Lab (4): Protein extraction from animal and plant source

Introduction:

Cell lysis is the first step in cell fractionation, organelle isolation and protein extraction and purification. As such, cell lysis opens the door to a myriad of proteomics research methods. Many techniques have been developed and used to obtain the best possible yield and purity for different species of organisms, sample types (cells or tissue) and target molecule or subcellular structure.

There is no universal protocol for protein sample preparation. Sample preparation protocols must take into account several factors, such as the source of the specimen or sample type, chemical and structural heterogeneity of proteins, the cellular or subcellular location of the protein of interest, the required protein yield. (1)

- PAUSE AND THINK ➔ How cellular location of the protein will affect the isolation protocol?

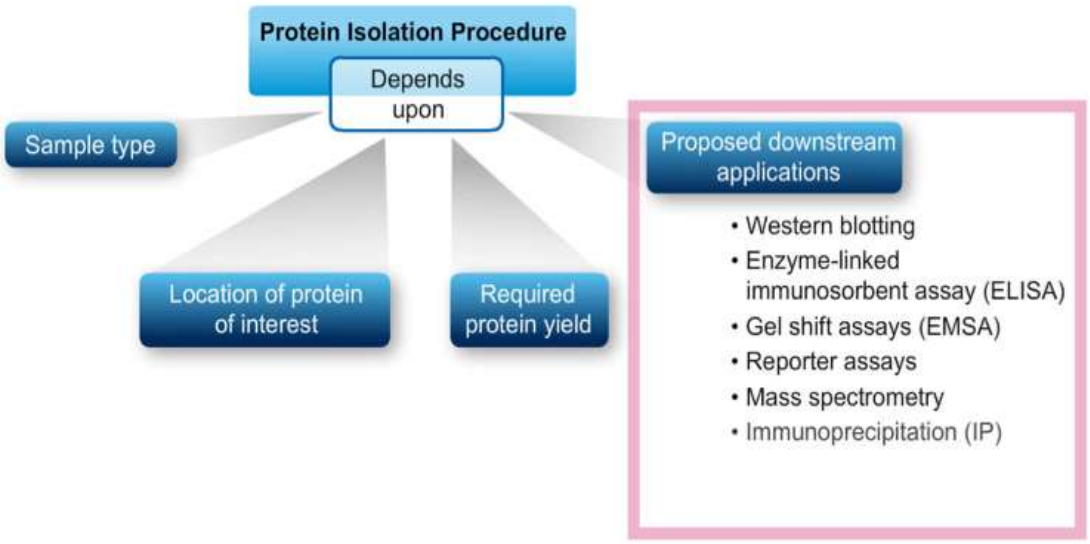

Figure 1. Isolation of high quality protein samples is the cornerstone to completing successful downstream applications. (1)

Principle:

The initial step of any purification procedure must, of course, be to disrupt the starting tissue to release proteins from within the cell. The means of disrupting the tissue will depend on the cell type. In animal cells, the plasma membrane is the only barrier separating cell contents from the environment, but in plants and bacteria the plasma membrane is also surrounded by a rigid cell wall. Plant cell walls consist of multiple layers of cellulose. These types of extracellular barriers confer shape and rigidity to the cells. Plant cell walls are

particularly strong, making them very difficult to disrupt mechanically or chemically, whereas the lack of an extracellular wall in animal cells makes them relatively easy to lyse.

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. Tris or phosphate buffers are most commonly used. While in the 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. (2)

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

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

Aim:

- To prepare crude extract from plant source.

Materials:

Chemical

Plant tissue, phosphate buffer 0.1 M (pH 7.0), distal water.

Equipment and Glassware

Measuring, centrifuge tube, measuring cylinder, cheesecloth, shaker, blade, blender, electronic balance, centrifuge.

Protocol:

1. Weight 12 g of the sample and place it in the blender with 200 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 10 min at 4 ◦C for the removal of any cell debris that remained in the preparation.
5. Measure the volume of the supernatant.

Results:

Volume of the supernatant (crude extract) = _____ ml

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

Aim:

- To prepare crude extract from animal source.

Materials:

Chemical

Animal tissue, 0.1 M Tris-HCl (pH 7.4), distal water.

Equipment and Glassware

Measuring cylinder, blade, blender, electronic balance, centrifuge.

Protocol:

1. Cut ~7.5 g of muscle tissue from the tissue source (record. Record the exact weight of tissue used).
2. Cut the tissue into small pieces. Discard the connective tissue and fat.
3. Add 38 ml of cold extraction buffer (0.1 M Tris-HCl, pH 7.4) in a blender with the sample. note: (20% weight/volume).
4. Transfer the homogenized tissue/buffer mixture into centrifuge tubes.Transfer the homogenized tissue/buffer mixture into centrifuge tubes (note: Balance the tubes.
5. Centrifuge your homogenate for 5 minutes at 7,000 rpm.
6. Measure the volume of the supernatant.

Results:

Volume of the supernatant (crude extract) = _____ ml

References:

1. https://www.thermofisher.com/sa/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-cell-lysis-and-protein-extraction.html
2. Wilson K, Walker J. (2010) Principles and Techniques of Biochemistry and Molecular Biology. Cambridge University Press. p 311-2.