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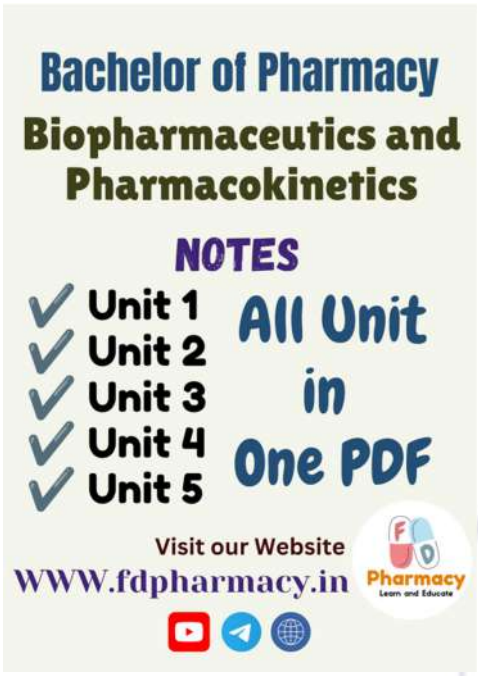
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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 4

TOPIC :

- a) Immuno blotting techniques- ELISA, Western blotting, Southern blotting.

Blotting Techniques

Blotting techniques are laboratory methods used to detect specific biological molecules such as DNA, RNA, and proteins. These techniques are widely used in molecular biology, biotechnology, genetics, and disease diagnosis.

Types of Blotting Techniques

Technique	Detects
Southern Blotting	DNA
Northern Blotting	RNA
Western Blotting	Proteins

Southern Blotting

- Southern blotting is a technique used to detect specific DNA sequences in a DNA sample.

Principle

- DNA fragments are separated by gel electrophoresis and transferred onto a membrane. A labeled DNA probe complementary to the target DNA sequence is then added. Hybridization between the probe and target DNA indicates the presence of the specific DNA sequence.

Procedure

1. Isolation of DNA from the sample.
2. Digestion of DNA using restriction enzymes.
3. Separation of DNA fragments by agarose gel electrophoresis.
4. Denaturation of DNA into single strands.
5. Transfer of DNA onto a nitrocellulose or nylon membrane.
6. Addition of labeled DNA probe.
7. Hybridization between probe and target DNA.
8. Detection of bound probe by autoradiography or color development.

Applications

- Detection of genetic disorders.
- DNA fingerprinting.
- Gene mapping.
- Paternity testing.
- Detection of mutations.

Northern Blotting

- Northern blotting is a technique used to detect specific RNA molecules in a sample.

Principle

- RNA molecules are separated by gel electrophoresis and transferred onto a membrane. A labeled probe binds to complementary RNA sequences and helps identify specific RNA molecules.

Procedure

1. Isolation of RNA.
2. Separation of RNA by gel electrophoresis.
3. Transfer of RNA onto a membrane.
4. Fixation of RNA on the membrane.
5. Addition of labeled probe.
6. Hybridization of probe with RNA.
7. Washing and detection of bound probe.

Applications

- Analysis of gene expression.
- Detection of viral RNA.
- Study of mRNA levels.
- Research in molecular biology.

Western Blotting

- Western blotting is a technique used to detect specific proteins in a biological sample.

Principle

- Proteins are separated according to molecular weight by gel electrophoresis and transferred onto a membrane. Specific antibodies bind to the target protein and allow its detection.

Procedure

1. Extraction of proteins.
2. Separation of proteins by SDS-PAGE.
3. Transfer of proteins onto a membrane.
4. Blocking of non-specific binding sites.
5. Addition of primary antibody.
6. Addition of enzyme-linked secondary antibody.
7. Addition of substrate.
8. Detection of protein bands.

Applications

- Diagnosis of HIV infection.
- Detection of viral proteins.
- Protein analysis.
- Biomedical research.
- Identification of biomarkers.

ELISA (Enzyme-Linked Immunosorbent Assay)

- ELISA is a laboratory technique used to detect and measure antigens or antibodies in a sample.

Principle

- ELISA is based on the specific antigen-antibody reaction. An enzyme-linked antibody reacts with a substrate to produce a colored product that can be measured for detection.

Components of ELISA

1. Antigen or Antibody
2. Enzyme-linked Antibody
3. Microtiter Plate
4. Washing Buffer
5. Substrate Solution

Types of ELISA

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA

1. Direct ELISA

- Direct ELISA is used to detect antigens using a single enzyme-linked primary antibody.

Principle

- The antigen is attached to the microtiter plate and detected directly by an enzyme-linked primary antibody. Addition of substrate produces a color change.

Procedure

1. Coating of antigen.
2. Blocking of unoccupied sites.
3. Addition of enzyme-linked primary antibody.
4. Washing.
5. Addition of substrate.
6. Observation of color development.

Applications

- Detection of antigens.
- Diagnosis of infectious diseases.
- Research laboratories.
- Hormone and protein analysis.

Advantages

- Simple and rapid.
- Fewer steps required.

Disadvantages

- Lower sensitivity.
- Limited signal amplification.

2. Indirect ELISA

- Indirect ELISA is used to detect antibodies in a sample.

Principle

- A known antigen is coated on the plate. The primary antibody from the sample binds to the antigen, and an enzyme-linked secondary antibody binds to the primary antibody.

Procedure

1. Coating of antigen.
2. Blocking.
3. Addition of sample containing primary antibody.
4. Washing.
5. Addition of enzyme-linked secondary antibody.
6. Washing.
7. Addition of substrate.
8. Observation of color development.

Applications

- Detection of antibodies in serum.
- Diagnosis of HIV.
- Diagnosis of Hepatitis.
- Detection of viral and bacterial infections.

Advantages

- High sensitivity.
- Signal amplification.

Disadvantages

- More time-consuming.
- Possibility of cross-reactivity.

3. Sandwich Elisa

- Sandwich ELISA is used to detect antigens using two antibodies.

Principle

- The antigen is captured between a capture antibody and an enzyme-linked detection antibody, forming an antibody-antigen-antibody sandwich.

Procedure

1. Coating of capture antibody.
2. Blocking.
3. Addition of sample containing antigen.
4. Washing.
5. Addition of enzyme-linked detection antibody.
6. Washing.
7. Addition of substrate.
8. Observation of color development.

Applications

- Detection of hormones.
- Detection of cytokines.
- Diagnosis of infectious diseases.
- Detection of viral and bacterial antigens.

Advantages

- Highly sensitive.
- Highly specific.

Disadvantages

- More expensive.
- Requires matched antibody pairs.

4. Competitive ELISA

- Competitive ELISA is used to detect and quantify antigens or antibodies in a sample.

Principle

- The sample antigen competes with enzyme-labeled antigen for binding sites on a specific antibody. Higher antigen concentration produces lower color intensity.

Procedure

1. Coating of antibody.
2. Addition of sample antigen and labeled antigen.
3. Incubation for competition.
4. Washing.
5. Addition of substrate.
6. Observation of color development.

Interpretation

- **More color = Less antigen present**
- **Less color = More antigen present**

Applications

- Detection of hormones.
- Detection of toxins and drugs.
- Measurement of small antigens.
- Clinical diagnosis.

Advantages

- Suitable for small molecules.
- High accuracy.

Disadvantages

- More complex procedure.
- Interpretation can be difficult.