Biology Pre-lab

 (a) Describe very briefly the procedure of isolating intact living cells from a green leaf.

To isolate intact living cells from a green leaf, start by sterilizing the leaf with 70% ethanol and distriled water to remove any surface contaminants. Next, macerate the leaf in a buffer solution, such as phosphate buffer, using a mortar and pestie or blender to break down the tissue and release the cells. Then, then the mixture through a cheese loth or nylon mesh to remove any debris and excess tissue.

After litering, centrifuge the litrate to separate the cells from the liquid. The cells will form a pellet at the bottom of the centrifuge tube. Resuspend this pellet in a suitable medium, such as Murashige and Sixog medium, which provides the necessary nutrients for cell growth. If desired, enrich the cell suspension with additional nutrients and growth regulators to promote cell growth and division.

Finally, culture the cells in a controlled environment, such as a tissue culture chamber, where they can grow and thrive. This environment should provide optimal temperature, humidity, and light conditions for the specific type of cells being isolated. By following these steps, you can successfully isolate intact living cells from a green leaf.

(b) Explain why the leaf tissue Is kept at low temperature during cell isolation

During cell isolation, leaf tissue is typically kept at a low temperature (around 4°C) to:

 Slow down metabolic processes: Low temperatures reduce cellular activity, minimizing changes in cellular composition and preventing degradation of organelies.

Inhibit enzyme activity: Cold temperatures inhibit the activity of enzymes that might break down cellular components, ensuring the integrity of the cells and their organelles.

Reduce cell wall degradation: Low temperatures prevent the breakdown of cell walls, making it easier to maintain cell structure and integrity during isolation.

4. Minimize oxidative stress: Cold temperatures reduce the production of reactive oxygen species (ROS), which can damage cellular components and compromise cell viability.

 Preserve cell membrane integrity: Low temperatures help maintain the stability and integrity of cell membranes, reducing the likelihood of membrane damage and cell lysis.

By keeping the leaf bissue at a low temperature, you can help preserve the cells' natural state, ensuring the isolation of healthy, intact cells.

2. (a) Name 3 method of disrupting cells before fractionation.

Here are three common methods of disrupting cells before fractionation:

- Mechanical disruption: This involves physically breaking open cells using techniques such as:
 - Homogenization (e.g., using a blender or homogenizer)
 - Sonication (using high-frequency sound waves)
 - Grinding (using a mortar and pestle)
- 2. Enzymatic disruption: This method uses enzymes to break down cell components, such as:

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