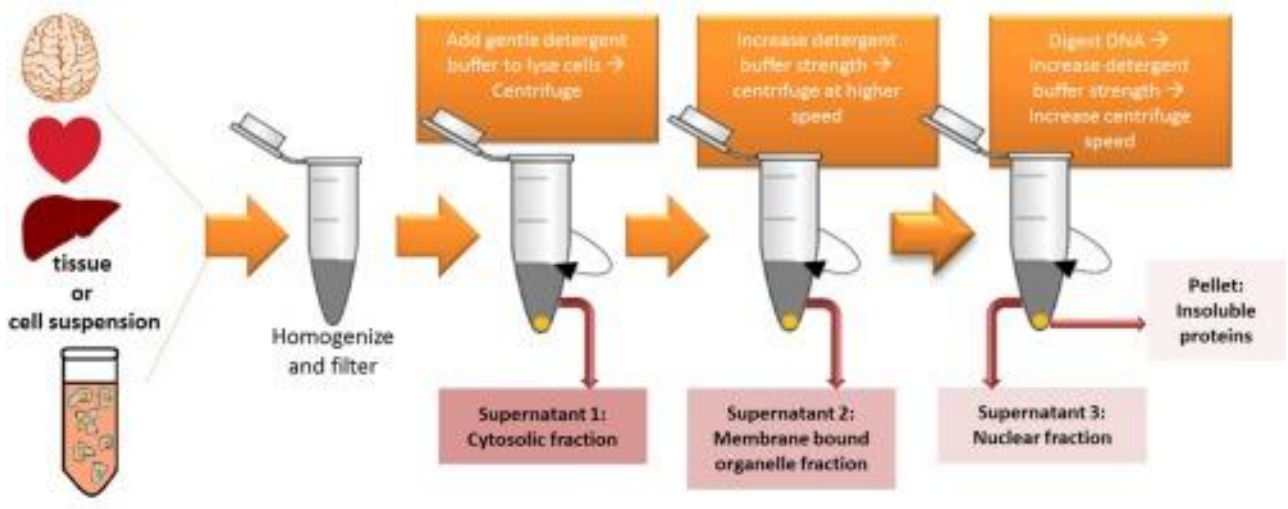
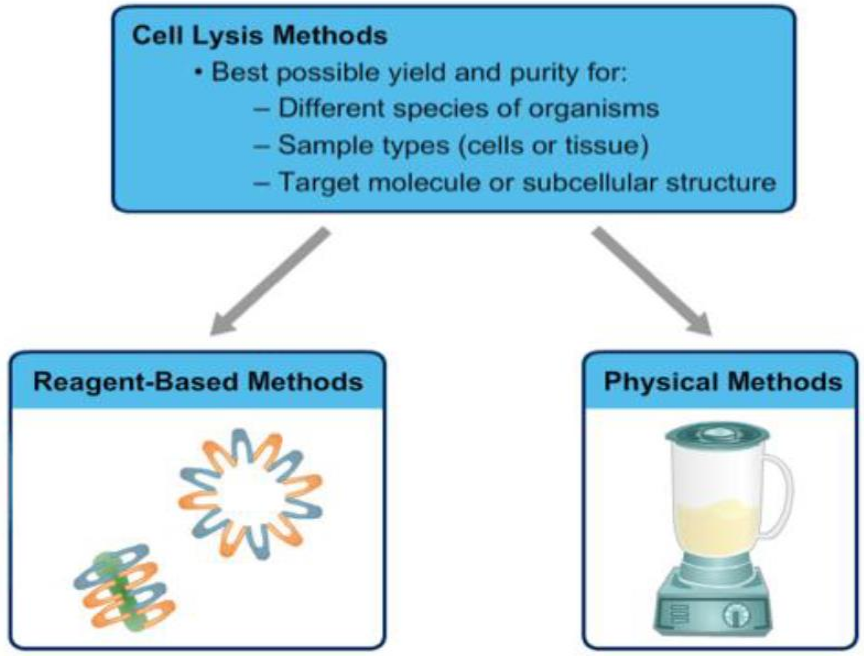
Protein extraction from animal and plant source

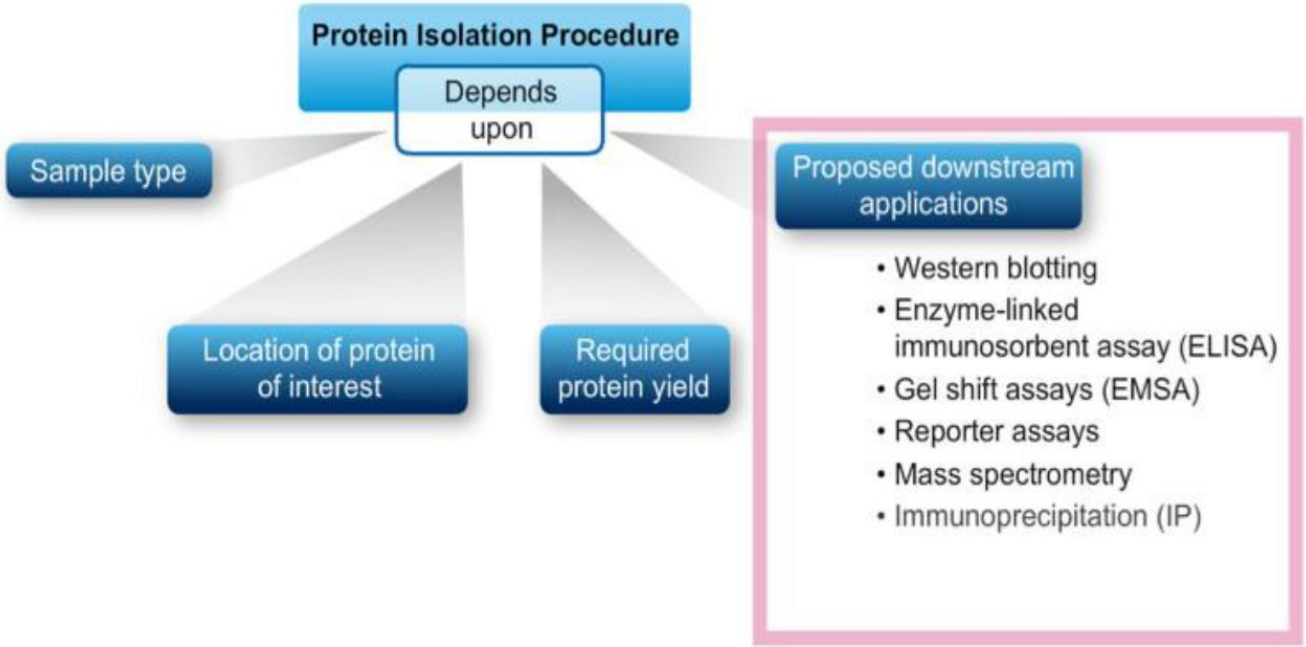


Cell lysis:

- is the first step in cell fractionation, organelle isolation and protein extraction and purification.
- Mechanical and non mechanical methods.



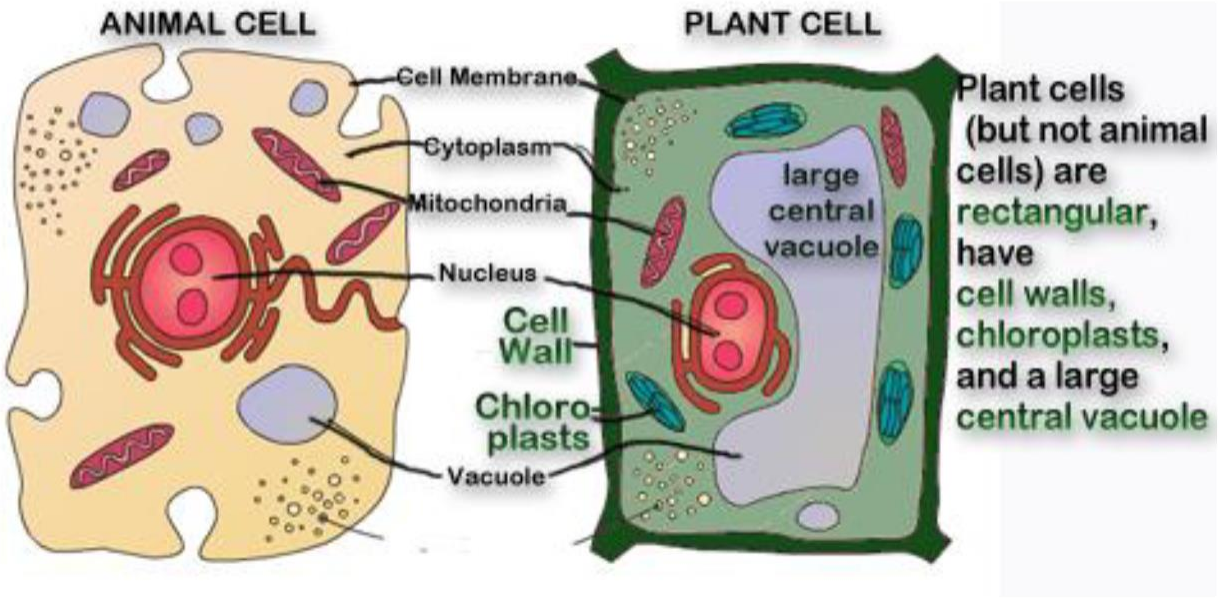
- There is no universal protocol for protein extraction. Why??



Practical part

Principle

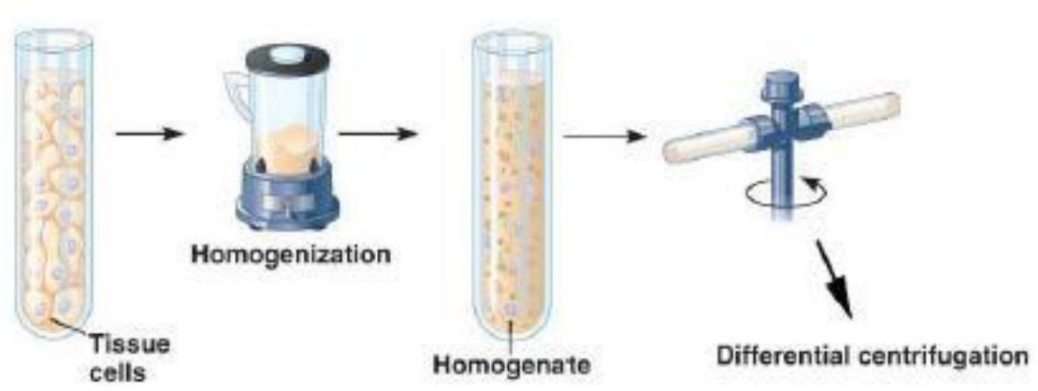
The initial step of any purification procedure is to disrupt the starting tissue to release proteins from within the cell. The means of disrupting the tissue will **depend on the cell type.**

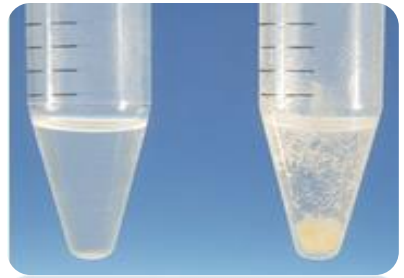


Cell disrupting will be achieved using both **mechanical and non-mechanical methods.**

In non-mechanical method, normally extraction buffers are at an ionic strength (0.1–0.2 M) and pH (7.0–8.0) that is considered to be compatible with that found inside the cell.

In mechanical method both animal and plant cells are susceptible to shear forces using blenders. Here, the tissue is cut into small pieces and blended, in the presence of buffer to disrupt the tissue, and then centrifuged to remove debris.





After extraction, protein concentration determination is a routine requirement during protein purification, which can be achieved by different method (next lab).

Experiment (1). Protease inhibitor extraction from plant source:

Objective:

To prepare crude extract from plant source.

Method:

1. Weight 12 g of the sample and place it in the blender with 50 ml of the extraction buffer (phosphate buffer 0.1 M, pH 7.0)
2. Incubate the homogenate at room temperature on a rotary shaker for 30 min at 150 rpm.
3. Filter the slurry through cheesecloth and then transfer to centrifuge tube.
4. Centrifuge the filtrate at 10,000 rpm for 15 min at 4 °C for the removal of any cell debris that remained in the preparation.
5. Measure the volume of the supernatant.

Experiment (2). Lactate dehydrogenase extraction from animal source:

Objective:

To prepare crude extract from animal source.

Method:

1. Cut ~15 g of muscle tissue from the tissue source. Record the exact weight of tissue used.
2. Cut the tissue into small pieces. Discard the connective tissue and fat.
3. Add 75 ml of cold extraction buffer (0.1 M Tris-HCl, pH 7.4) in a blender with the sample.
4. Transfer the homogenized tissue/buffer mixture into centrifuge tubes (note:. Balance the tubes.
5. Centrifuge your homogenate for 15 minutes at 3000 xg.
6. Measure the volume of the supernatant.

Questions :

There are different mechanical methods (other than using blenders) for cell disruption. Search for one method and describe it briefly.