

IMPORTANT QUESTIONS ON IMMUNOLOGICAL TECHNIQUES

Immuno and Histochemical Technique

1. Match the types of ELISA to their description.

A. Direct - (i) Detection of specific antibodies in serum.

B. Indirect - (ii) Capture antibodies are coated onto the surface.

C. Sandwich - (iii) Measures the concentration of an antigen by detection of signal interference.

D. Competitive - (iv) The antigen is directly immobilized to the surface.

A. A-(ii); B-(i); C-(iv); D-(iii).

B. A-(iii); B-(i); C-(ii); D-(iv).

C. A-(iv); B-(i); C-(ii); D-(iii).

D. A-(iv); B-(ii); C-(iii); D-(i).

2. How is radioimmunoassay (RIA) is different from the conventional enzyme-linked immunosorbent assay (ELISA)?

A. Use of antigen-coated plate

B. Use of antibody-coated plate

C. Use of radioisotopes

D. Use of secondary antibodies

3. Which of the given techniques is used in detecting and locating a specific DNA sequence on a chromosome?

A. FISH

B. GISH

C. Chromatography

D. ELISA

4. Which of the following technique should be used to study the protein-protein interaction?

A. ELISA

B. RAPD

C. Immunoprecipitation

D. Chromatography

5. Which one of the blotting techniques is used in the detection of the proteins among the mixture of proteins?

A. Northern blotting

B. Southern blotting

C. Western blotting

D. Eastern blotting

6. Which of the given techniques can be used to distinguish the difference between individual DNA-binding proteins and protein complexes that recognise the same DNA sequence?

A. Chromatography and Western blot

B. SDS and ELISA

C. EMSA and ChIP

D. ChIP and SDS

7. Which of the following is the quantitative technique to detect the antigen levels in the sample?
- A. Immunoelectrophoresis
 - B. Rocket Immunoelectrophoresis
 - C. ChIP
 - D. Chromatography
8. Which of the given tools is used for determining the origin of genomes or chromatin in hybrids?
- A. GISH
 - B. FISH
 - C. ELISA
 - D. RIA
9. Which one of the following is not an immunodiffusion test?
- A. Double-diffusion
 - B. Gel diffusion
 - C. Antigen layer diffusion
 - D. Ouchterloney technique
10. In rocket immunodiffusion, what does the length of the rocket signify?
- A. Proportional to the amount of antibody placed in each well
 - B. Proportional to the amount of antigen placed in each well
 - C. Inversely proportional to the amount of antibody placed in each well
 - D. Inversely proportional to the amount of antigen placed in each well

Answers:

- | | |
|------|-------|
| 1. C | 6. C |
| 2. C | 7. B |
| 3. A | 8. A |
| 4. C | 9. C |
| 5. C | 10. B |

SOLUTIONS

Solution:1

- 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.
- 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 labelled secondary antibody against the host species of the primary antibody binds to the primary antibody for detection. The method can also be used to detect specific antibodies in a serum sample by substituting the serum for the primary antibody.
- In sandwich ELISA, 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.
- Competitive ELISA also known as inhibition ELISA or competitive immunoassay, competitive ELISA assays measure the concentration of an antigen by detection of signal interference.

Solution:2

The key difference between RIA and ELISA is that radioimmunoassay (RIA) is an immunoassay technique that uses radioisotopes to detect antigen-antibody complexes while enzyme-linked immunosorbent assay (ELISA) is an immunoassay technique that uses enzymes to detect antigen-antibody complexes.

Solution:3

- Fluorescence in situ hybridization (FISH) is a laboratory technique for detecting and locating a specific DNA sequence on a chromosome.
- The technique relies on exposing chromosomes to a small DNA sequence called a probe that has a fluorescent molecule attached to it.
- FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification.
- FISH can also be used to detect and localize specific RNA targets (mRNA)

Solution:4

- For Protein-protein interactions: Individual protein immunoprecipitation is a procedure by using an antibody that is specific for a known protein to isolate that particular protein out of a solution containing many different proteins.

Immunoprecipitation (IP) is a method to isolate a specific antigen from a mixture, using the antigen-antibody interaction. Antigens isolated by IP are analyzed by SDS-PAGE or Western blotting.

Solution:5

- Southern blotting is a laboratory technique used to detect a specific DNA sequence in a blood or tissue sample.
- The northern blot, or RNA blot, is a technique used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA) in a sample.
- A western blot is a laboratory method used to detect specific protein molecules from among a mixture of proteins.

- The eastern blot, or eastern blotting, is a biochemical technique used to analyze protein post-translational modifications.
- ncRNA and miRNA) in cells, circulating tumour cells, and tissue samples.

Solution:6

- Related transcription factors are known to recognize identical or similar DNA sequences.

Antibody-based methods such as chromatin immunoprecipitation or EMSA supershift experiments are the only means to differentiate between proteins that bind the same DNA sequence, to a limited extent.

Solution:7

- Immunoelectrophoresis is a qualitative technique that combines two methods one after another:
 - (i) Gel electrophoresis – separation of components by charge
 - (ii) Immunodiffusion – diffusion of both antibody and antigen toward each other produce precipitin line.
- It is a strictly qualitatively technique to detect antibodies and antigens in samples.
- But also a quantitative technique exist called rocket immunoelectrophoresis to measure the antigen level in a sample.

Solution:8

- Genomic in situ hybridization (GISH), which uses total genomic DNA as a probe, is a powerful tool for determining the origin of genomes or chromatin in hybrids.
- This technique successfully detected barley chromosomes in hybrids with wheat

Solution:9

- Simple immunodiffusion (Oudin technique) in which one of the two reagents remains fixed (either the antigen or the antibody) and the other reagent moves.
- Double immunodiffusion (Ouchterlony technique) in which antigen and antibody are free to move towards each other.

Solution:10

In this rocket immunodiffusion method, antigen migrates from the well through agarose gel containing antiserum, forming rocket-shaped precipitin peaks. The height of this peak is proportional to the concentration of the antigen-loaded in the corresponding well.

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