

ACTIVITY 4

ELISA

1. What is ELISA test?

- ELISA is used in many laboratories to determine whether a particular antibody is present in a patient's blood sample. Although the procedure is routine and straightforward, it involves a number of variables, such as reagent selection, temperature, volume measurement, and time, which if not adjusted correctly can affect subsequent steps and the test outcome. The test measures whether a specific antibody associated with an illness can be found in a patient's blood. A positive result indicates that the antibody is there and implies that the person has encountered a particular disease.

2. Principle behind ELISA test?

- In ELISAs, the antigen antibody complex is exposed to the second antibody, which binds to the antibody portion of the complex (against which it was formed), creating a sandwich-type structure. The signaling system consists of an enzyme attached to the second antibody. When the appropriate chemical is added, the enzyme converts it to a colored substance that can be measured. This test quantifies how much enzyme is present by the amount of color produced. The more enzyme present, the more secondary antibody must be attached. The amount of secondary antibody present is determined by the amount of target, or first antibody, available. Finally, because the first antibody binds to antigen, the more antigen that is accessible, the more first antibody will be retained. The measure of color, therefore, reflects the amount of antigen initially present.

3. What are the four types of ELISA? Discuss each type.

a. Direct ELISA

- In a direct ELISA, the antigen is immobilized to the surface of the multi-well plate and detected with an antibody specific for the antigen. The antibody is directly conjugated to HRP or other detection molecules.

b. Indirect ELISA

- Indirect ELISA is a technique that uses a two-step process for detection, whereby a primary antibody specific for the antigen binds to the target, and a labeled secondary antibody, against the host species of the primary antibody binds to the primary antibody for detection. As for direct ELISA assays, the antigen is immobilized to the surface of the multi-well plate. The method can also be used to detect specific antibodies in a serum sample by substituting the serum for the primary antibody.

c. Sandwich ELISA

- Sandwich ELISA (or sandwich immunoassay) is the most commonly used format. This format requires two antibodies specific for different epitopes of the antigen. These two antibodies are normally referred to as matched antibody pairs. One of the antibodies is coated on the surface of the multi-well plate and used as a capture antibody to facilitate the immobilization of the antigen. The other antibody is conjugated and facilitates the detection of the antigen.

d. Competitive ELISA

- Also known as inhibition ELISA or competitive immunoassay, competitive ELISA assays measure the concentration of an antigen by detection of signal interference. Each of the previous formats can be adapted to the competitive format. The sample antigen competes with a

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Table of Contents Elisa Test Questions And Answers

1. Understanding the eBook Elisa Test Questions And Answers
 - The Rise of Digital Reading Elisa Test Questions And Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Elisa Test Questions And Answers
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Elisa Test Questions And Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Elisa Test Questions And Answers
 - Personalized Recommendations
 - Elisa Test Questions And Answers User Reviews and Ratings
 - Elisa Test Questions And Answers and Bestseller Lists
5. Accessing Elisa Test Questions And Answers Free and Paid eBooks
 - Elisa Test Questions And Answers Public Domain eBooks
 - Elisa Test Questions And Answers eBook Subscription Services
 - Elisa Test Questions And Answers Budget-Friendly Options
6. Navigating Elisa Test Questions And Answers eBook Formats
 - ePub, PDF, MOBI, and More
 - Elisa Test Questions And Answers Compatibility with Devices
 - Elisa Test Questions And Answers Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Elisa Test Questions And Answers
 - Highlighting and Note-Taking Elisa Test Questions And Answers
 - Interactive Elements Elisa Test Questions And Answers
8. Staying Engaged with Elisa Test Questions And Answers

- Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Elisa Test Questions And Answers
9. Balancing eBooks and Physical Books Elisa Test Questions And Answers
- Benefits of a Digital Library
 - Creating a Diverse Reading Collection Elisa Test Questions And Answers
10. Overcoming Reading Challenges
- Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Elisa Test Questions And Answers
- Setting Reading Goals Elisa Test Questions And Answers
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Elisa Test Questions And Answers
- Fact-Checking eBook Content of Elisa Test Questions And Answers
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
- Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
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 - Interactive and Gamified eBooks

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