

# Biology protein lab report



**ASSIGN  
BUSTER**

The objective of this lab was to measure the amount of protein from a piece of beef liver. This was done by taking the liver, blending it and then using a centrifuge to separate the supernatant from the pellet. Once that was completed, ammonium sulfate was added to the supernatant, chilled and then spun for a second time. Next, 20 mL of water is added to the pellet, stirred and the volume was recorded. The teacher calculated the total mass of liver to be 10.098g.

Lastly a spectronic 20 at a wavelength of 540 nm is used to measure the absorbance of protein at different concentrations of the liver extract. The results for this showed that an absorbance of 540 nm, as the amount of liver extract increases, the absorbance increases. Introduction The main objective of this lab was to measure the amount of protein from a piece of beef liver, and then use the spectronic 20 to discover the concentration of protein found in the beef liver. When a solution containing protein is saturated with a salt (ammonium sulfate, for example), the high salt concentration can alter the proteins' configuration (tertiary and quaternary structure) by competing with water for hydrophilic sites. Proteins are kept in solution normally by their hydrophilic interactions with water. " (43) When these interactions are disturbed, the molecules of protein change their shape and aggregate form a precipitate. If the salt is taken out of the solution, the proteins will go back into the solution and sometimes return to their normal shape.

Salting out is the method of causing " proteins to form a precipitate in the presence of high concentrations of salt. " (43) Most proteins have a particular concentration of salt which can be used to form a precipitate. By increasing the concentration of salt in the liver it will slowly increase the

amount of proteins that are salted out. After the proteins have been salted out and redissolved, they are assayed into different “ colorimetric reactions. ” (43) The way this is done is by using a biuret reaction.

The biuret reagent is an alkaline copper sulfate solution, the color is blue. The biuret reaction produced a purple complex between the copper ions and the peptide bonds. “ Molecules containing two or more peptide bonds– they’re called “ polypeptides”–will give a positive biuret reaction. Proteins give a particularly strong reaction because they have so many peptide bonds. All proteins have roughly the same number of peptide bonds per gram of protein. ]” (43-44) The strength of the color formed in the biuret reaction is measured using the spectronic 20.

A standard curve is constructed and used to determine the concentration of protein in the homogenized beef liver. The purpose of this lab was to determine the amount of protein contained in beef liver. To do this, the biuret method of protein was used along with a spectrophotometer in measuring the absorbance of different protein concentrations. The biuret method requires the solution being tested be mixed with a biuret reagent and run through a spectrophotometer. This was accomplished by first setting up two sets of six test tubes. The first six test tubes are used to generate a standard curve.

This required putting specific amounts of water, biuret reagent, and bovine serum albumin (BSA) into each of the six test tubes. These tubes were then added to the spectronic 20, the first tube was used as the blank. The results of these findings were recorded in Table 1. Next, a piece of liver

about 10 grams ( the groups weight of liver was 10. 274g) was cut up into small pieces and added to a blender with 20 mL of water (all of the classes liver and water was added to the blender at this time). The volume total for the whole class was 140 mL.

The mixture was then separated and returned to each group. At this point the mixture is homogenized, to remove the unhomogenized the fragments of the liver are added to a centrifuge for 15 minutes spinning at 10, 000 RPM. After the fragment was spun, the supernatant was poured into a graduated cylinder and the pellet was discarded into centrifuge to separate the supernatant from the pellet. The volume of the supernatant was recorded at a volume of 12. 5 mL. Once that was completed, the salting out step was completed by added ammonium sulfate to the supernatant.

For every 2 mL of the supernatant, one gram of ammonium sulfate are dissolved into the solution. Since the volume of the supernatant was 12. 5 mL 6 grams of ammonium sulfate were added. The centrifuge tube was then added to an ice bath for approximately 10 minutes. After the 10 minutes the liquid is spun in the centrifuge for a second time for 5 minutes at 10, 000 RPM, this time the supernatant was discarded and the pellet was kept. Lastly 20 mL of water is slowly added to the pellet, and carefully stirred. The volume of the liver protein extract was recorded at 22. 3 mL.