

Practical: Quantitative estimation of proteins in legumes.

Quantitative estimation of proteins in legumes is commonly achieved through colorimetric assays such as the Lowry Method or the Bradford Assay, alongside the standard Kjeldahl Method used for total nitrogen/crude protein calculations.

Below is the standard, lab-ready experimental protocol optimized for estimating soluble protein content in pulse/legume seeds using the spectrophotometrically Folin-Lowry approach.

1. Principle

The Lowry method combines the Biuret reaction (where copper ions react with peptide bonds under alkaline conditions) with the reduction of the Folin-Ciocalteu phosphomolybdic-phosphotungstic acid reagent by aromatic amino acids (tyrosine and tryptophan). This yields an intense blue-colored complex measurable at **660 nm**.

2. Reagents Required

- **Extraction Buffer:** 0.1M Phosphate buffer (pH 7.4) or 0.1N NaOH.
- **Standard Protein Solution:** Bovine Serum Albumin (BSA) at 200 µg/mL.
- **Reagent A:** 2% Na₂CO₃ in 0.1N NaOH.
- **Reagent B:** 0.5% CuSO₄·5H₂O in 1% Potassium Sodium Tartrate.
- **Reagent C (Alkaline Copper Solution):** Mix 50 mL of Reagent A with 1 mL of Reagent B right before use.
- **Reagent D (Folin-Ciocalteu Reagent):** Dilute commercial Folin reagent with distilled water in a 1:1 ratio prior to the assay.

3. Step-by-Step Procedure

Phase A: Protein Extraction from Legumes

1. **Grinding:** Dehull your chosen legume seeds (e.g., mung bean, chickpea, or lentil) and grind them into a fine flour using a mortar and pestle or lab mill.
2. **Homogenization:** Weigh **0.5 g** of the flour and homogenize it thoroughly in 10–20 mL of your extraction buffer.
3. **Centrifugation:** Centrifuge the slurry at **5,000–10,000 RPM** for 15 minutes.
4. **Collection:** Decant and collect the clear supernatant (crude protein extract). If the extract is highly concentrated, dilute it appropriately with distilled water.

Phase B: Assay Protocol

1. **Standard Preparation:** Set up 6 clean test tubes and pipette volumes of working BSA standard (0.2, 0.4, 0.6, 0.8, and 1.0 mL) into them.
2. **Sample Preparation:** Add **0.1 mL** or **0.2 mL** of your legume protein extract to an "Unknown" test tube.

3. **Volume Equalization:** Bring the volume of all tubes (standards, blanks, and unknowns) up to exactly **1.0 mL** using distilled water.
4. **Alkaline Incubation:** Add **5.0 mL of Reagent C** to all tubes. Mix well and let them rest at room temperature for **10 minutes**.
5. **Color Development:** Rapidly add **0.5 mL of Reagent D** while vortexing/shaking immediately. Incubate in the dark at room temperature for **30 minutes**.
6. **Spectrophotometry:** Calibrate the instrument with the blank tube to 0 absorbance, then record the optical density (OD) of all samples at **660 nm**.

4. Calculations & Standard Curve

Plot a graph featuring the absorbance values on the Y-axis against the known concentrations of BSA standard (μg) on the X-axis to generate your standard calibration curve.

Locate the absorbance value of your legume sample on the curve to deduce the exact protein concentration:

$$\text{Protein Content \%} = \frac{\text{Concentration from Curve (}\mu\text{g)} \times \text{Total Volume of Extract (mL)}}{\text{Volume of Extract Used (mL)} \times \text{Weight of Sample (g)}} \times 10^6$$

×100

Preparation of standard dilutions:

Setting up the Standard Curve Dilutions

Prepare a series of standard tubes using a BSA working stock solution (100 $\mu\text{g/mL}$). Dilute each to a final volume of 1.0 mL with distilled water:

- Tube 1 (Blank): 0.0 mL BSA + 1.0 mL Water – 0 μg Protein
- Tube 2: 0.2 mL BSA + 0.8 mL Water - 20 μg Protein
- Tube 3: 0.4 mL BSA + 0.6 mL Water – 40 μg Protein
- Tube 4: 0.6 mL BSA + 0.4 mL Water - 60 μg Protein
- Tube 5: 0.8 mL BSA + 0.2 mL Water - 80 μg Protein
- Tube 6: 1.0 mL BSA + 0.0 mL Water - 100 μg Protein
- Tube 7 (Legume Sample): 0.1 mL Unknown Extract + 0.9 mL Water

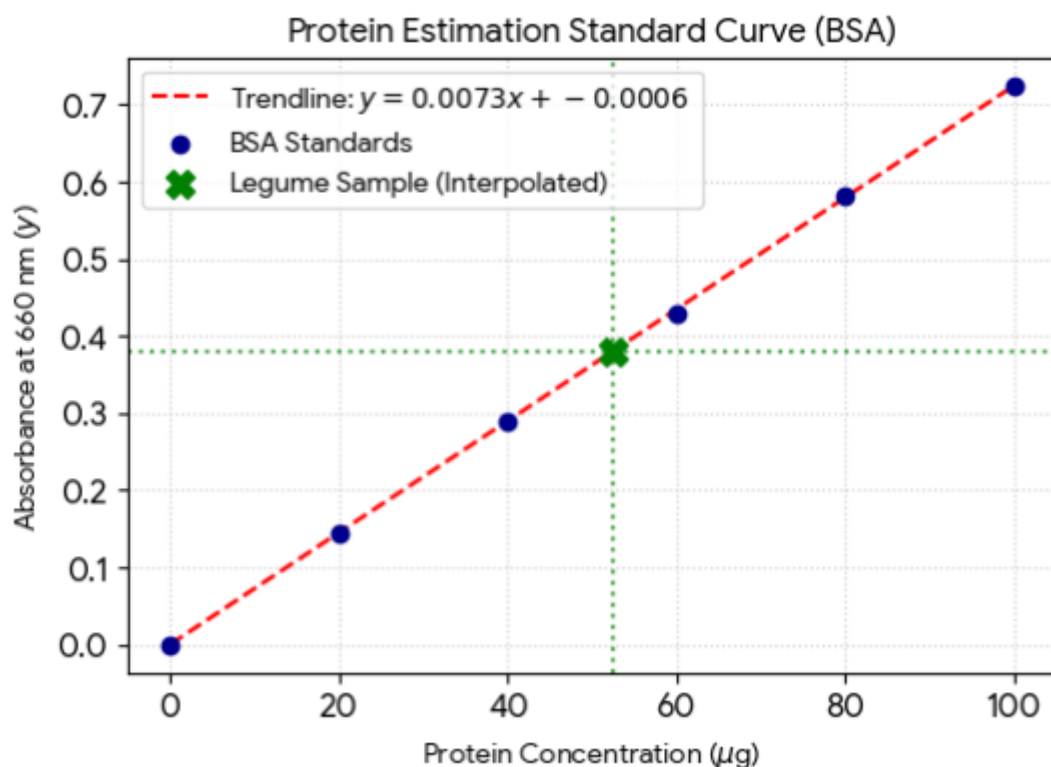
Step C: Color Development & Measurement

1. Add 5.0 mL of Alkaline Copper Reagent to all tubes. Mix well and incubate at room temperature for 10 minutes.
2. Rapidly add 0.5 mL of Folin-Ciocalteu Reagent to each tube while vortexing immediately.
3. Incubate in the dark for 30 minutes at room temperature to allow complete color progression.
4. Calibrate the spectrophotometer to zero using the Blank (Tube 1). Measure and record the absorbance (Optical Density) at 660 nm for all tubes.

3. Data Analysis & Standard Curve Generation

The concentration of protein is calculated by plotting a scatter plot using the standard values:

- X-axis: Concentration of BSA (μg)
- Y-axis: Absorbance at 660 nm



Finding the Unknown Concentration via Equation:

From the trendline generated by the standards $y = mx + c$

Protein Concentration(x) = $(y - c) / m$

Where:

- y = Absorbance of your legume sample
- m = Slope of the line
- c = Y-intercept (ideally close to 0)

Practical: Extraction of Essential oils from aromatic plants.

Experimental Procedure: Essential oil extraction from aromatic leaves using a Clevenger apparatus is performed via hydro distillation, where plant material is boiled in water and the vaporized volatile oils are condensed, separated, and collected

1. Prepare the Plant Material:

- Wash the aromatic leaves gently and cut or chop them into smaller uniform pieces (approx. 10–20 mm) to help release the volatile compounds. Weigh out 100 g of the prepared leaves.

2. Assemble the Setup:

- Place 500 mL to 800 mL of distilled water into a clean, appropriately sized round-bottom flask (e.g., a 2-liter flask).
- Add the weighed leaves directly into the water inside the flask.
- Mount the Clevenger-type receiving tube on top of the round-bottom flask, and attach the water-cooled reflux condenser on top of the Clevenger unit. Connect the rubber tubing for circulating cold tap water through the condenser.

3. Perform Hydro distillation:

- Turn on the heating mantle to heat the flask contents to a steady boil.

- As the water boils, the generated steam carries the vaporized essential oil compounds upward into the condenser.
- The mixture of water vapor and oil condenses and flows down into the graduated arm of the Clevenger apparatus.
- Excess condensed water automatically flows back (returns) into the boiling flask via the side return arm of the apparatus.
- Continue the distillation process for about 2 to 3 hours until no more distinct oil layer increases in the collection tube.

4. Collect and Recover the Essential Oil:

- Turn off the heating mantle and allow the apparatus to cool down safely to room temperature.
- Note that two distinct phases will be visible in the Clevenger tube: an aqueous lower phase (aromatic water/hydrosol) and a clear, less dense organic upper phase (essential oil).
- Drain or pipette out the upper essential oil layer carefully into a clean micro-vial.

5. Dry and Store:

- Add a tiny pinch of anhydrous sodium sulfate (Na_2SO_4) to the isolated essential oil to remove any residual trace moisture.
- Transfer the purified oil into a sealed, dark amber glass vial.
- Store the vial in a refrigerator at around 4°C protected from light to prevent chemical degradation.

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Practical: To study density and frequency of different plant species using quadrat method.

Principle: Density represents the numerical strength of a certain plant species in the community per unit area. The number of individuals of the species in any unit area is its density. The unit area may be as small as 5 square cm to as large as 10 square metre depending on the size and nature of the plant community under study. For herbaceous vegetation a metre square quadrat is normally used. Density which gives an idea of degree of competition is calculated as follows.

Total number of individual(s) of the species in all the sampling unit (S) Density= Total number of sampling units studied (Q) The value thus obtained is then expressed as number of individuals per unit area. When the measured unit area is divided by the number of individuals the average area occupied by each individual is obtained.

Requirement: Cotton/nylon thread (five meters), 4 nails and a hammer

Procedure

- In the selected site of study, make a 1 m X 1 m quadrat with the help of nails and thread. Hammer the nails firmly and make sure that the vegetation is not damaged while laying the quadrat.
- List the names of the plant species seen in the quadrat (if the name is not known mark these as species A or B etc., and the same species if seen in other quadrats assign the same alphabet).
- Count the number of individuals of each species present in the quadrat and record the data as shown in the table.
- Similarly make nine more quadrats randomly in the site of study and record the names and number of individuals of each species.

Observations: Record the total number of species seen in the ten quadrats. This will give an idea about the composition of the vegetation. There will be difference in the species composition in the quadrats made in shady areas, exposed areas with bright sunlight, dry or wet areas etc.

Table

Plant Species	Quadrats employed in study & no. of individuals in each quadrat										Total No. of individuals (S)	Total no. of Quadrats studied (Q)	Density (D)
	I	II	III	IV	V	VI	VII	VIII	IX	X			
A	2			5		7		10		3	27	10	$27/10 = 2.7$
Z	1	2	4	8	3		2				20	10	$20/10 = 2.0$

Discussion: Plants growing together exhibit mutual relationships among themselves and also with the environment. Such a group of plants in an area represent a community. The number of individuals of a species varies from place to place, making it necessary to take many random sample areas for reliable results. Density values are significant because they show relative importance of each species. With increasing density, the competition stress increases and the same is reflected in poor growth and lower reproductive capacity of the species. Data on population density are often very essential in measuring the effects of reseedling, burning, spraying and successional changes. Discuss the vegetation composition of the area (herbs/shrubs) and comment on the dominant component species.

Frequency calculation by quadrat method:

Frequency is concerned with the degree of uniformity of the occurrence of individuals of a species within a plant community. It is measured by noting the presence of a species in random sample areas (quadrats) which are distributed as widely as possible throughout the area of study. Frequency is the number of sampling units (as %) in which a particular species (A) occurs. The frequency of each species (sps. A or sps. B or sps. X etc) is expressed in percentage and is calculated as follows. % Frequency or Number of sampling units (quadrats) in which the species occurs = $\frac{\text{Frequency Index}}{\text{Total number of sampling units (quadrats) employed for the study}}$

Requirements: Cotton/nylon thread of 5 metres, 4 nails and a hammer

Procedure

(i) In the selected site of study, make a 1 m X 1 m quadrat with the help of nails and thread. Hammer the nails firmly and make sure that the vegetation is not damaged while laying the quadrat.

(ii) List the names of the plant species seen in the quadrat (if the name is not known mark these as species A or B etc. and if the same species is seen in other quadrats assign the same alphabet)

(iii) Similarly lay nine more quadrats randomly in the site of study and record the names of individuals of each species.

(iv) Calculate the percentage frequency of occurrence using the formula given.

Observations:

Record the total number of species seen in the ten quadrats. This will give an idea about the composition of the vegetation. There will be difference in the species composition in the quadrats made in shady areas, exposed areas with bright sunlight, dry or wet areas etc. Observe that the frequency of occurrence is not the same for all species

Table

Table 24.1: Frequency studies for the given vegetation

Plant Species	Number of quadrats employed in the study (Q)										No. of quadrats in which the species is present (N)	Percentage of frequency $F = N/Q \times 100$
	I	II	III	IV	V	VI	VII	VIII	IX	X		
A	√		√	√			√			√	5	$5/10 \times 100 = 50\%$
B		√									1	$1/10 \times 100 = 10\%$
C					√	√	√		√		4	$4/10 \times 100 = 40\%$

Discussion:

Variation in distribution of a species is caused by factors like soil conditions, quantity and dispersal of gemmules, vegetative propagation, grazing, predation, diseases and other biotic activities. Also, frequency values differ in different communities. They are influenced by micro-habitat conditions, topography, soil and many other environmental characteristics. Thus, unless frequency is not correlated with other characters such as density, frequency alone does not give correct idea of the distribution of a species. Frequency determinations by means of sample areas are often needed in order to check general impressions about the relative values of species. Many species having low cover or population density also rate low in frequency, but some may have high frequency because of their uniform distribution. Usually if the cover and population density are high, the frequency will be high. The plants with high frequency are wide in distribution.

Practical: To study the effect of temperature and pH on microbial growth of microorganisms.

Objective

Determine how different temperatures and pH levels affect the growth rate and survival of a chosen test microorganism (such as *Escherichia coli* or *Saccharomyces cerevisiae*).

Identify cardinal values, including the minimum, optimum, and maximum limits for growth.

Materials

Active microbial broth culture (*E. coli*, yeast, or similar)

Nutrient broth or growth medium

Buffer solutions or acid/base adjusters to prepare media at specific pH levels (e.g., pH 3, 5, 7, and 9)

Test tubes and racks

Incubators or water baths set to specific temperatures (e.g., 4°C, 25°C, 37°C, 50°C)

Spectrophotometer to measure optical density OD 600 or turbidity

Experimental Procedure

Part 1: Effect of Temperature

1. Label sterile nutrient broth tubes with the test organism name and target temperatures (e.g., 5°C, 25°C, 37°C, 50°C).

2. Inoculate each tube with an equal volume of the active microbial culture.

3. Incubate each designated tube at its set temperature for 24 to 48 hours.
4. Measure growth by recording the turbidity or optical density at 600 nm using a spectrophotometer. Higher absorbance indicates more growth.

Part 2: Effect of pH

1. Prepare nutrient broth media adjusted to distinct pH levels, typically pH 3 (acidic), pH 5, pH 7 (neutral), and pH 9 (alkaline).
2. Inoculate each pH-adjusted tube with an equal amount of the test microorganism.
3. Incubate all tubes at the organism's optimal temperature (e.g., 37°C for *E. coli*) for 24 hours.
4. Measure the resulting growth via optical density or visual turbidity grading (clear vs. cloudy)

Expected Results & Classification

- Temperature responses: Microbes perform best at an optimum temperature. Mesophiles thrive near 37°C, psychrophiles in the cold (0–15°C), and thermophiles at elevated heat (>50°C). Extreme deviations denature proteins and halt growth.
- pH responses: Neutrophiles prefer a pH near 7.0, acidophiles prefer acidic conditions approx 3.0, and alkaliphiles prefer basic conditions (8.0–10.5). Extreme pH levels disrupt cellular membrane potentials and enzyme structures.

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Practical: To demonstrate the technique of Microtomy.

Microtomy is the process of cutting extremely thin slices of tissue using a mechanical instrument called a rotary microtome for microscopic examination

Objective

To demonstrate the technique of microtomy by sectioning a paraffin-embedded tissue block and mounting the sections onto a glass slide.

Materials Needed

- Rotary microtome with a sharp steel or disposable blade
- Paraffin wax-embedded tissue block
- Water bath (maintained around 37°C–40°C)
- Ice tray or cooling plate
- Clean glass microscope slides
- Fine paintbrush and teasing needle

Step-by-Step Procedure

1. Preparation and Cooling

- Chill the paraffin wax tissue block on an ice tray or cooling plate. Cold blocks provide the firmness needed to prevent section compression or tearing.
- Secure the chilled tissue block firmly into the microtome chuck holder.

2. Facing the Block

- Set the microtome to the trimming mode (usually set to a thickness of 10 to 20 microns).
- Carefully advance the blade to face (level) the block until the target tissue surface is fully exposed and flat.

3. Sectioning

- Switch the microtome to sectioning feed mode, adjusting the thickness setting to 4 to 5 microns for routine histological observation.
- Turn the hand wheel smoothly and at a steady rhythm to produce a continuous ribbon of paraffin sections.
- Use a fine paintbrush to gently catch and support the emerging ribbon.

4. Floating and Flattening

- Separate individual sections or short ribbons using a teasing needle.
- Transfer the sections onto the surface of a warm water bath. The warmth melts out any minor wrinkles or folds in the paraffin.

5. Mounting

- Dip a clean glass microscope slide into the water bath underneath the flattened tissue section.
- Carefully lift the slide upward, trapping the tissue section flat against the glass surface.
- Drain excess water and place the slide on a warm slide warmer or hot plate overnight to dry and secure the tissue.
- If you would like, I can detail the staining process (like H&E staining) or explain common troubleshooting issues during microtomy like ribbon curving or crumpling.

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Practical: To estimate the total nitrogen content in a given plant assay by the Kjeldahl Method.

Principle:

The organic nitrogen present in the plant tissue is converted into ammonium sulfate by digesting the sample with concentrated sulfuric acid in the presence of a catalyst. The digested solution is then made alkaline with sodium hydroxide, releasing ammonia gas, which is distilled and trapped in a standard acid or boric acid solution. Finally, the amount of ammonia is quantified via acid-base titration

Requirements:

Apparatus: Kjeldahl digestion flask (or digestion tubes), distillation apparatus, burette, pipette, conical flask, analytical balance, and heating mantle or digestion block.

Chemicals/Reagents:

Concentrated sulfuric acid H_2SO_4

Digestion catalyst mixture (e.g., Potassium sulfate K_2SO_4 and Copper sulfate CuSO_4 in ratio ~9:1; K_2SO_4 raises the boiling point of H_2SO_4)

Sodium hydroxide NaOH , 40% w/v)

Standard hydrochloric acid (HCl) or Sulfuric acid H_2SO_4 (e.g., 0.1 N)

Boric acid solution (3%) with mixed indicator (methyl red and bromocresol green)

Procedure:

Step 1: Sample Preparation and Digestion

1. Weigh accurately about 0.2 g to 0.5 g of finely powdered, oven-dried plant material and transfer it into a clean Kjeldahl flask.
2. Add 2.5 g to 5 g of the catalyst mixture $\text{K}_2\text{SO}_4 + \text{CuSO}_4$ and 10–15 mL of concentrated H_2SO_4
3. Place the flask on a digestion rack/heater. Heat gently initially until frothing stops, then increase the heat until the mixture boils steadily.
4. Continue heating until the solution turns clear or pale green/blue, indicating complete oxidation of organic matter.
5. Allow the digest to cool, and then slowly dilute it with a small amount of distilled water.

Step 2: Distillation

1. Transfer the cooled digest quantitatively into a round-bottom distillation flask and set up the steam distillation unit.

2. Place a conical flask containing 25 mL of 3% boric acid with a few drops of mixed indicator under the condenser outlet (ensure the tip of the condenser dips into the boric acid solution).

3. Carefully add excess 40% NaOH solution through the funnel into the distillation flask until the mixture is strongly alkaline.

4. Heat/steam-distil the contents. The liberated ammonia gas passes into the condenser and is absorbed by the boric acid solution, changing its color (usually from pink/purple to green). Collect about 50–70 mL of distillate.

Step 3: Titration

1. Titrate the green distillate against standard 0.1 N HCl (or H₂SO₄) until the original color returns (sharp transition back to pink/pale purple endpoint).

2. Record the volume of acid used. Run a blank titration simultaneously using all reagents without the plant sample to account for any trace nitrogen impurities.

Calculation

% Nitrogen = $\frac{V_{\text{sample}} - V_{\text{blank}} \times \text{Normality of acid} \times 0.014 \times 100}{\text{Weight of plant sample (gm)}}$

(Where 1 mL 1N HCl equiv 0.014 g of Nitrogen)

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Practical: Isolation of Biomolecules.

Required Materials

- **Biological sample:** Strawberries or chopped onions work best for high yields.
- **Lysis buffer:** 100 mL water, 10 mL liquid dish soap (breaks down lipid membranes), and 1 teaspoon of table salt (neutralizes DNA charges so it clumps).
- **Precipitation agent:** Ice-cold 91% or 99% isopropyl alcohol or ethanol.
- **Equipment:** Blender or mortar and pestle, coffee filter or cheesecloth, funnels, test tubes or clear glasses, and a wooden skewer or glass rod.

Step-by-Step Procedure

[Mechanical Breakdown] → [Chemical Lysis] → [Filtration] → [Alcohol Precipitation]

1. Mechanical Breakdown

- Mash the strawberries in a plastic bag or blend the chopped onion with a splash of water.
- Purpose: Breaks the rigid plant cell walls.

2. Cellular Lysis

- Add 2 space-tablespoons of the **lysis buffer** to the mashed sample. Mix gently for 5–10 minutes. Avoid making too many bubbles.
- Purpose: The soap dissolves the cell and nuclear membranes, releasing the DNA. The salt helps the DNA strands come together.

3. Filtration

- Pour the mixture through a coffee filter or cheesecloth into a clean glass or test tube.
- Purpose: Removes the large cellular debris, cell walls, and un-lysed tissue, leaving a clear liquid (filtrate) containing the DNA.

4. Precipitation

- Tilt the glass and slowly pour an equal volume of **ice-cold alcohol** down the side so it forms a distinct layer on top of the filtrate. Do not mix.
- Purpose: DNA is insoluble in cold alcohol. It will precipitate out of the aqueous solution at the interface of the two liquids.

Observation & Result

Within a few minutes, you will see a **white, stringy, snot-like substance** rise into the alcohol layer. This is a collection of thousands of genomic DNA strands clumped together. You can spool it out using a wooden skewer or glass rod.

Practical: To measure the size of pollen grain using Micrometry.

The size of a microscopic spore is measured in a laboratory experiment using Micrometry: Calibration and Measurement Guide through a calibrated system combining a stage micrometer and an ocular micrometer.

Materials Required

- Light compound microscope
- Ocular micrometer (an eyepiece glass disc with arbitrary etched scale divisions)
- Stage micrometer (a special glass slide with a standardized fine scale, typically 0.01 mm or 10 μm per division)
- Slide containing the spore sample (such as a mushroom spore print suspension)

Experimental Procedure**Step 1: Install the Ocular Micrometer**

- Unscrew the upper lens of the microscope eyepiece.
- Place the circular ocular micrometer disc carefully onto the internal shelf of the eyepiece, etched side down/visible, and reassemble.

Step 2: Calibrate the Ocular Scale

Because the spacing of the ocular divisions changes depending on the magnification, you must calibrate it against the known standard scale of the stage micrometer for each objective lens (e.g., 40x or 100x oil immersion):

- Place the stage micrometer slide on the microscope stage.
- Focus using the target objective lens.
- Align the lines of the ocular micrometer scale with the lines of the stage micrometer scale by moving the mechanical stage or rotating the eyepiece.
- Note the number of overlapping ocular divisions (OD) and stage divisions (SD).
- Calculate the calibration factor (CF) for that specific objective lens using the formula:
Calibration Factor (CF) = $\frac{\text{Number of stage divisions} \times 10\mu\text{m}}{\text{Number of ocular divisions}}$

Step 3: Measure the Spore Size

- Remove the stage micrometer slide and replace it with your spore sample slide.
- Locate mature, well-dispersed spores under the same objective lens used during calibration.
- Align the ocular scale with the length, width, or diameter of a chosen spore.
- Count the number of ocular micrometer divisions (N) covered by the spore.
 - Calculate the absolute actual size of the spore:
 $\text{Actual Spore Size} = N \times \text{CF}$
- Repeat the measurement for at least three to ten mature spores to find an accurate average size range.

Practical: To make a Comparative account of reproductive Organs in *Riccia*, *Marchantia*, and *Funaria***1. Purpose & Scope**

This Standard Operating Procedure (SOP) provides detailed instructions for preparing, observing, and comparing the male (Antheridia) and female (Archegonia) reproductive systems of three representative bryophytes: *Riccia*, *Marchantia*, and *Funaria*. This document

is written in accessible language to guide laboratory personnel and students through proper sample handling, slide sectioning, and morphological analysis.

2. Required Laboratory Equipment

- **Specimens:** Plant thalli/gametophores of *Riccia*, *Marchantia*, and *Funaria* bearing reproductive structures.
- **Instruments:** Fine dissection needles, forceps, fresh single-edge razor blades, camel-hair brush.
- **Optics & Glass:** Compound light microscope, clean glass slides, coverslips.
- **Reagents:** Glycerin (10%), Safranin or Fast Green stain, distilled water.

3. Step-by-Step Experimental Procedure

Step 3.1: Identifying Reproductive Zones

- **Riccia:** Locate the dorsal midline furrow of the mature thallus where reproductive organs are embedded inside the tissue.
- **Marchantia:** Identify upright stalked structures called umbrella-like discs. Disk with scalloped margins indicates an Antheridiophore (male); star-like rays indicate an Archegoniophore (female).
- **Funaria:** Locate leafy shoots. The male shoot displays a flat, flower-like rosette of leaves at the top. The female shoot forms a protective, closed bud structure.

Step 3.2: Micro-Sectioning & Slide Preparation

1. Place the selected plant tissue target zone between a split piece of potato pith or hold it firmly flat on a cutting block.
2. Use a wet razor blade to slice exceptionally thin Vertical Longitudinal Sections (VLS) or Vertical Transverse Sections (VTS).
3. Use a moist camel-hair brush to gently transfer the thinnest transparent sections into a watch glass containing clean water.
4. Add 1-2 drops of Safranin stain, leave for 1 minute, and rinse out excess stain with water.
5. Mount the stained slice on a clean glass slide in a drop of dilute glycerin. Carefully lower a coverslip at a 45-degree angle to completely avoid trapping air bubbles.

Step 3.3: Microscopic Examination

Focus under low power (10x magnification) to navigate the primary tissues, then switch to high power (40x) to observe individual cell walls, sterile jackets, and egg/sperm chambers.

4. Comparative Data Reference Matrix

Use this standard reference matrix to verify morphological landmarks under the microscope and classify the structural evolution of the specimens.

Feature / Organ	<i>Riccia</i> (Liverwort)	<i>Marchantia</i> (Liverwort)	<i>Funaria</i> (Moss)
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Plant Body Type	Simple prostrate, dichotomously branched thallus.	Complex prostrate thallus with internal air chambers.	Erect leafy gametophore with stem-like axis and leaves.
Organ Distribution	Monoecious or Dioecious; scattered singly along the dorsal midline furrow.	Strictly Dioecious; localized on specialized vertical stalks.	Monoecious but Autoecious (male and female branches separate on same plant).
Organ Positioning			
Antheridium Structure	Deeply embedded inside the thallus tissue, opening via pore. Ovoid body with a short, simple stalk; single-layered sterile jacket.	Borne on receptacles: Antheridia on upper surface of disc; Archegonia on lower surface. Club-shaped body with a short stalk; multicellular jacket enclosing spermatocytes.	Superficial; clustered at the apex of the leafy stem protected by perichaetial leaves. Club-shaped body with a long multicellular stalk and a distinct operculum (cap cell).
Archegonium Structure	Flask-shaped; nearly sits directly in tissue. Neck has 4 vertical rows of cells; 4 neck canal cells.	Flask-shaped; suspended upside down. Neck has 6 vertical rows of cells; 4-6 neck canal cells.	Flask-shaped with a heavy multicellular stalk. Long twisted neck with 6 rows; 6 or more neck canal cells.
Sterile Appendages	Completely absent within the thallus tissue.	Absent inside the receptacles.	Present: Multicellular uniseriate sterile hairs (Paraphyses) mixed with organs.

5. Safety & Handling Prerequisites

- **Sharp Hazard:** Razor blades used for free-hand sectioning must be handled with care. Always cut away from your fingers.
- **Staining Safety:** Wear gloves when using Fast Green or Safranin stains to prevent skin discoloration.

Specimen Integrity: Fresh material should be kept moist in a petri dish prior to dissection. Fixed material (in FAA solution) must be rinsed thoroughly in water before sectioning.

Practical: To study and count the number of bacterial colonies using colony counter.

The Principle of Colony Counting

The method works on the biological rule that each viable bacterial cell present in a sample remains physically separated when spread across a solid nutrient agar surface. During incubation, this single parent cell undergoes exponential binary fission, eventually forming a visible, distinct cluster called a Colony Forming Unit (CFU).

A digital colony counter eliminates human tracking errors (like eye strain, skipping colonies, or double counting) through three coupled mechanisms:

- **Pressure-Sensitive Platform:** The plate sits on a spring-loaded, inverted translucent grid. Slight downward pressure from a probe or marker compresses a micro-switch underneath.
- **Electronic Ledger:** Every compression increments the built-in system tally by exactly **one (+1)**, instantly modifying the digital LED screen while emitting an audible verification beep.
- **Wolffhuegel Grid Architecture:** The stage features a defined square grid pattern. This lets the user scan the plate systematically from quadrant to quadrant, preventing structural counting drift.

MATERIALS AND EQUIPMENT

- Incubated agar Petri dishes containing target bacterial populations (ideal dense threshold: 30 to 300 colonies).
- Electronic pressure-sensor digital colony counter system.
- Conductive electronic sensor probe or standard contrasting permanent felt-tip marker pen.
- Surface disinfectant solution (70% v/v Isopropyl Alcohol or Ethanol) paired with lint-free wipes.

STEP-BY-STEP PROCEDURE

1. **Pre-Operational Sanitization** Dampen a clean wipe with 70% v/v alcohol. Thoroughly wipe the upper exterior surfaces, taking care not to pool moisture along the bezel borders. Inspect the power cable for physical wear, plug into the primary outlet, and toggle the power button to 'ON'. Verify sub-stage illumination illuminates the grid uniformly.
2. **System Reset Initialization** Depress the physical 'RESET' or 'CLEAR' push-button on the front interface panel. Check that the LED display panel rolls over to display an unambiguous, clean '000' reading before placing any samples down
3. **Sample Mounting & Contrast Selection**
Invert the incubated Petri dish so it rests **bottom-side up (agar side facing upward)** on the counting grid. Center it inside the circular metal alignment bracket. Toggle the background contrast selector (black/white background toggle) to match your colony morphology: use white contrast for dark chromogenic colonies and dark contrast for translucent, faint strains.
4. **Focus Adjustment** Swing the articulating flexible arm supporting the magnifying lens directly over the center of the sample dish. Slide the lens assembly vertically until the entire field of view shows sharp colony boundaries without geometric color fringing.
5. **Systematic Sector Counting Execution**
Hold the sensor marker pen perpendicular to the plate. Starting at the top-left quadrant of the Wolffhuegel grid, press the pen tip firmly against the plastic plate directly above a visible colony. Listen for the distinct audible 'beep' and verify that the numeric screen updates (+1). Move systematically across rows, marking each colony with an ink dot to visually lock it out from duplicate counts.
6. **Tally Verification & Logging** Scan the plate surface through the lens one last time to ensure every colony shows a corresponding ink dot. Lift the plate and transcribe the final absolute digit showing on the digital reader window straight into your laboratory workbook record.
7. **Post-Operational Shutdown** Remove the inked Petri dish. Click the reset button back to zero. Turn the primary power toggle to 'OFF', unplug the main line cord, wipe down the stage

clean of fingerprints using 70% alcohol, and swing the magnifier arm down into its safe storage profile.

MATHEMATICAL CALCULATIONS & DATA VALIDITY LIMITS

To convert raw numbers into standardized units, process the tally via the formula below:

CFU/mL (or CFU/g) = (Number of Colonies × Dilution Factor) / Volume of Inoculum Plated (mL)

Statistical Validity Threshold Rules:

- **Acceptable Range:** Only count plates displaying between **30 and 300 colonies** to ensure data reliability.
- **Too Few to Count (TFTC):** Tally values below 30 are statistically non-representative and vulnerable to disproportionate variance errors.
- **Too Numerous to Count (TNTC):** Tally values above 300 lead to colony overlap, under-counting, and nutrient exhaustion artifacts.

SAFETY AND QUALITY CONTROLS

- Never open the lids of the Petri dishes during the counting workflow to prevent environmental contamination and accidental pathogen inhalation.
- Treat all counted plates as active bio-hazards. Once recorded, stack them into an autoclave bio-bag and sterilize at 121°C for 15-20 minutes prior to final disposal.

Practical: To study the morphological and anatomical adaptations of xerophytes and hydrophytes.

Required Material & Equipment

- **Plant Specimens:**
 - **Xerophytes:** *Nerium* (oleander) leaves, *Casuarina* stems, or *Aloe vera* leaves.
 - **Hydrophytes:** *Hydrilla* stems, *Nymphaea* (water lily) petioles/leaves, or *Eichhornia* (water hyacinth) petioles.
- **Apparatus:** Compound light microscope, digital microscope camera (or smartphone adapter), fresh razor blades, watch glasses, fine paintbrushes, glass slides, coverslips, and droppers.
- **Reagents:** Safranin O stain (1%), Fast Green stain (0.5% optional for counterstaining), Glycerin (or water), and distilled water.

Step-by-Step Procedure

Step 1: Morphological Observation & Photography

1. **Examine the macroscopic features** of the given xerophyte and hydrophyte specimens.
2. **Document adaptations** such as leaf size, thickness, spine presence, cuticle texture, or spongy petiole structures.
3. **Capture high-resolution photographs** of the intact specimens using a digital camera or smartphone. Place a metric ruler next to the specimen for scale.

Step 2: Microscopic Section Cutting (Anatomy)

1. Hold the plant specimen (e.g., *Nerium* leaf or *Hydrilla* stem) firmly between your thumb and forefinger.
 - *Note: If the specimen is too thin, sandwich it inside a potato block (pith) before cutting.*
2. Wet the razor blade and the specimen with distilled water to reduce friction.

3. Cut extremely thin, transverse sections (T.S.) using a swift, smooth slicing motion toward yourself.
4. Transfer the cut sections immediately into a watch glass filled with distilled water using a fine paintbrush to prevent dehydration.

Step 3: Staining & Mounting

1. Select the thinnest, most translucent sections from the watch glass.
2. Transfer them into a watch glass containing Safranin O stain for 1–2 minutes.
3. Rinse the sections in clean distilled water to remove excess stain.
4. Place the section in the center of a clean glass slide.
5. Add a single drop of Glycerin (or water) over the section to prevent drying and clear the tissue.
6. Gently lower a coverslip at a 45-degree angle using a needle to avoid trapping air bubbles.
7. Blot away excess fluid from the edges of the coverslip using filter paper.

Step 4: Microscopic Observation & Photomicrography

1. Mount the slide onto the microscope stage.
2. Focus the tissue layer under low power (10x) to locate the best region of the section.
3. Switch to high power (40x) to observe specific cellular adaptations.
4. Align your digital camera or smartphone adapter with the microscope eyepiece.
5. Adjust the lighting (condenser/diaphragm) and capture clear photomicrographs of the key anatomical structures.

4. Key Anatomical Features to Identify

To ensure your practical report is accurate, look for and photograph the following diagnostic features:

Plant Type	Specimen	Key Anatomical Features to Document
Xerophyte	<i>Nerium</i> Leaf/ <i>Casuarina</i> Stem	• Thick, waxy cuticle • Multiple epidermal layers • Sunken stomata hidden in trichome-lined pits (sunken cavities) • Well-developed sclerenchyma (mechanical tissue)
Hydrophyte	<i>Hydrilla</i> / <i>Nymphaea</i> / <i>Eichhornia</i>	• Large, prominent air cavities (Aerenchyma) • Highly reduced, thin cuticle • Reduced or absent vascular tissues (especially xylem) • Astersclereids (star-shaped cells for mechanical support in water lily)

5. Data Recording & Reporting

- **Labeling:** Label every saved photograph with the specimen name, tissue section type (e.g., T.S.), and total magnification (e.g., 100x or 400x).
- **Analysis:** Create a comparative chart in your lab report contrasting how the observed internal structures help each plant manage water availability.

Safety Precautions

- Wear laboratory coats, gloves, and safety goggles.
- Handle razor blades and scalpels with extreme care during sectioning.
- Dispose of used blades in designated sharps containers.

- Handle chemical stains (*Safranin*, *Fast Green*) carefully to avoid skin and clothing stains.

Practical : To estimate the enzymes in given plant assay under stress.

Part A: Peroxidase Test

Principle:

Plants subjected to environmental stresses, such as temperature stress and oxidative stress, may accumulate reactive oxygen species (ROS). To minimize the harmful effects of ROS, plants activate antioxidant defense enzymes, particularly Catalase (CAT) and Peroxidase (POD). The activity of these enzymes can be evaluated by comparing stressed plant material with an untreated or control sample.

Requirements:

Potato tuber, knife, Petri dish, test tubes, test-tube stand, test-tube holder, cork borer, spirit lamp, hydrogen peroxide, beaker, 2%AlcoholicBenzidine solution, etc.

Procedure

1. Cut the potato tuber into small, thin pieces.
2. Place the potato pieces in a Petri dish containing water.
3. Select a few pieces and transfer them into two separate test tubes. Label them A and B.
4. Add a suitable quantity of water to both test tubes.
5. Keep test tube A undisturbed in the test-tube stand.
6. Boil the potato pieces present in test tube B.
7. Carefully remove the water from both test tubes.
8. Add sufficient 2% AlcoholicBenzidine solution to both test tubes to completely immerse the potato pieces.
9. Leave the test tubes undisturbed for approximately 10–15 minutes.
10. Remove the Benzidine solution from both test tubes.
11. Add a dilute solution of H_2O_2 to both test tubes and observe the colour changes.

Result

1. The potato pieces in test tube A develop a blue colour rapidly, indicating peroxidase activity.
2. The potato pieces in test tube B show no significant colour development, as boiling at high temperature denatures the Peroxidase enzyme and consequently inhibits its activity.

Conclusion

Peroxidase enzymes are widely distributed in plant tissues and catalyse the oxidation of various substrates, such as phenolic and amine compounds, using H_2O_2 as an electron acceptor. The development of blue colour therefore indicates the presence and activity of peroxidase in the plant tissue.

Part B: Catalase Test

Requirements:

Potato tuber, knife, Petri dish, test tubes, test-tube stand, test-tube holder, cork borer, spirit lamp, hydrogen peroxide, etc.

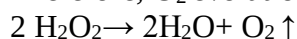
Procedure

1. Cut the potato tuber into small, thin pieces and place them in a Petri dish containing water.
2. Transfer a few potato pieces into two separate test tubes and label them A and B.
3. Add an appropriate quantity of water to both test tubes.
4. Keep test tube A undisturbed in the test-tube stand.
5. Boil the potato pieces present in test tube B.
6. Remove the water from both test tubes.
7. Add Hydrogen Peroxide solution, prepared by mixing 30 parts of water with 1 part of H_2O_2 , to both test tubes so that the potato pieces are completely immersed.
8. Observe and record the changes occurring in both test tubes.

Result

1. Test tube A: Formation of bubbles is observed, indicating the release of oxygen due to catalase activity.
2. Test tube B: No bubbles are produced because the high temperature during boiling denatures the Catalase enzyme, resulting in loss of enzyme activity.

Therefore, O_2 evolution is an indication of activity of enzyme Catalase



Practical: To demonstrate the phenomenon of a) Electrical adsorption b) Mechanical adsorption.

a) Electrical adsorption

Requirements : **Cellulose** (Whatman Filter Paper), Eosin Dye Methylene Blue, double distilled water, glass rod, tripod stand watch glass.

Principle : This experiment illustrates how electrostatic charges on a solid surface directs the selective attachment of charged dye molecules. Whatman filter paper develops a negative surface charge when immersed in water. Eosin Dye An acidic/anionic dye carrying a net negative charge. Methylene Blue is a basic/cationic dye carrying a net positive charge in solution.

Procedure :

1. Prepare a dilute aqueous solution of Eosin Dye (pink/red, anionic).
2. Prepare a dilute aqueous solution of Methylene Blue (blue, cationic)
3. Two equal sized long strips of Whatman filter paper are cut.
4. In one watch glass aqueous solution of Eosin Dye is poured and in other watch glass aqueous solution of Methylene Blue is poured.
5. Strips of Whatman filter paper are slightly moistened.
6. With the help of tripod stand one strip is hanged in watchglass with Eosin Dye and other strip is hanged in watchglass with Methylene Blue.

Observation:

1. After 10- 15 minutes eosin dye is observed to spread slowly on filter paper as compared to the strip dipped in Methylene Blue.

Result :

The affinity of negatively charged filter paper for positively charged Methylene Blue is more. Therefore electrical adsorption of positively charged methylene blue is faster than negatively charged eosin dye.

b) Mechanical adsorption

Requirements : Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), distilled water, soil sample (preferably clayey or loamy soil), Glass beaker (100 mL, 250 mL), glass funnel, filter paper (Whatman No. 1), glass rod, measuring cylinder, reagent bottles, weighing balance.

Procedure:

1. Take about 50 g of soil in a clean beaker.
2. Add 100 mL of distilled water to the soil and mix it well with a glass rod.
3. Prepare a dilute solution of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in a separate beaker.
4. Add the copper sulphate solution to the soil mixture and stir it properly.
5. Keep the mixture aside for about 15–20 minutes.
6. Filter the mixture through Whatman No. 1 filter paper using a glass funnel.
7. Collect the filtrate in a clean beaker.
8. Compare the colour of the filtrate with the original copper sulphate solution.

Observation:

The original copper sulphate solution is blue in colour. After mixing it with soil and filtering, the blue colour of the filtrate becomes lighter.

Result:

The soil retains some of the copper sulphate from the solution. This shows that soil has the ability to adsorb substances mechanically.

Precautions:

1. Mix the soil and copper sulphate solution properly.
2. Use clean filter paper while filtering.
3. Do not disturb the soil too much during filtration.

Practical: To isolate chloroplasts and mitochondria from fresh plant tissue using a homogenizer.

Principle:

Cellular organelles disintegrate when plant cells are gently homogenized in a cold separation buffer. After filtering, the homogenate is run through differential centrifugation. Due to variations in size and density, various organelles settle at different centrifugal forces. Being larger C

chloroplasts settle earlier. Since Mitochondria are smaller, Mitochondria need a larger centrifugal force.

Part A : Isolation of Chloroplasts:

Requirements: Fresh spinach leaves, Homogenizer, Cooling Centrifuge, Centrifuge tubes, Beakers, Measuring cylinder, Muslin cloth/nylon mesh, Glass rod, Pasteur pipette/dropper, Ice tray or ice bath

Reagents: Chilled isotonic isolation buffer (0.33 M sucrose buffer, supplemented with Tris-HCl, pH 7.5 and a small amount of EDTA), Distilled water

Procedure:

1. Take approximately 10–20 g of fresh spinach leaves washed thoroughly, cut into small pieces. And place in a chilled homogenizer.
2. Add an adequate volume of chilled isolation buffer.
3. Gently homogenize the plant tissue until a uniform green suspension is obtained, using short bursts (3 to 5 seconds at low/medium speed) to break rigid cell walls while minimizing damage to the organelles.
4. Filter the homogenate through 2–4 layers of chilled muslin cloth to remove large cell debris and unbroken tissue. Transfer the filtrate into centrifuge tubes.
5. Centrifuge at a low speed (1,000 rpm for 5–10 min at 4°C).
6. Transfer the supernatant to a fresh chilled tube and centrifuge the supernatant at a higher speed (3,000 rpm for 10 min at 4°C) so that sedimentation of chloroplasts is obtained.
7. After centrifugation green pellet containing crude chloroplasts is obtained.
8. Gently resuspend the pellet in a small volume of chilled isolation buffer.

Observation: A green pellet is obtained at the bottom of the centrifuge tube.

Result: Chloroplasts are isolated from fresh spinach leaves by homogenization followed by differential centrifugation.

Part B: Isolation of Mitochondria

Requirements: Fresh young/actively respiring plant tissue, Homogenizer, Centrifuge, Centrifuge tubes, Beakers, Measuring cylinder, Muslin cloth/nylon mesh, glass rod, Pasteur pipette/dropper, Ice tray or ice bath.

Reagents: chilled isotonic isolation buffer (0.25 M sucrose buffer supplemented with Tris-HCl, pH 7.5 and 1 mM EDTA), Distilled water

Procedure:

1. Take approximately 10–20 g of fresh spinach leaves washed thoroughly, cut into small pieces. And place in a chilled homogenizer.
2. Transfer plant tissue to a prechilled homogenizer, add an appropriate volume of cold mitochondrial isolation buffer.
3. Homogenize gently to release the cellular contents.
4. Filter the homogenate through chilled muslin cloth or nylon mesh and transfer to chilled centrifuge tubes. Centrifuge at low speed 1,000 rpm for 10 min at 4°C to remove cell debris and nuclei.
5. Transfer supernatant to a fresh tube. Centrifuge the supernatant at a higher speed i.e., 10,000 rpm for 15 min at 4°C to get sedimentation of Mitochondria.
6. Whitish or cream-coloured pellet is obtained. Gently resuspend the pellet in chilled isolation buffer. Repeat washing if a cleaner mitochondrial fraction is required.

Observation: A whitish/cream-coloured pellet is obtained.

Result: Mitochondria are isolated from fresh plant tissue by homogenization followed by differential centrifugation.

Precautions:

1. Fresh plant material should be used.
2. Plant tissues should be kept in chilled isolation buffer throughout the procedure.
3. Avoid prolonged homogenization of plant tissues.

Practical ; To separate the chlorophyll pigments from spinach leaves using column chromatography

Requirements:

Fresh spinach leaves, acetone, petroleum ether, silica gel or activated alumina, glass burette as the column, cotton or glass wool, mortar and pestle, beakers, test tubes, glass rod, measuring cylinder, filter paper and funnel.

Principle:

Different pigments present in spinach have different solubility and adsorption properties. When the pigment extract passes through a column containing silica gel or alumina, the pigments move at different rates and form separate coloured bands.

Procedure:

1. Take a few fresh spinach leaves and wash them with water.
2. Cut the leaves into small pieces and grind them in a mortar with a small amount of acetone.

3. Filter the extract to remove the leaf pieces.
4. Take a clean chromatography column (glass burette as the column) and place a small piece of cotton or glass wool at its bottom.
5. Fill the column carefully with silica gel or activated alumina.
6. Add the spinach extract slowly to the top of the column.
7. Allow the extract to pass into the column without disturbing the surface.
8. Add petroleum ether slowly as the solvent.
9. As the solvent moves down the column, different coloured pigment bands appear.
10. Collect the coloured fractions separately in test tubes.

Observation:

Different coloured bands are seen in the column. The pigments may appear as yellow, yellow-green, blue-green and green bands.

Result:

The photosynthetic pigments present in spinach are separated into different coloured bands by column chromatography.

Precautions:

1. Use fresh spinach leaves for the experiment.
2. Do not allow the column to become dry.
3. Allow the solvent to pass through the column slowly.