FREQUENTLY ASKED QUESTIONS (FAQs)

Q.No. 1: What is protein purification?

Ans: Proteins are the molecules that do interesting things in cells, they are involved in the production of all other molecules and cell structure. To purify the protein away from the other thousands of cellular proteins, so that its structure can be examined, or its interactions with other molecules studied more specifically, is called protein purification.

Q.No. 2: Who suggested the term protein?

Ans: The term "protein" comes from the Greek, "standing in front" and was suggested by Jons Jacob Berzelius in 1838.

Q.No. 3: Why proteins are important in biology?

Ans: They serve as catalysts that maintain metabolic processes in the cell, serve as structural elements both within and outside the cell. They are signals secreted by one cell or deposited in the extracellular matrix that are recognized by other cells and are receptors that convey information about the extracellular environment to the cell. They serve as intracellular signaling components that mediate the effects of receptors and are key components of the machinery that determines which genes are expressed and whether mRNAs are translated into proteins.

Q.NO. 4: Why protein purification is important?

Ans: They are involved in manipulation of DNA and RNA through processes such as DNA replication, DNA recombination, RNA splicing or editing. Beyond this, there are a number of important reasons for purifying proteins, and a brief list of these may serve to emphasize why individuals spend so much time and effort in this pursuit. By purifying a protein it can be clearly established that a particular biological activity (enzymatic activity, signaling capacity, etc.) actually resides in a unique protein. Purified proteins serve as extremely valuable biochemical reagents. It is remarkably valuable to be able to obtain things like purified growth factors or hormones, proteases, DNA polymerases, reverse transcriptases, ligases, phosphatases, or antibodies that recognize a particular epitope of interest. Once a protein is purified, it is possible to study its enzymology, understand its affinity for particular substrates, or dissect its

ability to catalyze enzymatic reactions.

Q.No. 5: What is the use of MALDi- TOF in protein purification?

Ans: MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight) is a technology that can provide much information about a protein, even when only small quantities of proteins are available.

Q.No. 6: What is 100 fold purification?

Ans: Purifying a protein to homogeneity, from a large volumes of highly viscous material, which can involve separating one milligram of the protein-of-interest (POI) from 100 mg of initial total protein is called a 100-fold purification.

Q.No. 7: Discuss briefly ion exchange chromatography technique?

Ans: Once viscosity has been largely eliminated and once the crude protein sample is particle free, it may be time to use ion exchange chromatography the most frequently employed chromatographic method for proteins. Early, small-scale testing with a relatively salt-free sample is advised. There are simple, syringe-operated ion exchange columns both anion exchange columns and cation exchange columns. These columns can be used to determine (within one-half of a pH unit) the isoelectric point of the protein. This is accomplished by equilibrating the two columns with low ionic strength buffers of varying pH values.

Q.No. 8: What are the hydrophobic ligands in HIC?

Ans: The hydrophobic ligands are usually attached to the porous hydrophilic gels via a 3-carbon spacer based on epichlorohydrin chemistry. From strongest binding to weakest binding ligands, the order is Phenyl >Octyl > Butyl > Methyl. Strongly hydrophobic ligands are appropriate for weakly hydrophobic proteins and weakly hydrophobic ligands for strongly hydrophobic proteins.

Q.No.9: What is Gel Filteration?

Ans: Gel filtration is the easiest chromatographic method in principle, but it is the hardest method to administer properly. Gel filtration separates macromolecules by size.

Q.No. 10: What is the difference between SEC and Gel filteration?

Ans: Size exclusion chromatography (SEC) is generally used as an analytical HPLC method while gel filtration is used primarily in preparative protein separations. Size exclusion HPLC utilizes small, rigid, uniform, spherical beads of 5 micrometer or 10 micrometer diameter. Small, porous, silica beads used in SEC provide higher resolution than low pressure gel filtration.

Q.No.11: What are the applications of HPLC?

Ans: The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include :

Pharmaceutical Applications

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

Environmental Applications

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

Applications in Forensics

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

Food and Flavour

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

Applications in Clinical Tests

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.

3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

Q.No. 12: What is the use of Batch methods in protein purification?

Ans: Batch methods are particularly useful in early stages when a sample is highly viscous or full of fine colloidal material. Such batch methods include ammonium sulfate precipitation, precipitation from other salt solutions, from aqueous solutions at low pH, or from organic solvents (usually acetone, ethanol, ethylene glycol, or polyethylene glycol). In some cases, recrystallization from salt solutions is possible. Even if crystals do not form, differential precipitation can be an effective purification method. A particularly effective batch method is isoelectric precipitation in which the pH of a dilute aqueous buffer is adjusted to the isoelectric point (pI) of the POI.

Q.No. 13: What do you mean by A free method?

Ans: While exploring repeated rounds of ammonium sulfate precipitation, the purification of rabbit-derived antibodies, goat anti-rabbit IgG, and chicken IgY. Because this process does not utilize Protein-A, and this method is called “A-Free.”

Q.No. 14: What is the cause of error in protein purity process?

Ans: Without achieving protein homogeneity, serious errors and experimental artifacts may arise. Even a 1% contaminant may contribute to erroneous observations. A minor contaminant (protein or otherwise) could significantly raise or lower an enzyme’s apparent activity level.

Q.No. 15: Discuss the criteria of evaluating the purity of proteins?

Ans: 1) Constant specific activity across a broad portion of the peak in the final preparative chromatography column.

2) Single, symmetric band by size exclusion HPLC.

3) Single band, in the correct MW region, on an SDS gel (or, for hetero-oligomers, the appropriate number of bands in the correct positions.

4) Unambiguous, single amino acid detected in N-terminal amino acid analysis. Inability to detect an N-terminal amino acid may also be taken as evidence of purity—not a very strong criterion as many other proteins have blocked N-

terminal amino acids.

5) Single band on a native polyacrylamide gradient gel (or appropriate number of bands of correct MW for heterodimers, heterotetramers, etc).

6) Single, sharp band by isoelectric focusing in an acrylamide gel or in a capillary isoelectric focusing system (or the appropriate number of bands for hetero-oligomeric proteins).

7) Unambiguous N-terminal peptide sequence by Edman degradation.

8) Single band by Western blot, if antibodies are available.

9) Single MW form by Maldi TOF (matrix assisted laser desorption time-of-flight mass spectrometry).