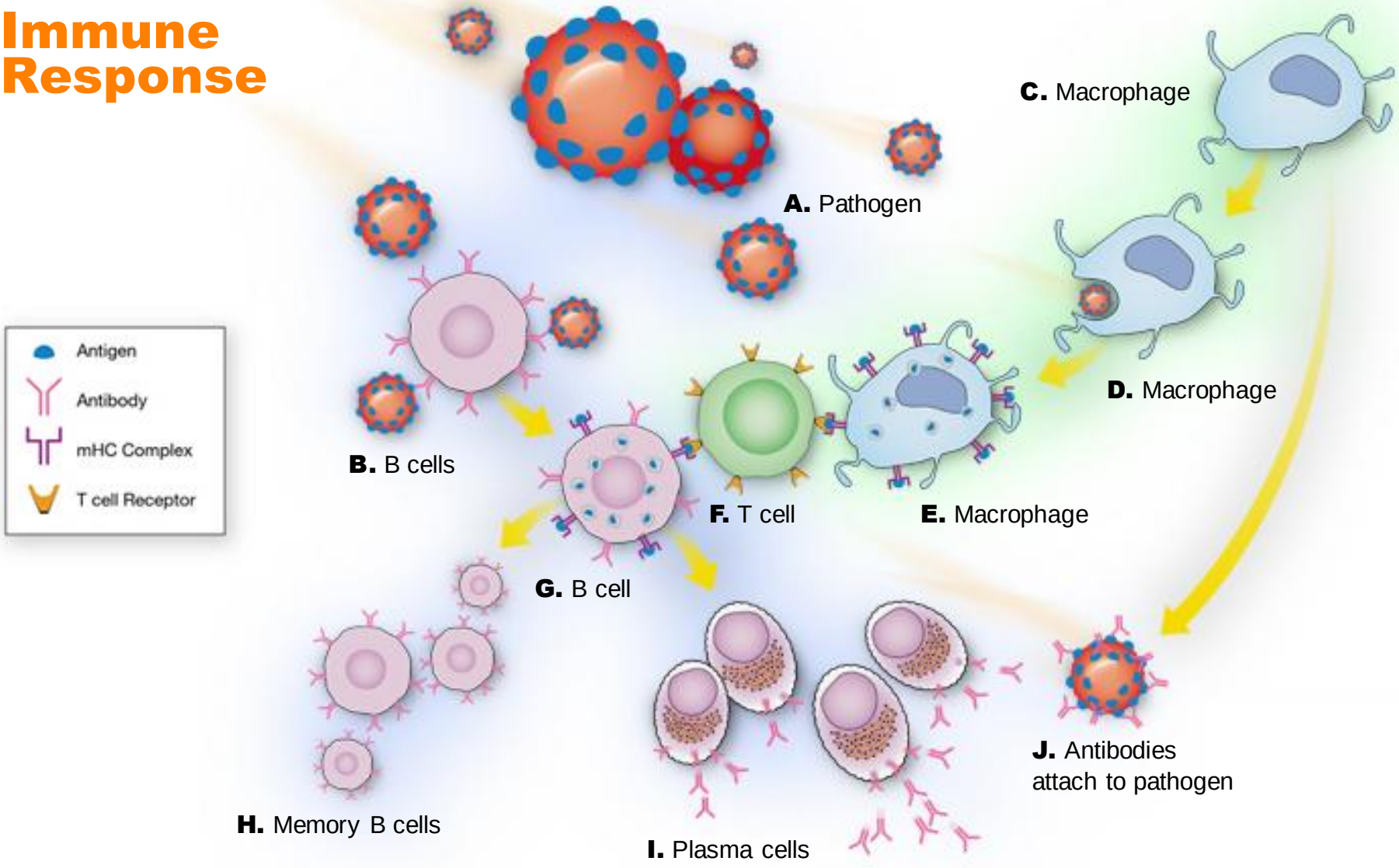
Enzyme-**L**inked
Immuno**s**orbant
Assay

- **Mammalian immune system**
- **Antibody specificity**
- **Biology's "magic bullet"**
- **Evolved over millions of years**
- **Harness nature's tool kit**
- **Imagine the applications!**

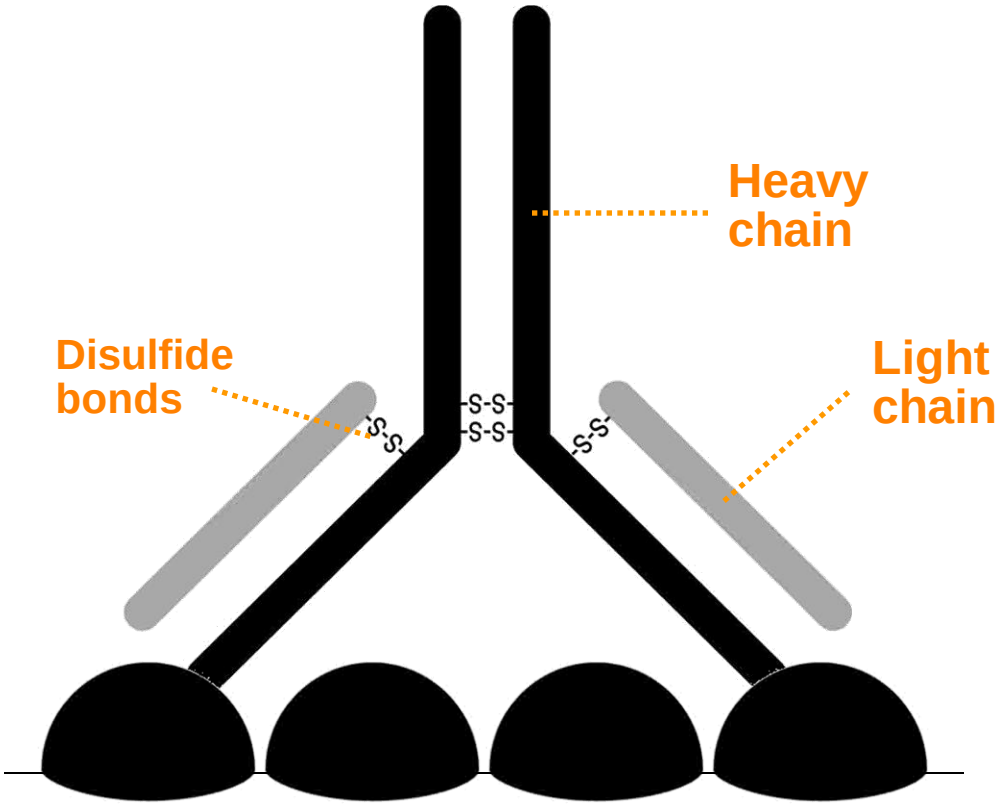
Links to the Real World

- **Mad Cow Disease, SARS, HIV**
- **GMO**
- **Drug and steroid testing**
- **Pregnancy / Reproduction**
- **Biodefense**
- **Cancer treatment**

Immune Response

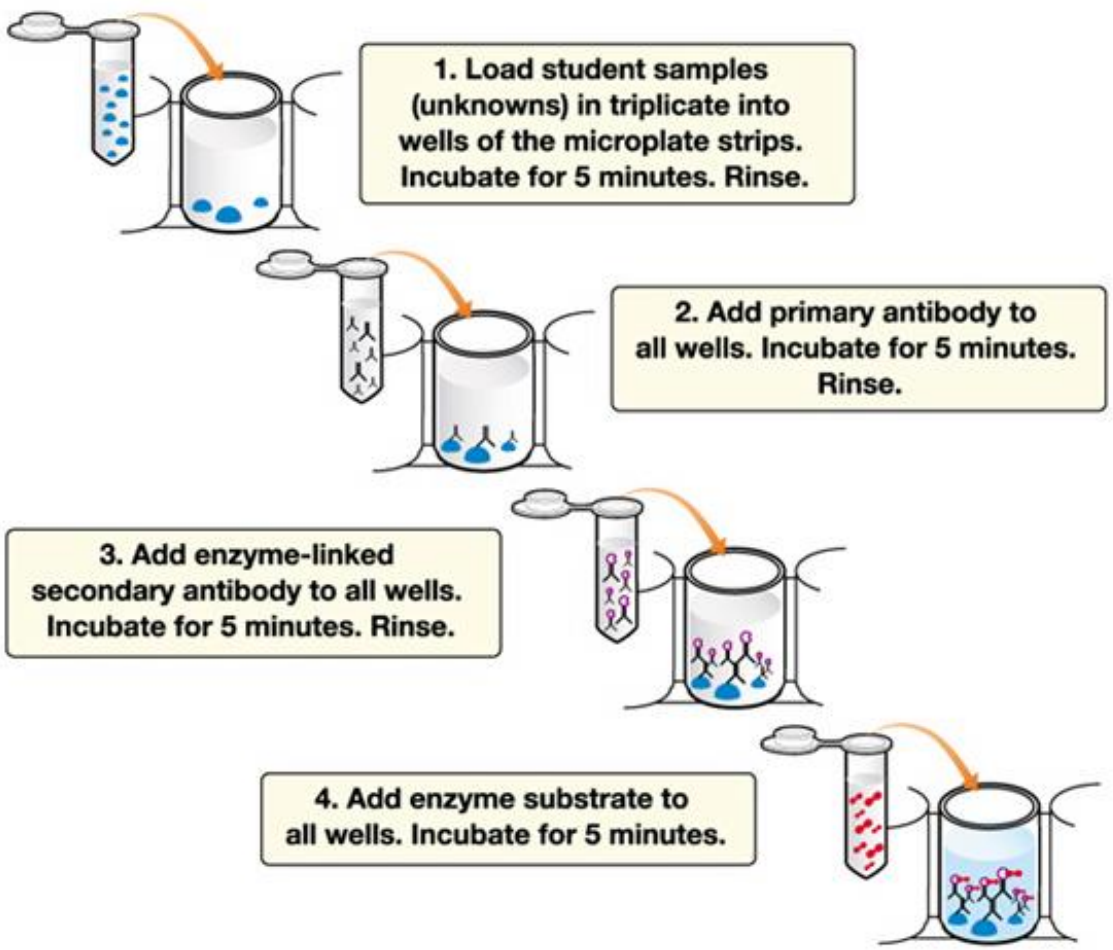


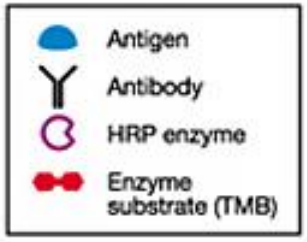
ELISA Antibody Structure



ELISA ANIMATION

ELISA Procedures Overview





ELISA Kit Workstation Inventory

Reagents:

Yellow tubes	Test samples	2
Violet tube (+)	Positive control	1
Blue tube (-)	Negative control	1
Green tube (PA)	Primary antibody	1
Orange tube (SA)	Secondary antibody	1

Lab Equipment and Supplies:

Microplate strips, pipettor, pipette tips, transfer pipette, wash buffer, paper towels, marking pen

Laboratory Quick Guide

Laboratory Quick Guide

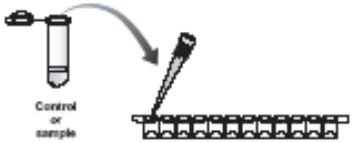
Antigen Detection ELISA

Student Workstation Checklist

One workstation serves 4 students.

Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.25 ml)	4	☐
Violet tube (+)	Positive control (0.5 ml)	1	☐
Blue tube (–)	Negative control (0.5 ml)	1	☐
Green tube (PA)	Primary antibody (1.5 ml)	1	☐
Orange tube (SA)	Secondary antibody (1.5 ml)	1	☐
Brown tube (SUB)	Enzyme substrate (1.5 ml)	1	☐
12-well microplate strips		2	☐
50 µl fixed-volume micropipet or 20–200 µl adjustable micropipet		1	☐
Yellow tips		10–20	☐
Disposable plastic transfer pipet		1	☐
70–80 ml wash buffer in beaker	Phosphate buffered saline with 0.05% Tween 20	1	☐
Large stack of paper towels		2	☐
Black marking pen		1	☐

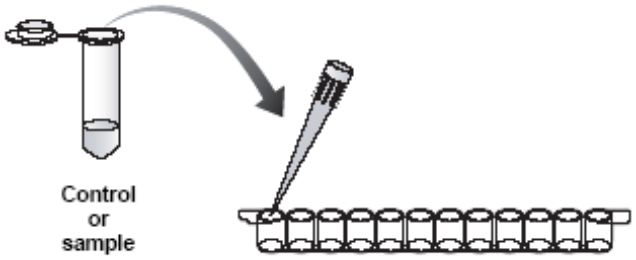
1. Label the yellow tubes with each student's initials.
2. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "–" for the negative controls. Label the remaining wells with your and your partner's initials (3 wells each).
3. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.
4. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.
5. Transfer 50 µl of each of your team's samples into the appropriately initialed three wells, using a fresh pipet tip for each sample.
6. Wait 5 minutes while the proteins in the samples bind to the plastic wells.



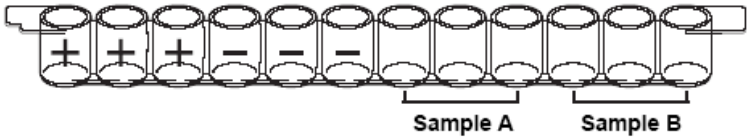
PROTOCOL II
Antigen Detection ELISA

Step One

Label and add controls

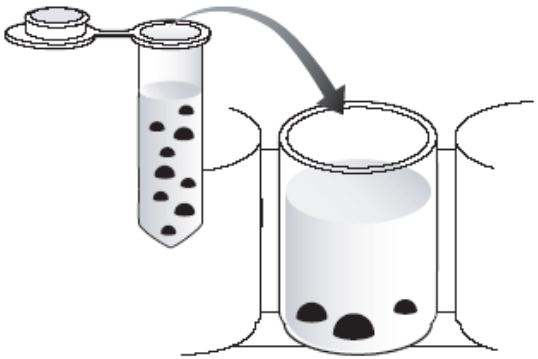


- **Obtain a test-sample**
- **Label the 12-well strip:**
  - **First 3 wells: positive controls "+"**
  - **Next 3 wells: negative controls "-"**
  - **Remaining wells to identify test-samples**



- **Add 50 ul of positive control to 1st 3 wells**
- **Add 50 ul of negative control to 2nd 3 wells**
- **Add 50ul of the student samples to the appropriately labeled wells**
- **Wait 5 minutes for the antigen to bind**

Microplate Strips

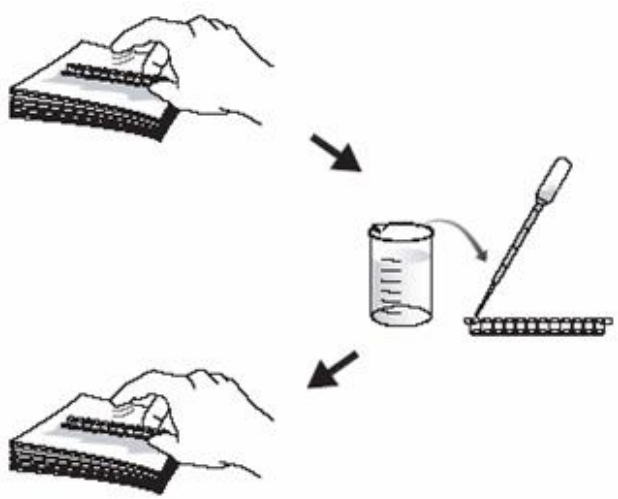


- **Microplate strips are made of polystyrene**
- **Hydrophobic side chains in amino acids bind to the polystyrene wells**

- **No coating is needed**

Step Two

WASH

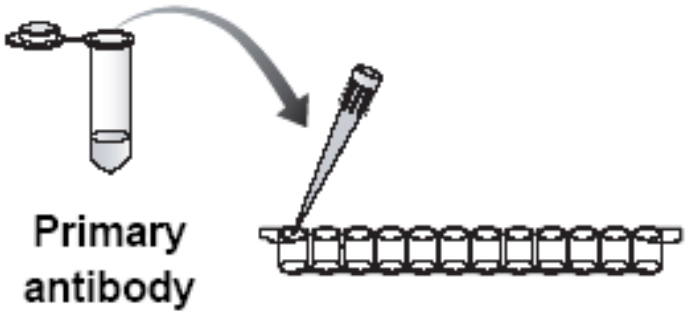


- Remove samples from wells by firmly tapping them on a paper towel
- Discard the top paper towel
- Using a disposable transfer pipette wash wells with wash buffer
- Remove wash buffer by firmly tapping the wells on a paper towel
- Discard the top paper towel
- Repeat wash step

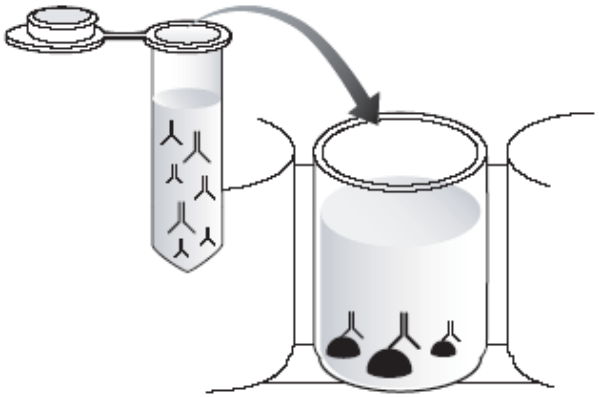
Step Three

Add (PA) Primary Antibody

- **Add 50 ul of the primary antibody (PA) to all 12 wells**
- **Samples are left in wells for 5 minutes**
- **After 5 minutes WASH 2X**



Wash Buffer

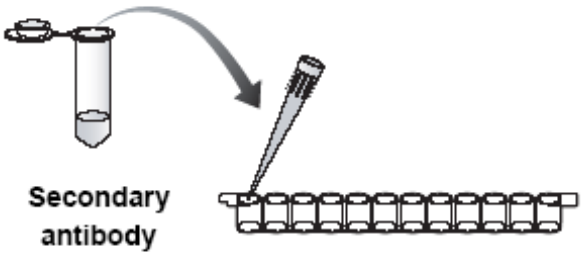


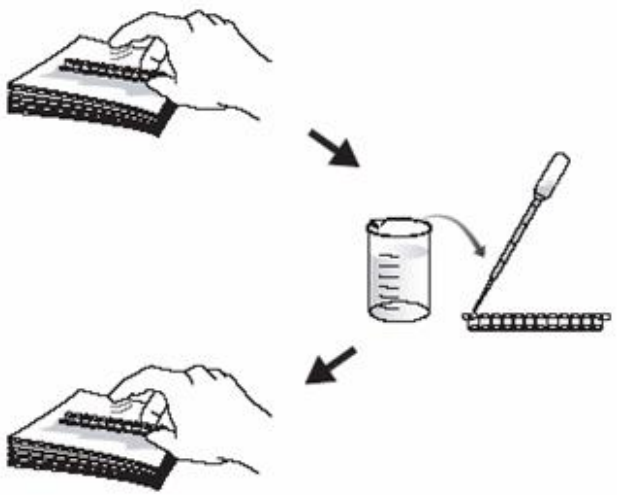
- **Wash buffer contains phosphate buffer saline (PBS) to keep antibodies in a stable environment that helps keep their structure**
- **Also contains Tween 20: a nonionic detergent removes non-specifically bound proteins and coats wells that acts as a blocking agent to reduce background**
- **Antibody will only bind to the antigen**

Step Four

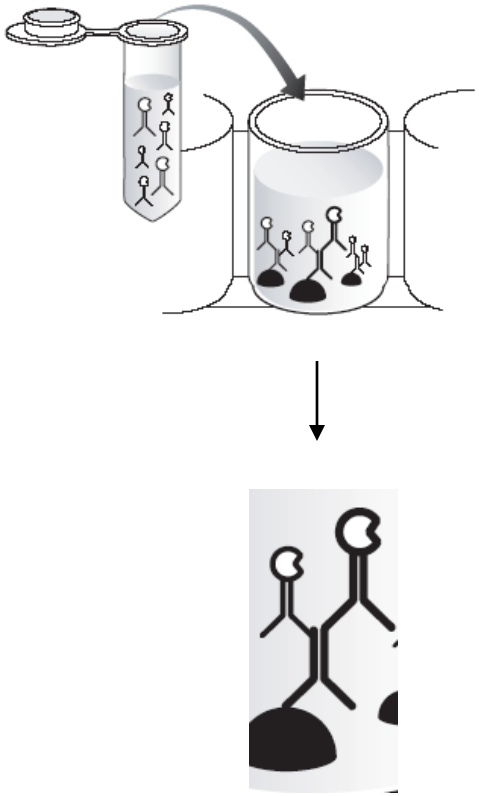
Wash antibody and add enzyme-linked secondary antibody (SA)

- Wash the primary antibody from polystyrene wells as before
- WASH 2X
- Add 50ul of the enzyme-linked secondary antibody to each well
- Wait 5 minutes

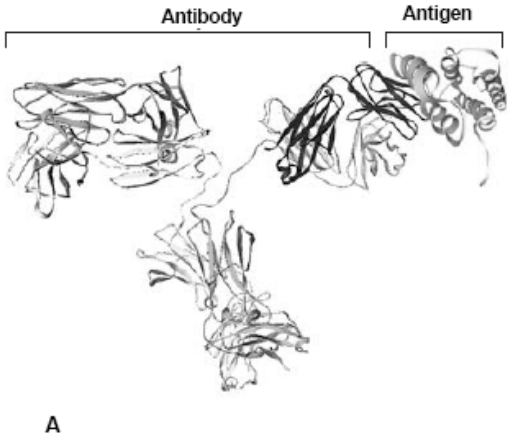


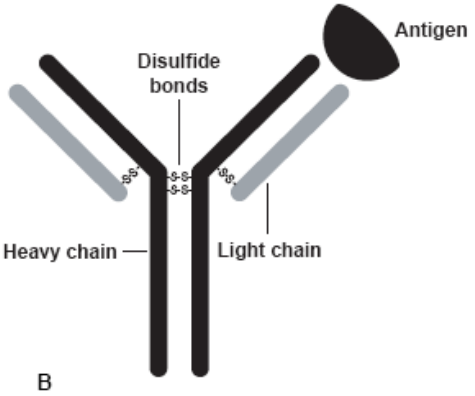


Antibody Specificity



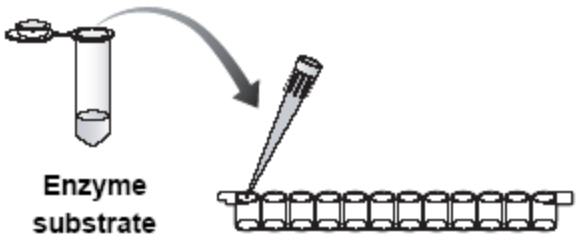
- **Secondary antibody (enzyme-linked antibody) will only bind to the primary antibody (serum antibody)**
- **Secondary antibody specifically recognizes the constant region of the primary antibody**
- **In which wells do you predict this is happening?**



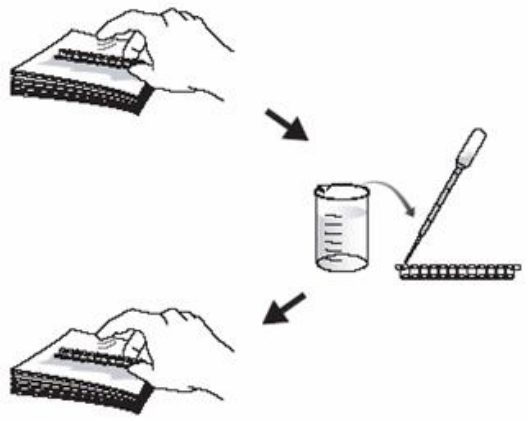


Step Five

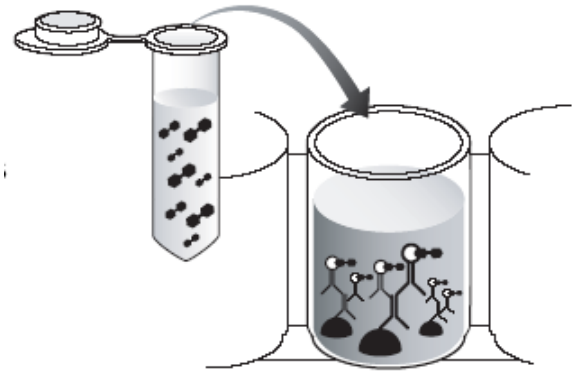
Add enzyme substrate (SUB)



- Wash the enzyme-linked secondary antibody from polystyrene wells as before
- Using a disposable transfer pipette wash wells with wash buffer
- WASH 3X
- Add 50ul of the enzyme substrate to each well
- Wait 5 minutes
- positive samples will begin to turn blue



What are the reagents?



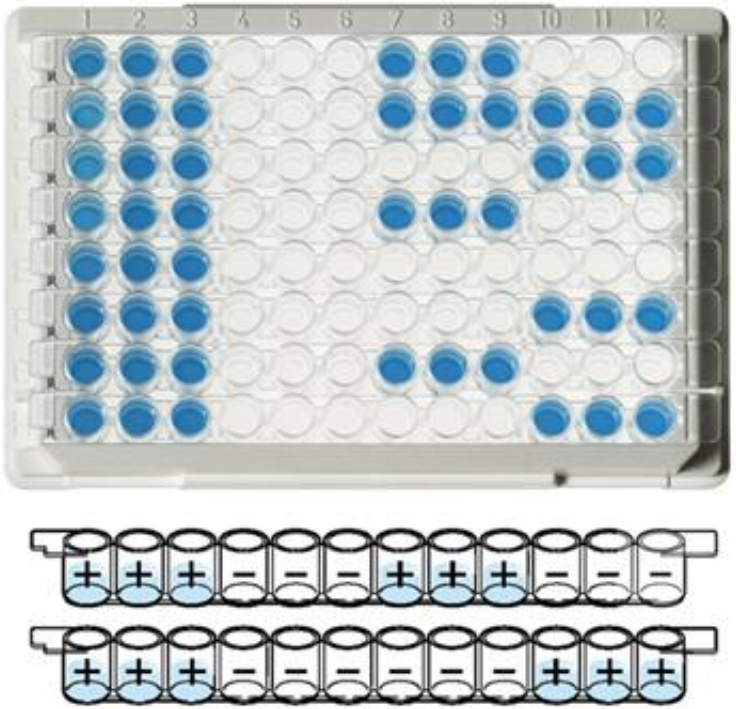
Antigen: Chicken gamma globulin

Primary antibody (PA): Polyclonal anti-chicken antibody made by rabbits

Secondary antibody (enzyme-linked) SA: Polyclonal anti-rabbit antibody made by goats linked (conjugated) to horseradish peroxidase (HRP)

Enzyme substrate (SUB): 3,3',5,5' – tetramethylbenzidine (TMB) – a colorless solution that when oxidized by HRP turns blue

ELISA Kit
Results



Ways The ELISA Kit Can Be Used

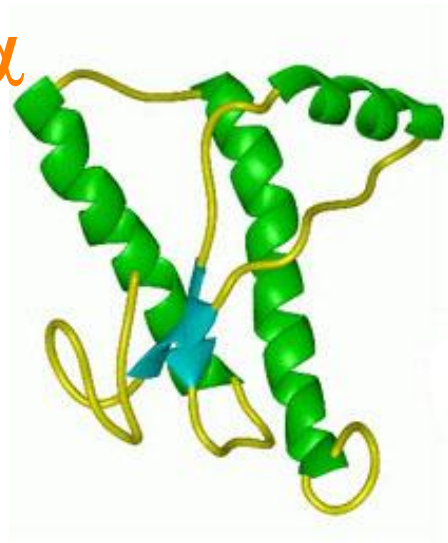
Protocol	Type of ELISA	Real-World Application
I	Tracking outbreaks of disease	HIV, SARS, smallpox & anthrax
II	Detecting antigens	GMO, BSE, pregnancy, drugs, (and all the above)
III	Detecting antibodies in serum	HIV, Lyme disease, smallpox and West Nile virus

ELISA test for Transmissible Spongiform Encephalopathies (TSEs)

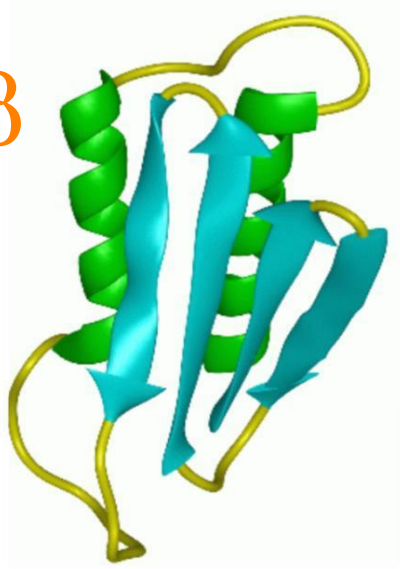
- Uses differences in diseased prions vs. normal prions to prepare sample.
- Proteinase K only digests normal, not diseased, prions .
- ELISA tests for any prion protein

Prion Proteins (PrP^{res} and PrP^{sens})

α



β



PrP^{sens}

- Proteinase K sensitive
- Soluble in detergent

PrP^{res}

- Proteinase K resistant
- Aggregates in detergent

TSE test sample preparation

1. Sample brain tissue
2. Homogenize brain tissue
3. Digest with Proteinase K (normal prions are digested, diseased prions are resistant)
4. Concentrate
5. Denature Proteinase K
6. Perform ELISA

Protocol II: Antigen Detection ELISA

Protocol - ELISA on simulated animal brain samples

Real-World Application – TSE Test

Tube Description	Actual Tube Contents	Simulated Tube Contents
Student samples	Antigen or PBS	Processed brain
Primary antibody	Primary antibody	Antibody against prion protein
Secondary antibody	Secondary antibody	HRP-linked antibody against primary antibody
Positive control	Antigen	Synthesized peptide with prion sequence
Negative control	PBS	Buffer

Real-world Applications of Antibodies

Applications
- **Dipstick tests/ELISA**
- **Immunostaining**
- **Western blotting**

Agricultural Uses
- **Crop-specific disease diagnosis**
- **Animal disease diagnosis**
- **Detection of GM crops**
- **Basic research**

ELISA to test for GMOs

"Genetically Modified Organism (GMO)"

an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination

DNA → RNA → Protein

- **ELISA can help farmers separate their GMO grain lots from non-GMO grain lots.**
- **ELISA tests are used to identify specific proteins**
  - **Delta-endotoxin Cry1Ab from Bt11**
  - **glyphosate from Round-up (RR)**

How to test for GMOs

ELISA:

Test for presence of proteins expressed from genetic modifications

Pro: Quick, inexpensive, low tech

Con: Crop specific, protein stability

PCR:

Test for presence of inserted foreign DNA

Pro: ID different GM crops, DNA stability

Con: Expensive, timely

Example: Pregnancy Test