BT312 - Analytical Biotechnology Laboratory - Introductory Class

Lab Equipments

Course Instructors:

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Syllabus

- Theory, operation and handling of instruments to be used in this lab course
- Identification and determination of concentration of amino acid using thin layer chromatography
- Determination of oligomeric status of the given protein by gel filtration chromatography
- Monitor equilibrium unfolding of a protein from the tryptophan fluorescence or Far UV-circular dichroism or enzymatic assay
- Determination of protein concentration by Bradford method
- Immuno-labeling and visualization of marker protein inside cells
- Screening of antibodies in radial immuno-diffusion assay
- Determination of concentration of antibody by ELISA
- Preparation of nanoparticle to detect bio-analyte
- Demonstration of flow cytometry with experimental sample
- Demonstration of real-time PCR with experimental sample
- Basics of microscopy with visualization of mammalian cells (unstained and stained sample)
- Demonstration of SEM with experimental sample
- Demonstration of AFM with experimental sample.

Microwave Oven

Principle:

- The microwave oven principle is based on the generation of electromagnetic radiation or microwaves in a specific frequency range, typically 2.45 GHz to heat food or other materials.
- This induces polar molecules in the item to rotate and produce thermal energy in a process known as dielectric heating.
- The generated heat is evenly distributed throughout the substance, as microwaves can penetrate a few centimeters into the substance before being absorbed completely.

Applications:

- Essential for accelerating Molecular Biology experiments.
- Efficient sample heating.

Water Bath

Principle:

- The sensor in water bath converts the temperature of the water into a resistance value, which is then amplified and compared by an integrated amplifier. This produces the control signal, which effectively regulates the average heating power of the electric heating tube and keeps the water at a constant temperature.
- Water Bath is essentially a container filled with heated water.
- Its primary purpose is to provide a controlled and constant temperature environment for incubating test samples submerged in water over an extended period of time.
- One of the advantages of using a water bath is that it allows for the heating of a small volume of liquid sample over an extended period without altering the concentration of its constituents through evaporation.

Applications:

- Essential for incubating biological samples, enzymatic reactions, and culturing microorganisms.

Magnetic Stirrer

Principle:

- Magnetic stirrers use a rotating magnetic field to move a stir bar around in liquid samples, and some are coupled with stirring hot plate.
- The movement of this stir bar mixes the samples thoroughly with rapid movement and agitation.
- The user controls the magnetic field's speed, so it can be customized to the specific sample that's being stirred.

Applications:

- Mixing samples under heating conditions

Analytical Weighing Balance

Principle:

- Analytical balance calculates weights based on the force required to balance the mass of a sample rather than utilizing actual masses.
- They produce a force to balance the sample using an electromagnet, then output the result by measuring the force required.
- A transparent enclosure with doors surrounds the measurement pan of an analytical balance, blocking external influences.

Applications:

- Used to measure the weight/mass of samples to a very high degree of accuracy.

Autoclave

Principle:

- The autoclave works on the principle of moist heat sterilization. The high pressure inside the chamber increases the boiling point of water for the sterilization of equipment, while ensuring the rapid penetration of heat into the deeper parts of equipment.
- The moisture present in the steam causes coagulation of proteins of microbes causing irreversible loss of their activity and functions. Thus, killing them and sterilizing the equipment.
- In general, an autoclave is run at a temperature of 121° C for at least 30 minutes by using saturated steam under at least 15 psi of pressure.

Applications:

- It is used to sterilize wide range of material including but not limited to laboratory glasswares, laboratory equipments and instruments, surgical material including needles, seizures etc.
- It is most commonly involved in the sterilization of biological media.

Gel rocker

Principle:

- Gel rockers use a platform that moves in a seesaw motion to create waves in liquid laboratory samples.
- It typically consists of a platform or tray that can oscillate back and forth, creating a gentle rocking motion.
- It allows uniform mixing of the samples without causing significant turbulence or disturbance.

Applications:

- Gels Rockers are commonly used for staining and de-staining gels after electrophoresis, hybridization, washing, blotting, cell culture and gentle mixing.

pH meter

Applications:

- pH meters measure water safety in pools and aquariums.
- They are used to monitor and control pH levels during food processing, brewing, wine-making, and dairy production.

Principle:

- A pH meter measures the acidity or alkalinity of a solution. Acidic solutions have more positively charged hydrogen ions, and alkaline solutions have less positively charged hydrogen ions (and more negatively charged hydroxide ions).
- The working principle behind pH meters is potentiometry.
- This is the measurement of a solution's electric potential (voltage).
- It measures electric potential using 2 electrodes inserted into the liquid to create an electrical circuit.

1. Reference electrode: will contain a substance with a known electric potential.
2. Sensor electrode, will be inserted into the solution being tested.

- The electric potential is the difference that results from comparing the reference electrode to the sensor electrode.

Ice Flaker

Principle:

- Water enters the water distribution tray from the evaporator's water inlet in a ice flake machine.
- Thin layer of ice on the inner wall is formed.
- The ice is broken into flakes by an ice blade and falls into the ice storage through the ice-dropping port.

Applications:

- Ice flakes are used for maintaining the conditions of proteins, enzymes and other reagents during lab experiments,
- For cooling and transporting of different biomedical products, food, etc.

Centrifuge

Principle:

- A centrifuge is a device, generally driven by an electric motor, that puts an object in a rotational movement around a fixed axis and separates mixtures by applying centrifugal force.
- **The sedimentation principle**: substances are separated based on their density under the influence of gravitational force.
- When spun rapidly, lighter particles stay at the top and heavier particles go to the bottom during centrifugation.

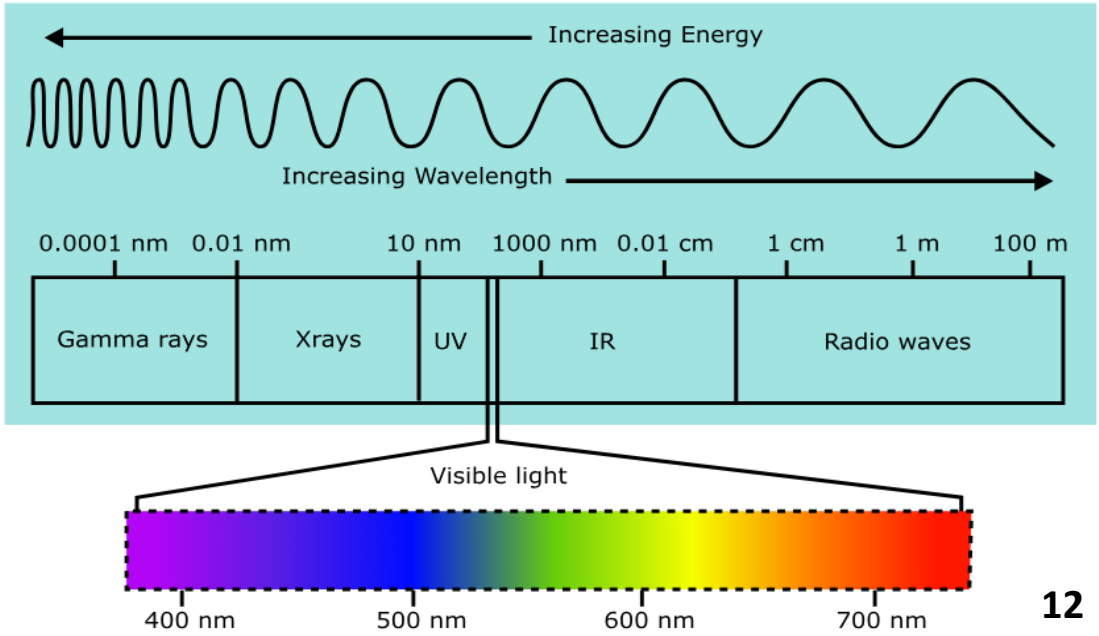
Applications:

- To separate a mixture of two different miscible liquids
- To pellet down cells
- Separation of blood components from blood samples

UV-Vis spectroscopy

Principle:

- UV-Vis spectroscopy measures the absorption of ultraviolet (UV) and visible (Vis) light by molecules.
- The principle is based on the interaction of light with electrons in the sample, causing electronic transitions between energy levels.
- The resulting absorption spectrum provides information about the sample's molecular structure, concentration, and presence of certain functional groups or chromophores.



Applications:

- It is used to observe the optical behavior of chemical compounds, identification of various species, and quantification of specific analytes.

Sonicator

Principle:

- The principle of sonication is based on the use of ultrasonic waves to agitate or disrupt particles, cells, or molecules in a liquid medium.
- The sound waves are delivered using an apparatus with a vibrating probe that is immersed in the liquid cell suspension.
- Mechanical energy from the probe initiates the formation of microscopic vapor bubbles that form momentarily and implode, causing shock waves to radiate through a sample

Applications:

- Used to break open cells and release intracellular contents such as proteins, nucleic acids and organelles.
- Helps in the efficient extraction and purification of proteins from cells or tissues.

Ultra low temperature freezer (-80℃)

Principle:

- The principle of a -80 degree Celsius (°C) refrigerator, also known as an ultra-low temperature (ULT) freezer or deep freezer, is to create and maintain a stable and extremely low temperature environment for the long-term storage of biological samples, chemicals, and other temperature-sensitive materials.

Applications:

- Primarily used for the long-term storage of biological samples, including DNA, RNA, proteins, cell lines, tissues, and other biological materials.
- Certain enzymes and chemicals remain stable at very low temperatures, making -80°C freezers suitable for their long-term storage

Compound Microscope

Principle:

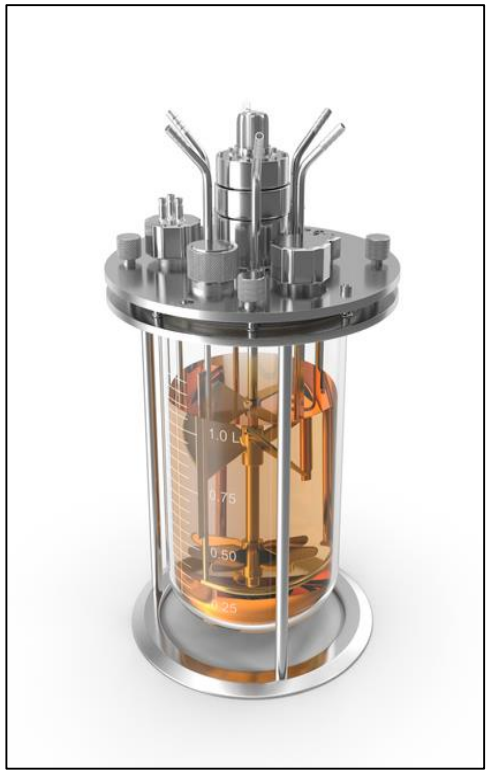
A compound microscope uses two lenses to make an image look bigger

- Eyepiece, Objective lens, Light source, Diaphragm
- **Diaphragm** gathers light on object,
- **Objective lens** creates real inverted image of object, which again acts as object for eye piece.
- **Eye Piece** creates a virtual inverted and magnified image

Applications

- Viewing samples at high magnification (40 - 1000x)
- Used for medical diagnosis, in visualizing tissue or cell samples

Bioreactor



Principle:

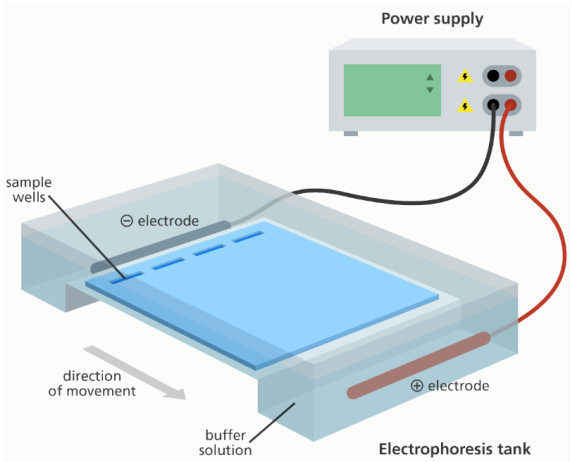
- The bioreactor is the heart of any biochemical process as it provides an environment for microorganisms to obtain optimal growth.
- The reactors can be engineered or manufactured based on the growth requirements of the organisms used.

Applications:

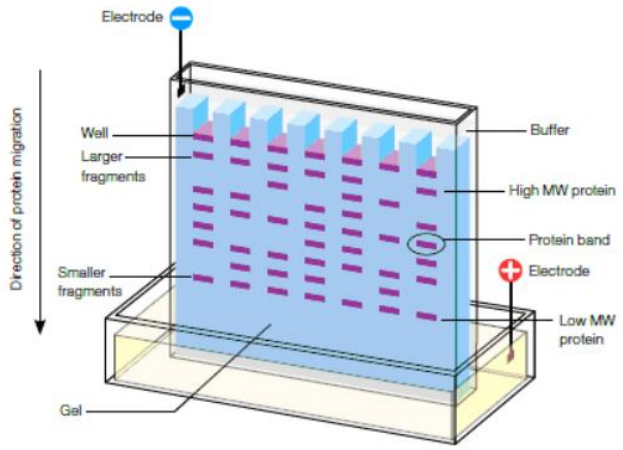
- Production of antibiotics, organic acids, enzymes
- Waste water treatment

Electrophoresis Unit

AGAROSE GEL



SDS-PAGE GEL



Principle: *Migration and separation of charged particles (ions) under the influence of an electric field*.

- On application of electric charge, each molecule having different size and charge will move through the gel at different speeds. The porous gel used in this technique acts as a molecular sieve that separates bigger molecules from the smaller ones.
- Components: consists of a gel (agar/polyacrylamide), electrophoretic chamber with a cathode (negative terminal) at one end and an anode (positive terminal) at the opposite end.
- Types : Agarose and SDS-PAGE

Applications:

- Separate DNA, RNA or protein molecules based on their size and electrical charge.
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting

Incubator

Principle:

- All incubators work based on a simple principle that microorganisms require an optimum environment for their growth and development. An incubator provides an optimum level of temperature, humidity, oxygen, and carbon dioxide so that the microorganism can multiply and increase their numbers.

- Incubator contain a thermostat which maintains the inside temperature of the incubator. This temperature is monitored outside via the thermometer. By utilizing the heating and no-heating cycles the inside temperature of the incubator is maintained.

Applications:

- It promotes the growth of micro-organisms by providing a favorable environment.

Hot Air Oven

Principle:

- Hot air ovens work based on the principle of dry heat sterilization, where hot air is used to kill microorganisms and eliminate contaminants from various items and materials.
- The hot air circulates within the oven, ensuring even distribution of heat, which helps achieve a consistent sterilization process throughout the load.
- The temperature in a hot air oven typically ranges from 50°C to 300°C.

Applications:

- Used in scientific and research laboratories to sterilize glassware and other laboratory equipment, preventing cross-contamination between experiments.

Laminar Flow Hood

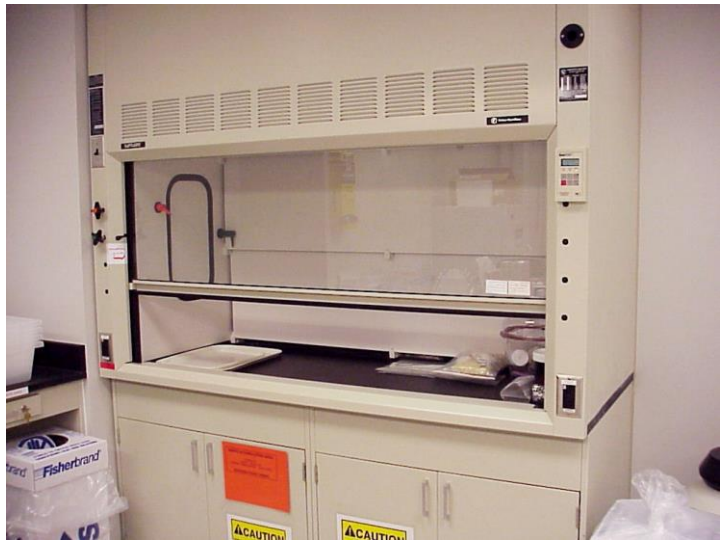
Principle:

- A laminar hood consists of a filter pad, a fan and a HEPA (High Efficiency Particulate Air) filter
- The fan sucks the air through the filter pad where dust is trapped.
- After that the prefiltered air passes through the HEPA filter which removes all airborne contamination to maintain sterile conditions.

Applications:

- Used in laboratories to prevent contamination of cultures, media, and samples by providing a clean workspace.

Fumehood



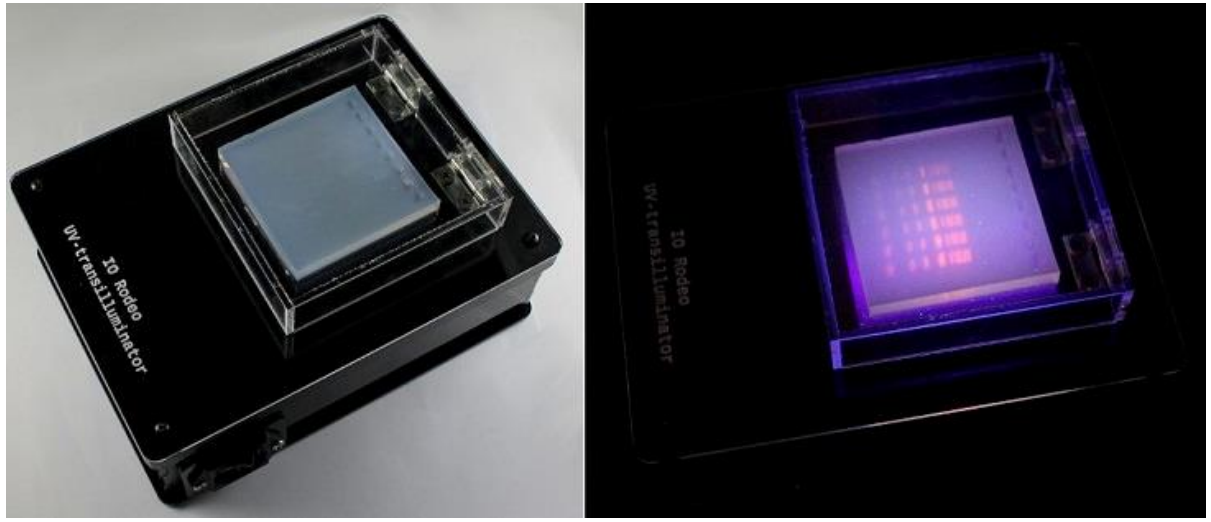
Principle:

- A fume hood is a ventilated enclosure in which gases, vapors and fumes are captured and removed from the work area.
- An exhaust fan situated on the top, pulls air and airborne contaminants through connected ductwork and exhausts them to the atmosphere.
- It is designed to ensure safety for the user and other inhabitants of the room, and it should be able to filter out any harmful gasses.

Applications:

- Fumehood has the ability to strongly suck the gasses out of the working area within the enclosure.
- For usage of chemicals which gives out strong, toxic fumes.

UV Transilluminator



Principle:

- UV transilluminators are used in molecular biology laboratories to view DNA or RNA that has been separated by electrophoresis through an agarose gel.
- Exposing the stained gel to a UV light source causes the dye (commonly ethidium bromide) bound to DNA to fluoresce and become visible.

Applications:

- This instrument is useful for viewing samples to size a PCR product, purify DNA segments after a restriction enzyme digest, quantify DNA, or verify RNA integrity after extraction

IMPORTANT NOTE

DO NOT USE ANY MACHINE
IF YOU DO NOT KNOW HOW TO USE/OPERATE