Extraction of total protein from the sample requires an optimized protocol. Protein extraction from the cell requires suitable reagents and technique that can yield a better and efficient result. The protocol once followed should be able to increase the net content of protein in the lysate.

**Learning Objectives:**

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

- Understand the mechanism of TRIzol extraction.
- Examine the technique involved in culture growth.
- Interpret the results of the experiment.
- Troubleshoot at various steps in the experiment.

**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.
Inoculation of Bacterial culture

Take a tissue paper and wipe the bench of the laminar hood thoroughly with 70% ethanol.

Now, switch on the UV light of the hood and leave it for 5 minutes for complete sterilization.

The laminar hood is now ready for use. Switch the air flow first followed by switching the light. Now, open the door and place the materials required for inoculation.
Inoculation of Bacterial culture

Take the master plate containing the bacterial colony to be inoculated from the refrigerator and place it in the laminar hood.

Also, remove the autoclaved broth from the autoclave and place it in the laminar hood for inoculation in aseptic conditions.

Pick a bacterial colony using the toothpick from the master culture and inoculate the bacterial colony to the sterile broth. Always keep the burner on during inoculation and perform the action close to burner to avoid any contamination.
Inoculation of Bacterial culture

Place the inoculated tube in the shaking incubator.

Set the parameters like temperature, rpm and time for the incubator and switch it on.
Inoculation of Bacterial culture

Transfer the bacterial culture into the clean centrifuge tube under aseptic condition for further processing.
Centrifugation followed by Buffer Treatment

Now, place the centrifuge tube containing bacteria in the centrifuge. Balance it by placing a tube opposite to it filled the same volume of water.

Set the parameters to 10 minutes, 12000 rpm and 4 degree C and run the centrifuge.
Centrifugation followed by Buffer Treatment

Carefully remove the tubes out from the centrifuge.

Pipette out the supernatant without disturbing the pellet. Subject the pellet to further processing.
Centrifugation followed by Buffer Treatment

Add phosphate buffer to the pellet and proceed with washing to remove any excess broth. Proceed for cell lysis, once washing is completed.
Cell Lysis by Sonication

Place the sample on ice and set the parameters of the sonicator at 6 cycles of pulses for 5 seconds and 20% amplitude with 5 seconds gap. Since the technique uses ultrasonic sound waves, the user is advised to plug the ears with cotton plugs to prevent any damage to the ear drums. Sonication helps protein extraction by cell lysis.
Cell Lysis by Sonication

High frequency sound waves from the sonicator hit the cell, causing its lysis. The contents as a result of this lysis, are released into the buffer medium.
Cell Lysis by Sonication

Centrifuge the contents at 12000 rpm, 4 degree C for 1 minute.

The cell debris can be seen as a pellet. Pipette out the supernatant carefully into a new tube. Do not disturb the pellet and take out as much supernatant as possible.
Trizol Treatment

The supernatant obtained is a mixture of all the biomolecules that make up the cell and predominantly consists of proteins, DNA and RNA. TRIzol - chloroform is added to separate proteins from the nucleic acids.

TRIzol reagent consists of Guanidium isothiocyanate and phenol. While TRIzol helps in keeping the RNA intact, chloroform brings about separation of the components into three distinct phases.
Trizol Treatment

Add TRIzol reagent to the supernatant.

Vortex the tube thoroughly to ensure uniform mixing of the supernatant with the reagent.
Chloroform Treatment

Add chloroform to the sample and mix thoroughly till a milk-shake like solution is formed.

Keep the tube undisturbed for 5 minutes till separation of the three phases is seen.
Chloroform Treatment

Centrifuge the tube at 12000rpm, 4 degrees C for 10 minutes.

A clear separation of the three phases can be seen after centrifugation is over. The topmost layer consists of RNA, the middle layer consists of proteins while the lowermost layer is made of DNA.
Absolute Alcohol Treatment

Pipette out the upper aqueous layer containing RNA without disturbing the other two layers. Depending on the need, RNA can either be stored in a new tube or be discarded.

Now, add absolute alcohol to the tube and mix gently till the middle layer dissolves completely. Keep the tube undisturbed at room temperature for 3 minutes.

Centrifuge the tube for 5 minutes at 2000 rpm.
Acetone Treatment

Upon alcohol addition followed by centrifugation, the DNA forms a pellet in the tube while the proteins remain a part of the supernatant.

Carefully pipette out the supernatant and transfer it to a fresh tube without disturbing the DNA pellet.

Add chilled acetone to the tube containing the supernatant and vortex thoroughly to form a homogenous mix.
Acetone Treatment

Place the tube in a -20 freezer for at least an hour.

After an hour’s incubation remove the tube from the freezer. Subject the contents of the tube to centrifugation at 12000 rpm for 10 minutes and 4 degrees C. The proteins are seen as a pellet in the tube now.
Wash Buffer Preparation

Clean the surface of the balance. Now place a butter paper and tare the weight of paper before weighing.
Wash Buffer Preparation

Now weigh the contents for wash solution. Weigh the required amount of Guanidine Hydrochloride and transfer it to the tube.

Take the required amount of ethanol in a measuring cylinder and add it to the tube containing guanidine hydrochloride.

Vortex the tube to dissolve the salt and to form a uniform solution.

Place the tube in a refrigerator and store it at 4 degree C for further use.
Rehydration Buffer Preparation

For making rehydration buffer, weigh 0.02 g of CHAPS and 0.06 g of Urea and dissolve in water. Add 0.05% of Bromophenol Blue to the rehydration buffer. This helps in tracking the sample during electrophoretic run.

Add required amount of water and vortex the tube for complete mixing of the salts.

Store the tube at 4 degrees C for later use.
Rehydration Buffer Preparation

Discard the supernatant obtained in the tube without disturbing the protein pellet. Air dry the pellet to remove traces of acetone which could interfere with the washing step. Now, proceed with washing to remove the pink color of the pellet. Add wash buffer to the tube containing the pellet.

Vortex the contents thoroughly and centrifuge the tube at 12000rpm for 10 minutes.

Again, carefully discard the supernatant without disturbing the pellet and carry out wash step with the pellet till the pink color disappears to white.
Rehydration Buffer Preparation

To the pellet obtained after washing, add about 0.4 ml of rehydration buffer.

Rehydration buffer contains CHAPS which solubilise the proteins, while Urea present helps denature the proteins.
Rehydration Buffer Preparation

Vortex the tube to thoroughly mix the contents.
Sample Storage at -20°C

The sample can be stored at -20°C for use later. When needed for protein quantification, the contents of the tube can be thawed and used.

Please go through the following IDD's for more information.
Definitions of the Components/Keywords

Methodology for the Extraction of Bacterial Protein

1. **Protein**: Are the biomolecules, composing of amino acid, which forms the building block of the system and performs most of the function in the system.

2. **Bacterial Protein Extraction**: The process by which proteins from the cell are recovered for analysis purpose is called protein extraction. The chemicals involved in the extraction are:

   a) **Luria-Bertani Broth (LB)**: The LB broth consists of yeast extract as carbon source, peptone as amino acid source, NaCl to maintain osmo-regulation and water.

   b) **Trizol Reagent**: The reagent consists of phenol, guanidium thiocyanate and chloroform. Phenol and chloroform helps in phase separation while guanidium thiocyanate acts as a RNase inhibitor.

   c) **CHAPS**: 3-(Dimethyl[3-(4-[5,9,16-trihydroxy-2,15-dimethyltetracyclo[8.7.0.02,7.011,15]heptadecan-14-yl]pentanamido)propyl]azaniumyl)propane-1-sulfonate ("CHAPS") is a zwitterionic detergent and a constituent of rehydration buffer that is used to solubilize the proteins including membrane proteins.

   d) **Urea**: It is an organic compound in rehydration buffer that is used to denature protein.
What is the amino acid source in LB broth?

- Glucose (X)
- Amino acid
- Peptone (√)
- NaCl

I am sorry, the correct answer is Peptone.
What is the purpose of sonication?

- To mix the culture
- To denature the protein
- To denature the nucleotides
- To lyse the cells

I am sorry, the correct answer is To lyse the cells.
The reagent that separates the proteins and DNA-RNA from the cell is

- Phenol
- Glucose ✗
- Rehydration buffer
- Trizol ✓

I am sorry, the correct answer is Trizol.
Question: What is the constituent(s) of the upper layer after trizol separation?

Options:
- RNA
- DNA and RNA
- DNA, RNA and proteins
- Proteins and DNA

Feedback:
I am sorry, the correct answer is RNA.
The chemical that inactivates the RNAses is

- Trizol
- Phenol
- Guanidium -HCl
- NaCl

I am sorry, the correct answer is Guanidium -HCl.
References

Papers:


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