Are You What You Eat?
Detection of Body Fluid Contamination of Food

Emily Faulconer

Teacher Section

Overview

An antigen is any substance that triggers an immune response and production of antibodies. Antibodies (also called immunoglobulins) are produced by the immune system in response to an antigen. Antibodies reversibly bind to antigens through interactions such as hydrogen bonds and hydrophobic interactions. This biological process can be used to detect the presence of antigens using ELISA (enzyme linked immunosorbent assay).

Students will investigate the use of ELISA as a qualitative assay for detection of body fluid contamination of foods using protein L. Through the laboratory activities, students will see how antibodies interact with antigens with high affinity and specificity. The stude

as the conjugated antibody. The protein in the milk binds to any unused

Advance Preparation

Antigen-Antibody Game

- Materials:

- 15 mL Blocking solution
- 16.5 mL TMB Membrane Peroxidase Substrate System (c, c', 5, 5'-tetramethylbenzidine)
- pH meter
- 2L Beaker
- 100mL Beaker
- 1 L Graduated Cylinder
- 2 96-well plate (additional ones can be used for pipetting and serial dilution practice)
- Reagent boats
- Microfuge tubes 0.5-2mL with rack
- Pipette tips
- Pipettes: 1mL, 5-r

Student Prior Knowledge and Skills

Students should be able to:

- pipette
- use linear algebraic equations to solve concentration/volume calculations
- convert between metric unit prefixes, including nano-
- understand basic laboratory safety procedures
- locate safety equipment such as an eyewash station, first aid kit, telephone, spill kit, and fire extinguisher
- wear appropriate laboratory attire and come dressed accordingly
- take volume and mass measurements, checking twice for accuracy
- tare the balance before taking mass measurements
- keep a clean laboratory bench
- clean all glassware before and after a laboratory exercise

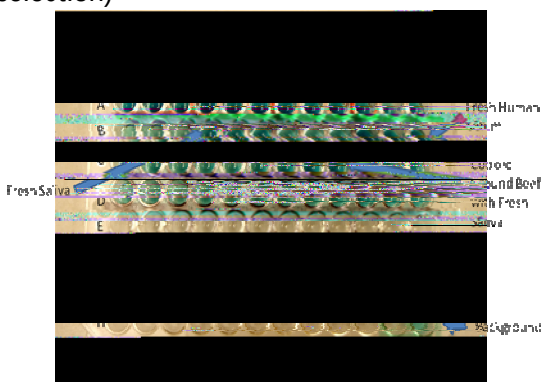
What is Expected from Students

Students should:

- actively participate in the Antigen-Antibody Game
- complete the Protein L laboratory exercise
- analyze the data and submit the results in the form of a laboratory report
- design a laboratory protocol to test for a specific antigen or antibody as a means of studying a new disease

Anticipated Results

- Example results for the Mammalian IgG Lab (individual results will vary based on sample selection)



- Source of error: letting substrate sit too long
  - The TMB substrate is light sensitive. Overexposure to light or prolonged incubation causes nonspecific color development or the TMB precipitates out of solution.

Have students predict and sketch the placement of the antibody
Have students brainstorm why there is a color change and what it might mean

154 mM NaCl

- Follow the procedure to prepare the carbonate buffer and PBS

- Exploration:
  - Serial Dilution Example using blue food coloring:
    Fill the first well with 55 µL of initial solution
    Fill the rest of the row with 45 µL of water
    Transfer 5 µL from the first to the second and mix. Transfer 5 µL to the next well, continue.
  - [illegible] rest

Student Section

Rationale

We have all had to get vaccines. A vaccine introduces a small or innocuous amount of a virus into our bodies so that we will initiate an immune response to it. The vaccine helps most people avoid the disease because the immune system remembers that "bug" and can attack immediately if it meets it again. So what does that have to do with detecting mammalian blood or saliva on food?

Introduction

The immune system contains five classes of immunoglobulins (antibodies) that bind with specific antigens. Once a new antigen is exposed to an organism, the immune system will launch an attack and maintain a memory of the attack. This process allows scientists to manufacture antibodies against very specific antigens.

Over the next few days, you will learn how this biological process is used in ELISA (enzyme linked immunosorbent assay) to detect antigens or antibodies. You will see this process in the laboratory exercise using the antigen Mouse IgG and HRP-Protein L. The HRP stands for horseradish peroxidase, whic026Tm0.0037Tj/TT01Tf0.00011Tcantigen

any antigen is present in the well, it will bind. After washing the plate to remove conjugate that has not bound to the antigen, the substrate is added. If any conjugate remains in the well, it will produce a color change in the substrate.

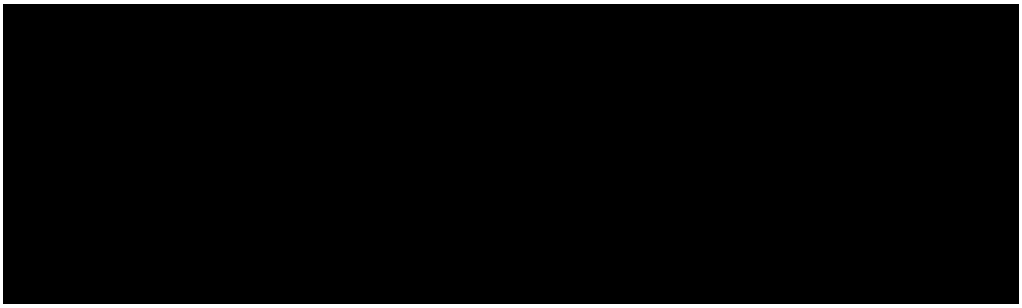


Figure 2: Major Steps of Antibody Capture ELISA

Name:_____ Date: _____

Vocabulary:

Affinity: _____

Aliquot: _____

Antibody: _____

Antigen: _____

Assay: _____

B Cell: _____

Chromogenic: _____

Contamination: _____

Dilution: _____

Enzyme Linked Immunosorbent Assay: _____

Helper T Cell: _____

HRP: _____

Hydrophobic: _____

Immune

Name:_____

Name:_____ Date: _____

Name:_____ Date: _____

Detection of Contamination using ELISA

Reagent List

5 µL Mouse Serum

Equipment List

5-50 Pg8M21Tf0.00079Tc10.9800121629.03998Tb998983Tm605

2. Pipette 50 µL of VR 4 into wells A3 and A4
   Pipette 50 µL of VR 3 into wells A5 and A6
   Pipette 50 µL of VR 2 into wells A7 and A8
   Pipette 50 µL of VR 1 into wells A9 and A10
   Pipette 50 µL of carbonate buffer into the blank and background (wells A1 and A2)
   Pipette 50 µL of samples, recording well location and description below:

Sample #	Location	Description	Visual Rating	Approximate Concentration
				ng/mL
				ng/mL
				ng/mL

Name:_____ Date: _____

Student-Created ELISA Protocol

If you could detect any virus, bacteria, or drug in the world, what would you choose? (Examples: HIV, SARS, Lyme's Disease)

Describe how you would make an antibody for this assay:

What would your microplate well look like with the antigen and antibody bound? Label the antigen and antibody.

Develop a protocol for your ELISA assay. Be sure to include a graphic that illustrates the plate arrangement. (see rubric)

Discussion Questions:

1. Why would you use ELISA to detect an allergy? What molecule would it graps:

Student-Created ELISA Protocol Rubric