The 4 Basic Steps Of Protein Expression And Purification Explained

Protein expression and purification is the process of manufacturing recombinant proteins from recombinant DNA and is a vital component in biomedical research.

Proteins are the building blocks of our DNA and facilitate the majority of biological processes that take place in a cell such as cell growth, gene expression, intercellular communication, nutrient uptake and apoptosis (cell death). Coded instructions which act as a template for protein production are stored in DNA which is then transferred to message RNA (mRNA) which is responsible for protein production by followed defined sequences of amino acids.

The processes of expression and purification are used in the biotech industry to produce recombinant protein, these are proteins encoded with recombinant DNA and cloned in an expression vector which facilitates the gene expression and translation of mRNA. In addition, it is possible to utilize recombinant DNA and protein technology to increase protein production, modify gene sequences and manufacture useful products for commercial use. Essentially, recombinant proteins are manipulated forms of the original proteins that have been modified for various uses in the medical and biotechnology fields.

Proteins expressed or recreated using translated code from mRNA, share the same characteristics and functions as the native version they originated from. For this reason, recombinant protein and DNA technologies are vital tools in healthcare, medical research and biotechnology production for several reasons:

1. Manufactured proteins are an essential component in vaccine development and production
2. The development of more accurate, effective and faster diagnostic kits and monitoring devices
3. Development and production of synthetic human insulin needed to treat diabetes
4. Various bacteria, enzymes and hormones can be manufactured and reproduced
5. Recombinant proteins are used in create drugs for treating a range of diseases and health conditions including AIDS, asthma, cancer, cerebral apoplexy, Crohn's disease, heart failure, cystic fibrosis, dwarfism, hepatitis, multiple sclerosis and rheumatoid arthritis

In addition to being prominent in the healthcare and research sectors, recombinant proteins also play an important role in other areas such as:

1. Agriculture; the health and productivity of animals can be improved by increasing the nutritional value of feed by adding enzymes
2. Biomining and bioremediation; lab-produced microbes such as bacteria or archaea are used to extract valuable metals from lower-grade ores
3. Environmental issues; the conversion of waste into biofuels, cleaning up oil spills and toxic waste, and detecting contaminants in drinking water,
4. Food production; manufactured enzymes like amylases and lipases can be tailored to different food processing solutions

Manufacturing recombinant proteins via the processes of expression and purification may seem complex, difficult and time-consuming. However due to the right expertise, large capital investment and specialized laboratory equipment many protein manufacturers have been operating successfully for several years.

The production of proteins may seem overly technical, although it can generally be broken down into four stages, which you can read more about below.

Picture 1 of The 4 Basic Steps Of Protein Expression And Purification Explained

1. Protein Expression

Protein expression is the process of modifying, regulating and synthesizing proteins inside living organisms that act as a DNA vector containing a template for cells with the desired protein to be cultured on. The type of DNA vector depends on the protein being manufactured, for example, a Pichia pastoris expression system is based on methylotrophic yeast to grow specialized enzymes used in the production of insulin. Other systems for expressing proteins include algal, bacterial, cell-free and mammalian.

During expression, cells are transfected with a DNA vector containing a coded template of a protein to be transcribed before being lysed or burst to extract specific proteins.

2. Extraction and Stabilization

Proteins can be extracted using various techniques which are determined by the location and type of protein being synthesized, the kind of DNA vector and its final application. Typically, a gentle detergent based solution is used to lyse cells allowing for the parsing of subcellular structures without causing physical disruption to the proteins being extracted.

During cell lysis, certain structures such as the cell membrane and organelles can be disrupted causing unregulated enzymatic activity resulting in decreased protein function and yield. Therefore, to avoid this issue, manufacturers add inhibitors to lysis reagents that can block or deactivate proteases and phosphatases thus stabilizing cells and their protein production. Formulas containing protease and phosphatase inhibitors are available in both liquid and tablet forms for all types of extraction system.

Picture 2 of The 4 Basic Steps Of Protein Expression And Purification Explained

3. Purification

When recombinant proteins are extracted they can also be tagged to enable more effective and faster purification which involves a series of processes to isolate and separate specific proteins from a biological mixture consisting of cell components, whole cells and organic tissues.

The technique used in protein purification is referred to as chromatography and the procedure involves flowing a solution containing protein through a column made of different materials that the proteins interact with making them easier to separate.

There are several kinds of chromatographic procedures typically used to separate proteins, these include:

1. Affinity chromatography; proteins bind to molecules on the surface of specialized resins
2. Hydrophobic interaction chromatography; separation of proteins depending on surface hydrophobicity (how attracted proteins are to water molecules)
3. Ion exchange chromatography; Anion exchange resins with a positive charge are used to attract and separate negatively charged proteins
4. Immunoaffinity chromatography; targeted proteins bind to immobilized antibodies
5. Tagged protein purification; engineer antigen peptides tags can be added to proteins which can then be attracted and bound to immobilized antibodies
6. High-performance liquid chromatography uses high-pressure liquid to force the solution containing proteins through the column faster

4.Cleaning

The final step in the process of extracting and purifying proteins is to clean the sample, this is necessary because the various detergents and salts used in the extraction stage can have negative effects on the function and stability of proteins.

Contaminants can be reduced or removed using several procedures including:

1. Protein dialysis; unwanted compounds and molecules are removed by diffusing the solution through a semipermeable membrane
2. Protein desalting; gel filtration is used to remove salts by using a resin with pores large enough for salt molecules to pass through but too small for proteins to penetrate
3. Protein concentrators; centrifugation is used to force molecules through a membrane to separate proteins and reagents

Protein extraction and purification are essential processes in the biotechnology and medical research industries due to their applications in many areas of life. Even though the process of manufacturing proteins may seem complicated, there are in fact proteins that have become simple to produce by following the four steps of expression, extraction and stabilization, purification and cleaning.