Home

Methodology for the Extraction of Plant Protein

Extraction of total protein from the sample requires an optimized protocol. Many protocols have been developed to increase the amount of protein in the extract form different samples. The method explained here focuses mainly on extracting protein from plant tissue.

Learning Objectives:

After interacting with this learning object, the learner will be able to:

- Describe extraction of plant protein using lysis buffer.
- Solubilise the plant protein using rehydration buffer.
- Interpret the results of the experiment.
- Troubleshoot at various steps in the experiment.

Click "Start" to begin » Start

Note: The current IDD exists in two modes- interactive and automatic. Students taking lab course should select interactive (set as default), while the automatic mode may be selected for general users.

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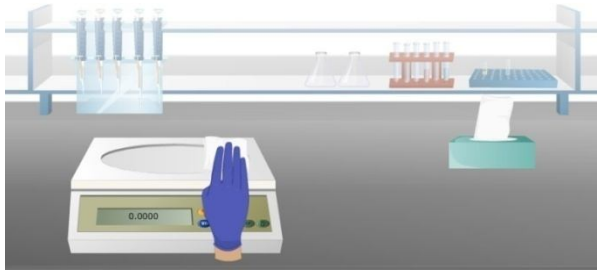
Developed by : IIT BOMBAY

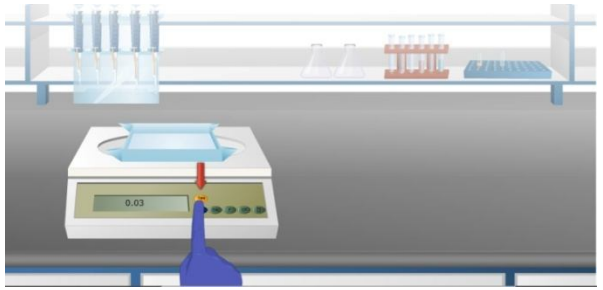
Demo

Reagents Preparation

Clean the surface of the balance with a tissue.

Place a butter paper and tare the weight of the paper.



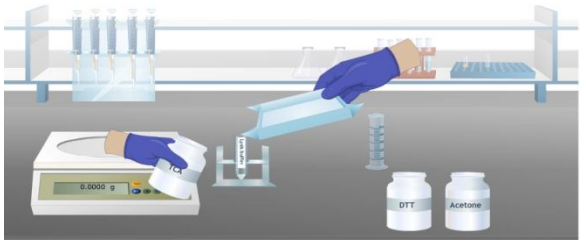


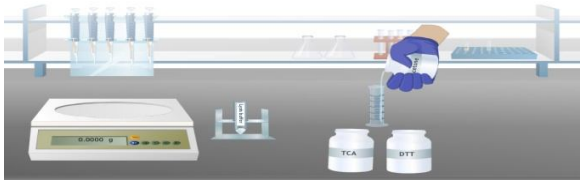
Demo

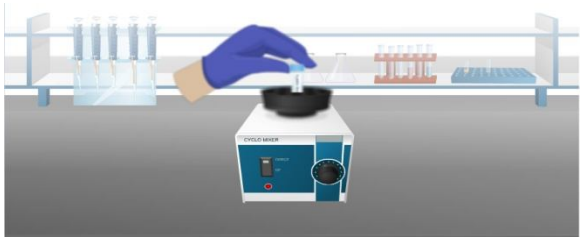
Reagents Preparation

Weigh 1g of Trichloro acetic acid, 0.007 g of Dithiotreitol and add 10 ml of Acetone to prepare lysis buffer.

Vortex the tube well to completely dissolve the contents and store it at 4 degree C for use later.





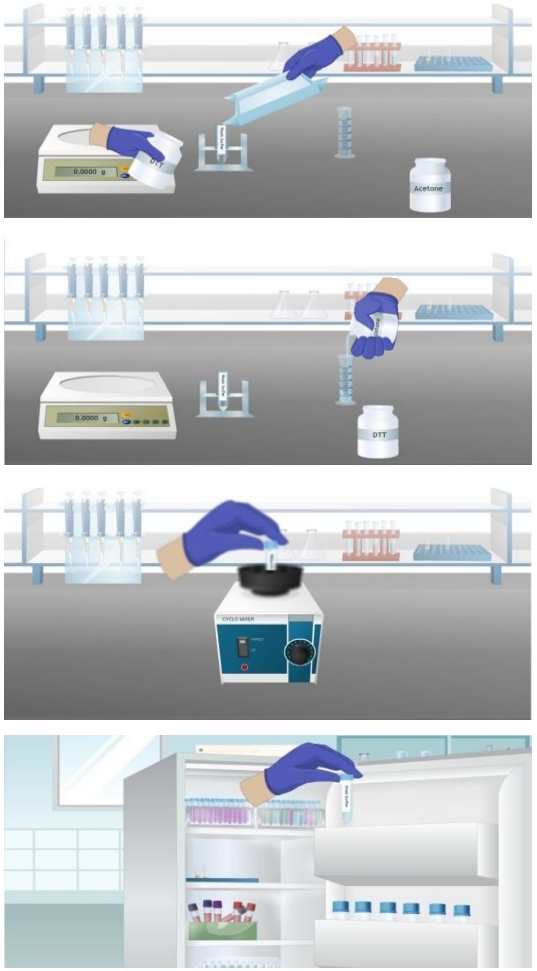


Demo

Reagents Preparation

Prepare wash buffer by weighing 0.07 g of Dithiotreitol and dissolve it in 10ml of acetone.

Vortex the contents of the tube to mix thoroughly. Store the wash buffer so formed at 4 degree C for further use.

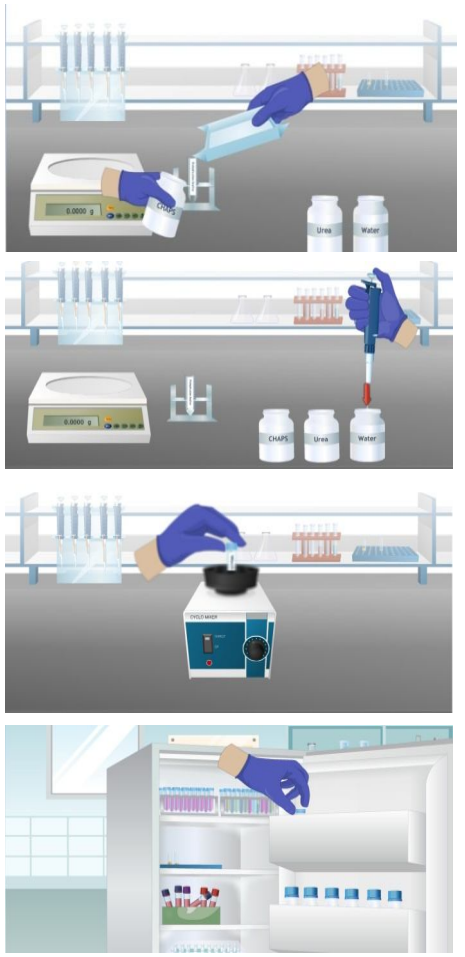


Demo

Reagents Preparation

Weigh 0.02 g of CHAPS, 0.6 g of Urea and dissolve it in water.

Vortex the contents to mix thoroughly.

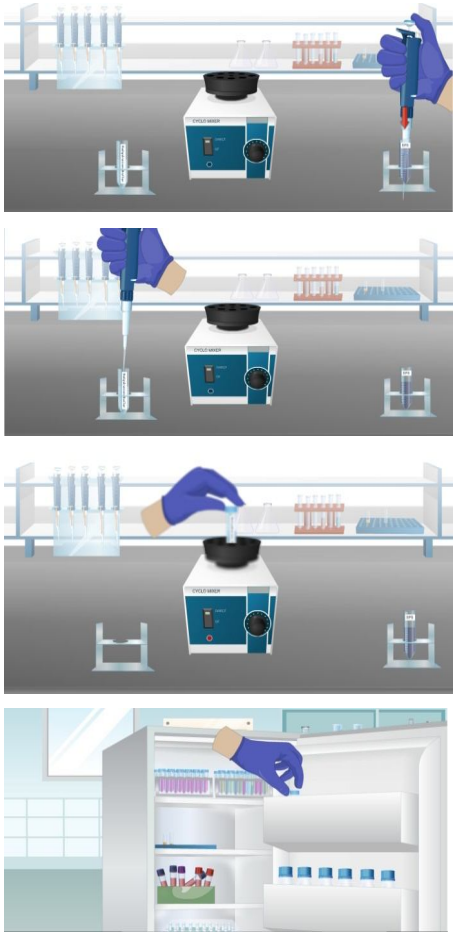


Demo

Reagents Preparation

Add 0.05% bromophenol blue to the rehydration buffer. It helps in tracking the sample during electrophoretic run.

Vortex the contents again for thorough mixing.



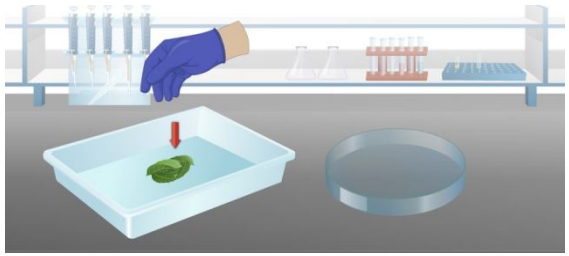
Demo

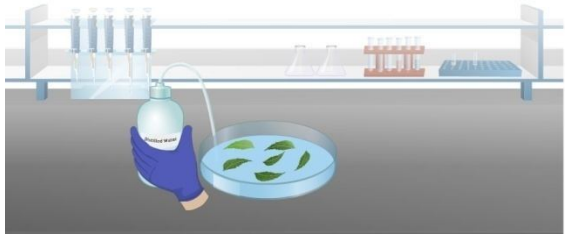
Cleaning and weighing of Leaves

Place the isolated leaves in a petriplate.

Wash the leaves with distilled water to remove all the particulate matter on the surface. Repeat it as many times as required without being too harsh till the leaves are completely clean.

Once clean, dry the leaves on a tissue paper without applying excess pressure.





Demo

Cleaning and weighing of Leaves

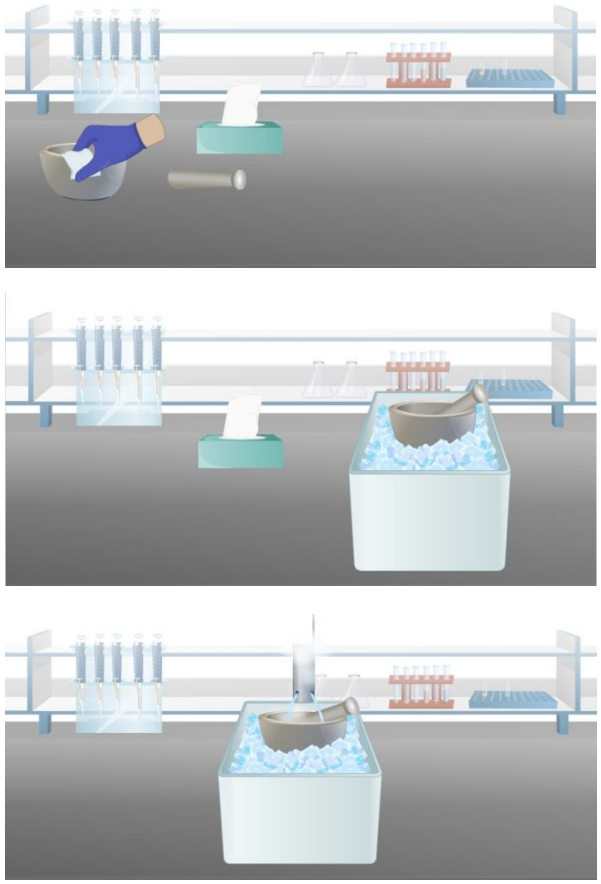
After the leaves are totally dry, weigh the required amount on a balance.

Demo

Liquid Nitrogen Treatment

Take a mortar and pestle. Wipe the inside of the mortar and the surface of the pestle with a tissue paper and place it on ice.

Pour liquid nitrogen into the mortar. This is done to pre-chill the mortar for further treatment.



Demo

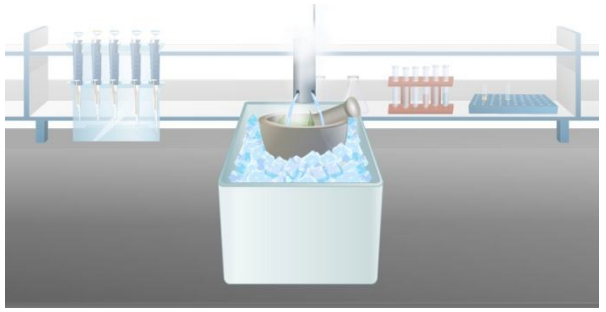
Liquid Nitrogen Treatment

Transfer the leaves into the pre-chilled mortar.

Demo

Liquid Nitrogen Treatment

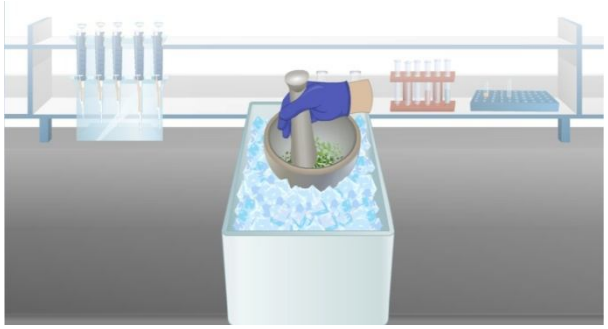
Add around 10 ml of liquid nitrogen to the mortar containing leaves, all at one go. A crackling sound is heard on addition of liquid nitrogen to the leaves.



Demo

Grinding the Leaves

Grind the leaves till a fine powder is seen. The finer the powder the better it is for protein extraction.

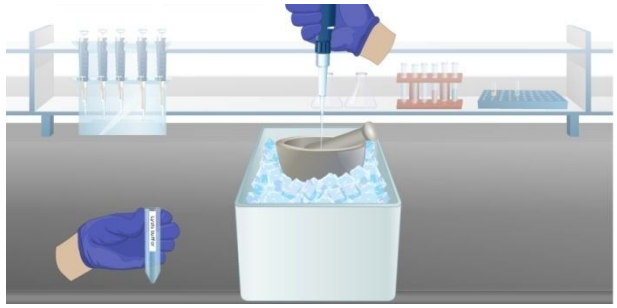


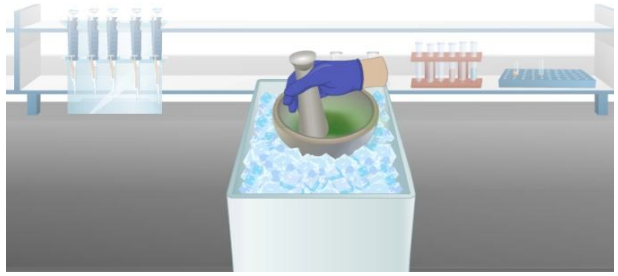
Demo

Lysis Buffer Treatment

To the powdered leaves, add lysis buffer and grind thoroughly.

Lysis buffer helps in complete lysis of the cells and hence bears a lot of significance in the protocol.





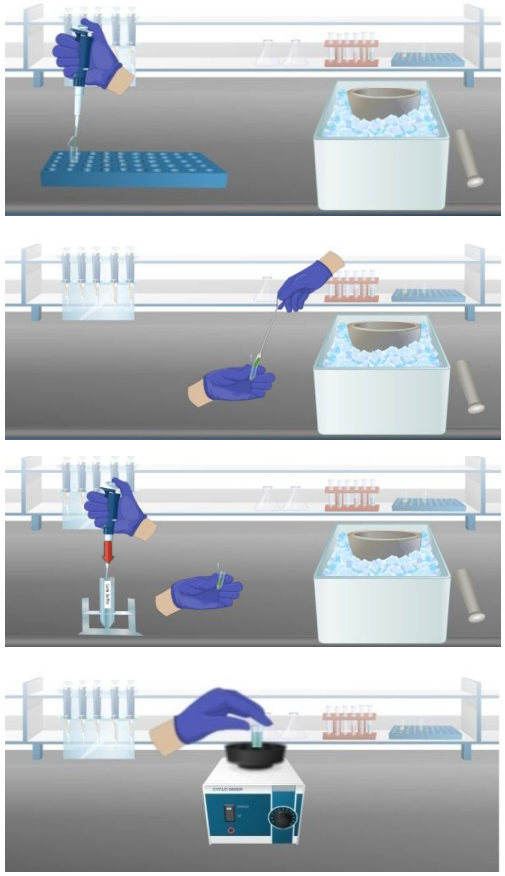
Demo

Lysis Buffer Treatment

To the thick paste in the mortar, add lysis buffer and grind thoroughly.

Transfer the lysate from the mortar to fresh eppendorf tubes. Scrape out the mortar to remove any tissue left out.

Add some more lysis buffer to the tube containing the lysate, to ensure proper lysis of cells. Vortex the tube thoroughly to mix the contents uniformly.



Demo

Protein Precipitation at -20°C

After vortexing the contents of the tube, incubate the tube at -20°C for one hour.

The proteins at the end of this incubation, get precipitated in the buffered solution.

Demo

Centrifugation and Acetone Wash

Take the tube out of -20 degree C freezer. Now, place the tube in the centrifuge.

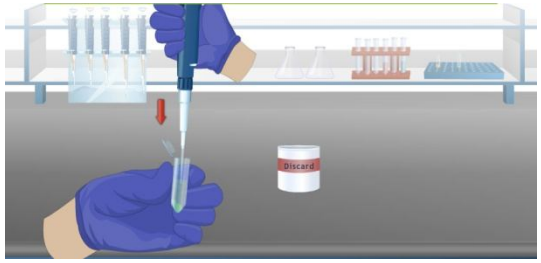
Set the parameters like speed, temperature and duration to 14000rpm, 4 degrees C and 30minutes, respectively.

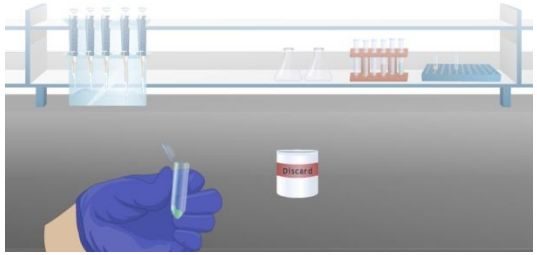
Demo

Centrifugation and Acetone Wash

Take the tube out of the centrifuge. The tube can be seen to have a clearly demarcated pellet and supernatant.

Discard the supernatant carefully without disturbing the pellet.

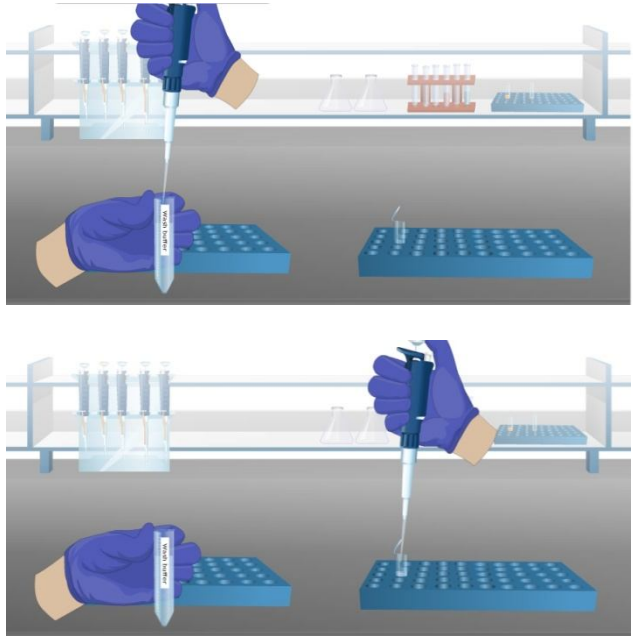




Demo

Centrifugation and Acetone Wash

To the pellet, add 1ml of wash buffer. Wash buffer helps in removing the color of the pellet.



Demo

Centrifugation and Acetone Wash

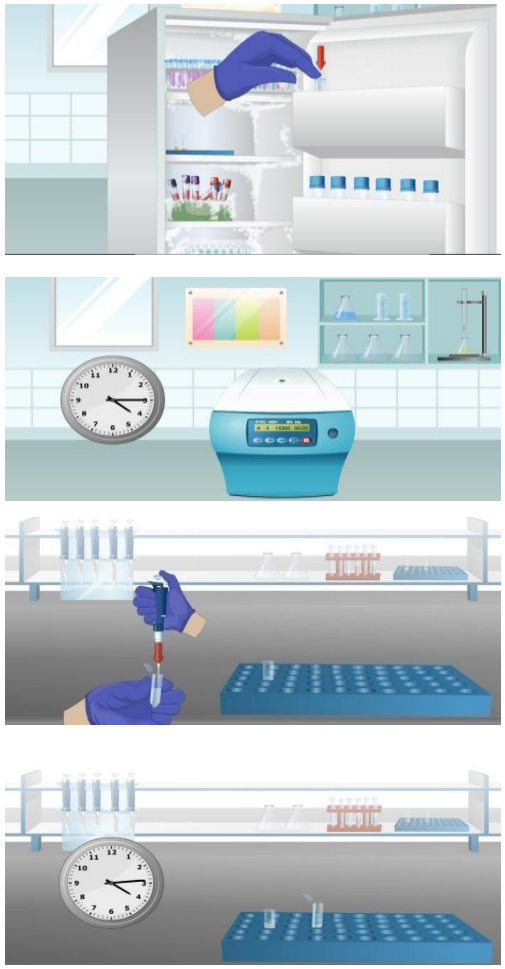
Vortex the tube to thoroughly let the pellet dissolve into the buffer. Incubate the tube in the -20 degree freezer for 30 minutes.

Demo

Centrifugation and Acetone Wash

Remove the tube from the freezer carefully. Centrifuge the tube at 14000rpm, 4 degrees C for 15 minutes.

Discard the supernatant carefully without disturbing the pellet. Air dry the pellet to remove even traces of acetone.



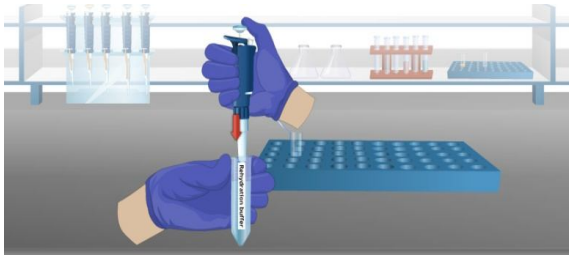
Demo

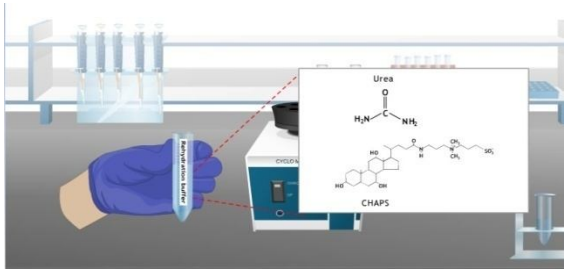
Rehydration Buffer Treatment

Add 400 µL of rehydration buffer to the air-dried pellet.

Vortex the tube till the pellet dissolves in the rehydration buffer.

Rehydration buffer consists of CHAPS which solubilises the proteins and Urea which denatures the proteins.





Demo

Rehydration Buffer Treatment

Incubate the tube overnight at 4 degree C for complete solubilisation of proteins in the rehydration buffer.

Demo

Sample Storage

The sample if not required immediately can be stored at -20°C for later use.

For more information and continuity please go through the following IDDs.

Glossary

Definitions of the components/Keywords

Methodology for the Extraction of Plant Protein

1. **Protein:** Proteins are the biomolecules, composed of amino acid, forming the building block of the system and performs most of the biological functions of the system.
2. **Protein extraction:** The process by which the proteins from the cell are recovered for the analysis purpose is called protein extraction. The chemicals involved in the extraction are
3. **Lysis Buffer:** A cocktail of reagents used for cell lyses.
   a) **Trichloro acetic acid:** The acid used in the lyses buffer for lyses of the cell and helps to precipitate the protein.
   b) **Acetone:** One of the constituent of lyses buffer used to denature the protein.
   c) **Dithiothreitol:** Constituent of lyses buffer used to reduce the disulfide bonds in the protein.
4. **Rehydration buffer:** A cocktail of reagents used for sample solubilization and used for sample storage.
   a) **CHAPS:** is a zwitter-ionic detergent, constituent of rehydration buffer that is used to solubilize the proteins including membrane proteins.
   b) **Urea:** It is a organic compound in rehydration buffer that is used to denature protein.
5. **Bromophenol blue (BPB):** Used in rehydration as color marker and acid -base indicator.

Quiz

Question: 1 2 3 4 5

Which among the following acts as a "cell lysing and precipitating agent" in the lysis solution?

- Trichloroacetic acid and acetone ✓
- Water
- Acetone
- Dithiothreitol

HIDE FEEDBACK

Congratulations, you have chosen the correct answer.

Back Next

Quiz

Question: 1 2 3 4 5

The reagent that denatures protein is

- Water
- Liquid nitrogen ✗
- Urea ✓
- Trichloroacetic acid

HIDE FEEDBACK

I am sorry, the correct answer is Urea.

Back Next

Quiz

Question: 1 2 3 4 5

What is the use of prechilled acetone?

- Washing the pellet ✓
- Drying the pellet
- Removing the pellet
- Washing the tube

HIDE FEEDBACK

Congratulations, you have chosen the correct answer.

Back Next

Quiz

Question: 1 2 3 4 5

Role of CHAPS in the rehydration buffer

- Detergent that dissolves protein ✗
- Detergent that disrupts the cell
- Detergent that dissolves membrane proteins ✓
- Detergent that removes the stain from the pellet

HIDE FEEDBACK

I am sorry, the correct answer is Detergent that dissolves membrane proteins.

Back Next

Quiz

Question: 1 2 3 4 5

Which one of the following is the cooling agent?

- Acetone
- Urea
- Liquid nitrogen ✓
- Water

HIDE FEEDBACK

Congratulations, You have chosen the correct answer.

Back Next

References

Papers:

1) Granier, F., Extraction of plant proteins for two-dimensional electrophoresis. *Electrophoresis* 1988, **9**, 712-718.

2) Méchin, V., Damerval, C., Zivy, M., Total protein extraction with TCA-acetone. Methods Mol Biol. 2007, **355**, 1-8.

3) Saravanan, R.S., Rose, J.K., A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues. *Proteomics* 2004, **4**, 2522-2532.

Books:

1) GE Handbook 2D-Electrophoresis: principle and methods

2) Biochemistry by Stryer et al., 5th edition

3) Biochemistry by A.L.Lehninger et al., 3rd edition

4) Biochemistry by Voet & Voet, 3rd edition