Laboratory Experiments

Serum contains proteins, antibodies, peptides, hormones, cytokines, glucose, fats, and inorganic substances. You are required to isolate the protein albumin from a horse serum sample. What methods would be useful to isolate albumin from the serum sample?

Some points you should consider before you select a method are the following:

a. The molecular weight of albumin is about 60 kDa
b. A few other proteins in the serum sample have molecular weights in the range of 50-70 kDa
c. The chosen purification method should yield a high purity product
d. The serum albumin binds to a reactive blue dye molecule (Cibacron Blue F3GA)
e. Another molecule, sodium dodecyl sulfate (SDS), has higher affinity to bind the blue dye molecule

The following material can be useful in the purification of horse serum albumin:

f. A slurry of the agarose-Cibacron Blue F3GA matrix
(*Albumin binds to the Cibacron Blue dye through ionic, hydrophobic, and steric interactions at the fatty acid binding site. SDS disrupts these interactions of albumin with the Cibacron Blue allowing for the elution of the protein.)*
g. Sodium phosphate buffer containing SDS
h. Sodium phosphate buffer
i. Plastic gravity column
j. Pipettes
k. Test tubes
l. The purity of the albumin can be checked using SDS-PAGE analysis.

A handout with a possible method for performing this purification can be accessed by clicking the link below.

Lab Handout (PDF)

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The following are additional laboratory experiments which reinforce the concepts of affinity chromatography:

1. Branchini, B.; Ziolkowski, R., The separation of chymotrypsin and chymotrypsinogen: An affinity chromatography experiment for biological chemistry students, Journal of Chemical Education, v56 n4 p281-A179 Apr 1979.

2. Bering, C.L.; Kuhns, J. J.; Rowlett, R., Purification of Bovine Carbonic Anhydrase by Affinity Chromatography: An Undergraduate Biochemistry Laboratory Experiment, Journal of Chemical Education, vol. 75, Issue 8, p.1021

3. Hunt E. A.; Deo S. K., Board-Game Gel Filtration and Affinity Chromatography, Journal of Chemical Education, v86 n1 p19, 2009.