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Protein Purification (Column Cromotography)

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

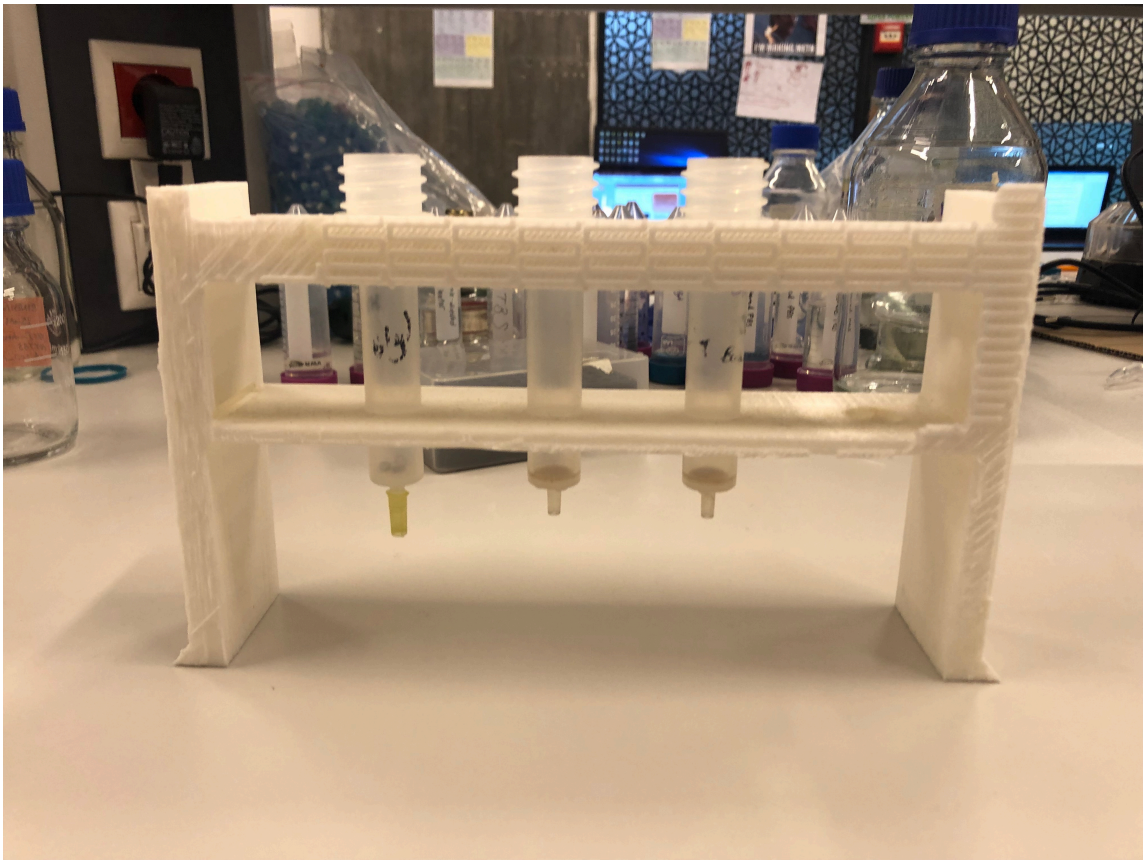
It is very difficult to purify a desired protein from a cell and tissue in a pure state. If the concentration of this protein is low, it is necessary to select the purification techniques appropriate for this protein to separate it from thousands of different proteins and to obtain it in pure form. The methods used in the purification of proteins are quite advanced.

Today, many enzymes and non-enzyme proteins have been isolated in pure and crystalline form. To study the amino acid composition and sequence, molecular weight, and other physical properties of a protein, it is first necessary to purify the protein. In order to isolate proteins from a group of tissues and cells, there are certain procedures that should be done first. In the first stage, the tissue or cell group where the protein we want to work at most is selected. The tissue and cells are homogenized by using buffer solutions to keep the protein we are interested in. The cell membrane and the nucleus membrane must be broken down. This homogenate is then subjected to centrifugation at certain rates to remove cell particles. The resulting protein mixture is then removed in the supernatant state to purify the proteins in pure form using one or more of the purification methods.

Guidelines

Binding buffer : 5 ml Immidiazole, 20 ml TBS, ddw up to 200 ml.
Wash Buffer : 8 ml Immidiazole, 20 ml TBS, ddw up to 200 ml.

1 2 ml of resin (Ni-beads) should be put onto columns. Wash column with double distilled water before using resin. 2000 µL Resin



2 1X Binding Buffer should be added until fill the whole column.

3 When all Binding Buffer is leaked, samples should be put into columns.

4 Wash columns and sample until all residues are gone. In the washing step take from sample and from bradford and put into 96 well plate.

10 µL sample

200 µL Bradford

5 As a last step, to keep the protein that is purified from samples, Elution Buffer is added and
transferred to new centrifuge tubes.

1000 µL Elution Buffer