KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Pollachi Main Road, Eachanari Post, Coimbatore – 641 021. INDIA

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B.SC., BIOCHEMISTRY

19BCU112	CELL BIOLOGY PRACTICAL	3H-2C

Semester I

Instruction hours/week: L:0 T:0 P:4

Marks: Internal: **40** External: **60** Total: **100** **End Semester Exam:** 3 Hours

Course Objectives

- To train the students in the preparation of biological material for staining, staining procedure and visualization of stained slides
- To identify the different stages of cell division in a biological material
- To acquire knowledge in the techniques involved with the sub cellular fractionation of cell organelles

Course outcomes (CO's)

1. Able to prepare slides for staining procedure and visualization of materials
2. Interpret various stages of cell division
3. Understand the principle behind the sub cellular fractionation of organelles and the techniques involved.

Experiments

1. Preparation of onion root squash and observation of cell
2. Visualization of animal and plant cell by methylene blue staining
3. Identification of different stages of mitosis in onion root tip.
4. Identification of different stages of meiosis in grasshopper testis.
5. Cell size determination using ocular stage micrometer
6. Micrographs of different cell components (dry lab).
7. Sub-cellular fractionation.
8. Visualization of nuclear fraction by acetocarmine stain.
9. Staining and visualization of mitochondria by Janus green stain.

SUGGESTED READING

1. Cooper, G.M. and Hausman, R.E., (2009). The Cell: A Molecular Approach 5th ed., ASM Press & Sunderland (Washington DC), Sinauer Associates, MA, ISBN:978-0-87893-300-6.

2. Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D. and Darnell. J., (2012) Molecular Cell Biology 7th ed., W.H. Freeman & Company (New York), ISBN:13:978-1-4641-0981-2 / ISBN:10: 1-4641-0981-8.

3. Alberts, B., Johnson,A., Lewis, J., andEnlarge, M., (2008) Molecular Biology of the Cell 5th ed., Garland Science (Princeton), ISBN:

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Subject : Cell Biology Practical **Subject code:** 19BCU112

Details of the Experiments

Exp. No.	Name of the Experiment	Support Materials
1.	Preparation of onion root squash and observation of cell	
2.	Visualization of animal and plant cell by methylene blue staining	
3.	Identification of different stages of mitosis in onion root tip	T1: 459-465
4.	Identification of different stages of meiosis in grasshopper testis	
5.	Cell size determination using ocular stage micrometer	homepages.gac.edu/cellab/chpts/chpt1/ex 1-3 html
6.	Micrographs of different cell components (Dry lab)	ib.bioninja.com.au/standard-level (topic-/-cellbiology/12-ultrastructure-of-cells/cell micrograph html
7.	Sub- cellular fractionation	W1
8.	Visualization of nuclear fraction by acetocarmine stain	
9.	Staining and visualization of mitochondria by Janus green stain	T2: 141-144

References Book:

T1: David L Nelson and Michael M Cox (2008). Lehninger Principles of Biochemistry (5th Ed) freeman and company

T2: Sadasivam S and Manickam A. (2009). Biochemical methods, New age International Publishers, New Delhi

R2: Singh S.R. (2014) Introductory practical Biochemistry, Narosa publishing house, New Delhi

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DEPARTMENT OF BIOCHEMISTRY

Subject	**:**	**Cell Biology-Practical**	**Semester**	**:**	**I**
Subject code	**:**	**19BCU112**	**Class**	**:**	**I B.Sc Biochemistry**

Experiment: 1

Preparation of onion root squash and observation of cell using acetocarmine stain

The roots can be easily grown if a onion placed on a water filled conical flask, In such a way that the onion disk touches water or else making the onion to sit on wet sand, In such a way that the bulb is buried partially in sand. In two days time nearly 1cm long roots developed, which can then be cut, fixed and stored.

Materials required:

Onion root tips, 1 N HCL, 1:3 acetomethanol fixative, 45% acetic acid, Slide, Cover glass, Sealing wax**.**

Procedure:

- 1. The root tip was cut using sterile blade and treat within HCL for 1 minute. This will soften the call wall
- 2. Transfer the root tip from 1N HCL to Acetocarmine stain and stain for 30 minutes take a drop of 45% acetic acid and place it on root tip. Leave for 1-2 minutes.
- 3. Place a cover glass on the root tip and squash it using a rubber end pencil.
- 4. Seal the edge of the cover glass to prevent drying. Observe the slide under microscope.

Result:

The plant cells have been visualized using light microscope (Acetocarmine stain).

Experiment No: 2

Preparation of onion root squash visualization of Plant cell by methylene blue

The root can be easily grown. If a onion placed on a water filled conical flask. In such a way that the onion disk touches water or else making the onion disk to sit on wet sand. The bulb is buried partially in sand. In two days time nearly 1cm long roots develop. Which can then we cut, fixed and stored.

Materials required:

Onion root tips, 1N HCL, Methylene blue, Slide, cover slip and sealing wax.

Procedure:

- The root tip was cut using sterile blade and treat within 1 HCL for 1 Minute. This will soften the call wall.
- Transfer the root tip from 1N HCL to methylene blue stain and stain for 30 minutes.
- Place a cover glass on the root tip and Squash it using a rubber end pencil.
- Seal the edges of the cover glass and prevent drying
- Observe the slide under microscope.

Result:

The plant cells have been visualized using light microscope (Methylene Blue).

Visualization of Animal Cell by Methylene Blue

Aim:

To visualize the animal call using methylene blue.

Materials required:

- Take a blood sample in the slide
- Add a few drops of methylene blue in the slide and keep the slide undisturbed for 30 minutes
- Observe the slide under the microscope.

Result:

The animal call has been visualized under the light microscope (methylene blue).

Experiment No: 3

Study of Different Stages of Mitosis in Onion Root Tip Cells

Onion root tip has meristamatic tissue just behind the root cap. Have this serves as a good material for studying various stages of mitosis. The roots can be easily grown if a onion is placed on a water filled conical flask, in such a way that the onion disk touches water or else making the onion to sit on a wet sand .In such a way that the bulb is burried partially in sand. In two days time nearly 1cm long roots developed, which can then we cut, fixed and stored longer grown roots are not good as it will have more of non - dividing tissue.

Materials required:

Onion Root Tips, 1N HCL, 1:3 acetomethanol fixative, 70% and 90% ethanol, 2% acetocarmine stain (2 grams of carmine mixed with 100ml of 45 % acetic acid, and boiled using reflux condenser for 1 hour to dissolve carmine) slide, cover glass, sealing wax or nail polish, 45% of acetic acid.

Procedure:

- Fix the freshly cut 1cm long root tips in acetomethanol fixative for overnight in a specimen tube.
- Remove fixative and add 90% ethanol. Leave for 2 hours.
- Decant 90% ethanol; add 70% of ethanol .The root tips can be stored in 70% ethanol for a long period of time. If the tube is tightly closed strong at 4 degree celsius is even better.

Staining and making squash preparation:

- Treat the root tips with 1N HCL for 1 minute. This will soften the cell wall.
- Rinse the tips once in water transfer to acetocarmine stain and stain for 30 minutes
- Take a drop of 45% acetic acid drops become colored, it can be decanted and a fresh 45% acetic acid drop is added.
- Place a cover glass on the root tip and squash it using rubber end pencil under the folds of blotting paper.

- Seal the edges of the cover glass with molten wax or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.
- The slide is ready for observation under microscope.

Experiment No: 4

Identification of different stage of Meiosis in grasshopper testis

Grasshopper testis is an ideal material for studying various stage of meiosis. Grasshopper is of good choice because it is easy available in lawns and fields. Males can be easy to distant. In addition is has fewer number of chromosomes. (locally available species contain seventeen or nighteen or twenty one chromosome in males add number of chromosomes due to xx or xo sex chromosomes system) . All chromosomes are of one type i.e acrocentric facilitating unambiguous of different stages.

Temporary squash preparation:

Material required:

Male grasshopper, insect saline (0.67% Nacl) 1:3 acentomethanol fixative, 70% and 90% ethanol, 2% acetocaremine stain (2gm of carmine mixeed with 100ml of 45% of acetic acid and boiled using a reflex condenser for 1hr to dissolve carmine), 45% acetic acid, slide cover glass, sealing wax or nail polish

Procedure:

Fixation of grasshopper testis:

- Hold a male grasshopper in hand, give a small incision with scissors at the junction of thorax and abdomen and press the abdomen gently. The testis covered in yellow fat bodies will pop out. Dissect them out and put in insect saline remove yellow fat with the help of forceps as much as possible. A pair of testis (Each having a brunch of white tubules) will be seen.
- Transfer the tubules in a test tube and fix in acetomethanol fixative, close the test tube and leave it for overnight.
- Remove the fixative and add 90% ethanol, leave for 2 hours.
- Decant 90% ethanol and add 70% ethanol for a long period of time. If the tube is lightly closed storing at 4° C is even better.

Staining and making squash preparation

- Stain the fixed testis in acetocarmine stain for 30 min.
- Take a drop of 45% acetic acid on slide place a few tubules of testis in the drop, leave for 1-2 minutes. If acetic acid drop can be added.
- Place a cover glass on the tubules and squash using a rubber and pencil under the blotting paper**.**
- Seal the edge of the cover glass with molten wax or with the nail polish. Immediately to prevent drying of acetic acid film and entry of air bubbles.
- The slide is ready for observation under a microscope.

Materials required

Male grasshopper, insect normal saline (0.61% Nacl) fixative (1:3 acetic acid) ,methanol 60% acetic acid, centrifuge tubes, slides, cover glass, gimsa stain.

Procedure:

- Dissect out testis from male grasshopper.
- Keeping the testis in normal saline, remove the yellow fat.
- Fix the testis in fixative in a centrifuge tube for 30 min.
- Remove the fixative and add about 0.5ml of 66% acetic acid, leave for 2-3 min till the testis appearance nearly dissolved.
- Add 5-6 ml fixative to the tube without removing the acetic acid.
- Centrifuge at 1200 RPM for 5 minutes.
- Gradually, remove the suspensible and add a few drops (0.2 ml) of fresh fixative and make a suspension.
- Drop a few drops of cell suspension on a slide and the flame dry.
- Stain the slides with giems a stain rinse in water dry and observe under a microscope.
- The slide can be mounted with DPX before observing the oil immersion lens.

Experiment No: 5

Measurement of the size of cells and Sub – cellular components in light microscope

Introduction:

The diameters of the cell are length/diameter of sub cellular components can be easily measured using an ocular micrometer which has graduation in arbitrary units. This arbitrary graduation of the ocular micrometer is calibrated, using a stage micrometer by supermetasm the two stage.

Materials required:

Light microscope, ocular and stage micrometer, slide having cell preparation, whose size is to be estimated.

Procedure:

- The ocular micrometer is placed on the circular shelves inside the eye piece. In such a way that the graduations sketched on the ocular is visible when an observation is made using the microscope.
- Place the stage micrometer on the stage of a microscope and focus the graduation using low power objectives the graduation on stage micrometer are space 0.01mm (10mm) apart.
- Superimposed the two scales and accord the number of ocular division coinciding exactly with the number of divisions of stage micrometer.
- The calibration factor are the least count of the ocular micrometer is calculated as fellows:-
- If 13 ocular divisions coincides with two division (2x10mm = 20mm) of stage micrometer then one ocular division = 20mm /13 divisions =1.54mm.
- Now remove the slide having cell preparation and a low power magnification. Position the cell using absorbed in such a way that the ocular micrometer is able to measure the diameter of cell or the length/diameter of the cell component in arbitrary unit.

Calculate the size as shown below:

- If the diameter is occupying 5 division of ocular the diameter of the cell will be 5 division x 1.54mm = 4.5mm.
- Similarly for high power objective the ocular micrometer calibration has to be done again following the same procedure and then cell diameter can be measured focusing the cell in high magnification.

Experiment No: 6

Sub cellular Fraction

Aim:

To practice cell fractionation and isolate chloroplast from plant cells.

Principle:

The green color of the leaves and sometimes the stems of plants are due to presence of the green pigments chlorophyll a and chlorophyll b in the sub cellular organelles called chloroplasts. The remainder of a green cell is typically colorless. The green organelles be free in the cytoplasm. Unattached to other cellular components such as the wall the membrane, the nucleus and the mitochondria. When the cell wall is disrupted, the cell membrane breaks, and the sub cellular components are released, as separate particles of various sizes and densities.

Materials:

- Freshly collected green leaves.
- Sterile and pre chilled mortar and pestle.
- Ice cold 0.5 m sucrose solution
- Clean and sterile blades
- Phosphate buffer
- Microscope

Preparation:

2.4g of sodium hydrogen phosphate was dissolved in 10ml of sterile distilled water prepare 0.2 m of monobasic salt .Similarly, 2.8 g sodium di-hydrogen phosphate was dissolved in 100 ml of sterile distilled water to prepare 0.2 m dibasic salt. 39 ml of monobasic salt was mixed with 61 ml of dibasic salt and the volume was made to 200 ml.

Procedure:

- Obtained 8 grams of de veined leaf tissue rinsed in ice water, blotted and cut into pieces about km.
- Place leaf pieces in a pre chilled blender up containing 40 ml of ice cold, 0.5 m sucrose. Blend for 15 seconds then blends again for 10 seconds.

- Remove the ice from the 100 ml beaker and the squeeze the leaf homogenate through 4 layers of pre chilled cheese cloth into the cold water by twisting the top corners of the cloth around each other.
- Pour 14 ml of the homogenate into each of two centrifuge tubes and centrifuge at 200g for 5 minutes.
- Using a Pasteur pipette, transfer each supernatant (containing the chloroplast) to a second centrifuge tube and centrifuge at 1000g at 7 minutes (save the pipette).
- Using the pipette, discard the supernatant but be careful not to disturb the pipette and gently suspend it by moving it up and down in the pipette.
- Using a clean Pasteur pipette add buffer until you have a total volume of 8 ml and mix the diluted suspension using the pipette .
- This is your chloroplast suspension you should examine it in the microscope.

Experiment No: 7

VISUALIZATION OF NUCLEAR FRACTION

Aim:

To visualization of nuclear fraction by microscope.

Materials required:

- Fresh rat liver
- 0.25m sucrose
- 50ml of centrifuge tube
- Table top and high speed centrifuge
- Vortex
- Micropipettes and tips
- Ice buckets

Procedure:

- Chop rat liver into approximate few mm^3 pieces.
- Add 0.25 m sucrose [10% w/v]
- Homogenize with hand blender
- Centrifuge the homogenate to remove the cell debris at 800g, 5 minutes.
- Collect the supernatant; this is your whole homogenate. Save 5 ml for the next experiment [tube #1] and record the volume of the rest.
- Centrifuge the rest of the homogenate for 15 minutes at 500 g.
- Resuspend the nuclear pellet in 0.25 m sucrose [save the suspension ,tube #2]
- Centrifuge the 10 minute at 24,000g
- Resuspend the nuclear pellet in 0.25 m sucrose [save the suspension tube #3]
- Rename tube#2 as tube N and tube #3 as M

Microscopic examination of nuclear fraction:

The nuclear fraction that has been isolated that previous experiment. [tube N] will be examined microscopically to identify the nuclei, in addition approximate size of the nuclei will be measured.

The nucleus is separated from the cytoplasm by an envelope consisting of two membranes the entire chromosomal DNA is held in the nucleus, packaged into chromatin fibrous by its association with equal mass of histone proteins. The nuclear contents communicate with cytosol by means of openings in the nuclear envelope called nuclear pores.

Nucleoli all large, round or oval structure in which ribosomal subunits are assembled. Thus are rich in RNA and protein. For the observation on nuclei the stain to be used in aceto-orcin which stains chromatin red. The nucleoi stand out. Since they do not stain with the orcin. Each nucleolus appears as a prominent round clear area.

Experiment No: 8

Staining and Visualization of Mitochondria Janus Green Stain

Introduction:

Mitochondria are considered as "power house of the cell". As it produces ATP by a process called oxidative phosphorylation. Each cell contains large number of mitochondria and they can be observed under a light microscope. It stained with Janus green this stain is bluish green in color. When oxidized and colorless. When reduced a dilute solution of the stain is applied to stain the cell, it enters the cytoplasm as well as in mitochondria. Since mitochondrial inner membrane cytochrome oxidized enzyme, which can keep the stain in oxidized state in mitochondria appeared stain while in rest of the cytoplasm. The stain gets reduced and this appears colorless.

Material's required:

Ethanol soaked tooth pick, Slide, Cover glass, 0.01% Janus green stain.

PROCEDURE:

- Try the ethanol soaked tooth pick in air and scrap gently the inner side of check a large member of cell will come on the tooth pick.
- Gently rule on the toothpick on side in one direction to make a spread of cells. Dry the cells on side .So that the cells will not get washed away while staining.
- Put a few drops of Janus green stain and leave for 5-10 minutes for staining.
- After 5 minutes of staining, rinse cells once with distilled water. So that complete stain is not gone and distilled stain remains mount the cell in a drop of distilled water with a cover glass and observe under bright field students microscopes. The cells can alternatively be mounted in the stain itself. A few air bubbles remaining inside the cover glass give a back ground stain that makes the viewing easily. The slide can be observed under the high magnification of student microscope.

Experiment No: 9

Micrography

Aim:

To use the photography to capture images in a microscopy. Dates back to the invention of the photographic process.

Introduction:

The technique of making photographic images through the microscope using intact film give microscopes an invaluable tool for capturing and conveying the images revealed through the microscope. In many ways, camera and film tend to be more demanding than the human eye. Similarly good photomicrophy consist of more than good visual microscopy. Instant photography through the microscope is performing to document microscopic data in quality form. It is designed to help the photo micrography use the techniques to achieve the highest quality instant imaging result.

Camera's for photo micrography:

Producing a real image of the specimen and the film plane. Facilitate focusing and forming of the specimen providing a mean to control exposure of the film. A variety of camera option is available for photomicrography.

Photo shoots procedure:

The first should be taken at what appears to be the best focus through the viewing eyepieces.

For the 2^{nd} shot, move the fine focus and raise the subject up toward the objective list the slightest amount. So that it now appears very slightly "out of focus". Through the viewing eyepiece.[your real point of visual best focus is now place slightly below the subject"].

For the 3^{rd} shot, go back and set visual best focus. Then using the fine focus, very slightly "lower" the subject stage. Now appears very slightly "out of focus". Through the viewing eyepiece [your point of visual] "Best "focus is now placed slightly "above the subject".