College of the Canyons: Introduction to Biotechnology

ELISA Post Lab

1. What is the term ELISA short for? Enzyme Linked Immunabsorbent Assay
2. What is the ELISA test used to detect? It can be used to detect very dilute concentrations of proteins, nucleic acids and other substances to which an antibody can be created.
3. Describe antigens and antibodies. A compound could be both an antigen and an antibody. Explain how this possible. An antigen is something that is being exposed to someone's immune system. As the antigen builds up in the person, that person will make antibodies to this antigen. The antigen is usually a small piece of protein that is found on the surface of a larger substance that can invoke an immune response (bacteria, virus, pollen, etc). Even anti-bodies themselves (which are large proteins) could be an antigen to someone's immune system. If you inject a new or different antigen into the bloodstream of a person, that person's immune system will make antibodies to it. If this new protein is an antibody, than the antibody is also an antigen with respect to the person's bloodstream to whom you are injecting the solution.

4. In the space below, diagram the position and interaction of: IgA, anti-IgA, Anti-IgA-HRP, BSA, OPD, enzyme….Hint…look at lab cover.

5. Why is ELISA often done in conjunction with serial dilution? Give two reasons.
The serial dilution provided a series of comparable standards for analysis of the sample (which is usually an unknown).
The serial dilutions also serve to act as a series of controls. Their concentration is compared to their expected absorbance to see how well the ELISA test reagents are with respect to standard results.

6. Which sample of saliva (the 1 to 100 or 1 to 500 dilution) would have the strongest reaction with the OPD? Defend your choice. The 1:100 should have the strongest reaction with the OPD. Referencing question 4, the amount of Anti-IgA-HRP is a function of the amount of IgA present. A 1: 100 dilutions should have more of the IgA present as compared to the 1:500 sample (5 times the amount). So more Anti-IgA-HRP, more reaction with the OPD.

7. What is the purpose of each of the following reagents in ELISA

a. BSA: Bovine Serum Albumin is a blocking agent. It is used to coat the remaining surface of the plastic well after adding the initial anti-IgA (see figure in question 4).	b. Anti-IgA-HRP Binds to IgA and with it carries the enzyme Horse Radish Peroxidase. This enzyme catalyzes the conversion of OPD from a colorless substance to a colored substance (with help of hydrogen peroxide) to create absorbance which is basis of the spectrophotometers' quantification of absorbance.
c. Anti-IgA: This is the "capture anti-body. Along with BSA, it is the only molecule that coats the surface of the plastic well. Only substances complementary to Anti-IgA will bind (IgA). It is this antibody that lends it specificity to the ELISA test.	d. TBT This is short for" Tris-Borate-Triton. This is a buffer solution (TB) and a soap (Triton). The buffer keep the proteins intact, stable, etc. The Triton helps to prevent the proteins from "arbitrary binding", as they are somewhat "sticky" and may give false results if becomes to sticky, too much, etc.

8. Treating an antibody with acid will result in a single large molecule being converted into 4 smaller proteins. Describe why this is occurring . Hint: Look the structure up in your textbook. Even slight treatment with acids will alter an anti-body's ability to bind specifically. Diagram and /or example both of these ideas.

Acidic conditions will reduce the sulfide bridges in the molecule. Specifically, the S---S link is converted to a SH HS and the S---S bond is broken. This then separates the light and heavy chains of the antibody, created 4 separate smaller proteins from the original larger one.

Acidic conditions can also alter the individual amino acids of the antibody. The binding sites are mosaics of small bumps and grooves complete with many functional groups. These functional groups have specific charges that are often the result of reduction state. For instance; COOH is acidic and COO – is alkaline, NH3+ is acidic and NH2 is alkaline. As the pH changes, so does the charge on these functional groups. This affects the ability of the antibody to bind specifically as bind ing is a function of shape AND charge.