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Quantitative Determination of Serum Protein by Bradford method using UV spectrometer

Aim: To perform quantitative determination of Serum protein by Bradford method.

Apparatus Required:

1) Bradford Reagent
2) Phosphate Buffered Saline
3) pipette
4) Microcentrifuge tube
5) Milk Sample
6) Cuvettes
7) Different protein standards
8) ~~Spectrometer~~ Spectrometre.

Theory:

Protein quantization is one of the most commonly performed procedures in a biotechnology laboratory. The protein concentration and the milk will be measured using Bradford Assay.

Procedure:

1) The 2-200µl. pipette was set up to 98 µl. The PBS sample was labelled into an empty microcentrifuge tube
2) Then, we switched to 2-200 µl pipette and the pipette was set to 2 µl.
3) The 2µl pipette was labelled with milk sample.
4) The milk sample and PBS was mixed either by pipetting or vortexing.
5) This mixture had diluted the milk to one part in 50.

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6) The two cuvettes were labelled as control and sample. The two cuvettes should not be handled otherwise light passes through and made certain levels well above the area.
7) The pipette 20 µl. diluted milk sample was added into the cuvette labelled sample and 20 µl. of 1× PBS was added to the cuvette labelled control.
8) Then, the protein standards were set up and protocols were observed for correct concentration.
9) Each cuvettes were labelled and marked with the corresponding concentration.
10) 20 µl of protein standard were added into the corresponding cuvette.
11) Make sure to use a clean pipette tip for each sample.
12) Now you are ready to add the Bradford Reagent to all the cuvette you have prepared including the sample and control.
13) Add 1ml of 1× Bradford reagent to each of the cuvettes.
14) Mix completely by pipetting up and down with the micropipette.
15) Incubate the cuvettes at room temp for 5 mins.
16) After 5 mins visually compare the cuvette containing the milk sample to the cuvettes containing the protein standards.
17) Determine the standard that most closely matches the colour of the milk sample. Estimate the protein concentration of the milk sample based upon the visual comparison.
18) To determine the protein conc. using a spectrophotometer select for the Bradford assay.
19) When asked, insert blank into the spectrophotometer insert the cuvette labelled control. Use the blank to set the spectrometer to zero absorbance or 100% transmittance.

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20) If instructed read the absorbance of the seven protein standard and record the absorbance values.

21) Remove the cuvette labelled blank and insert the milk sample into the spectrophotometer.

OBSERVATION

The readings were noted 0.548 and 0.978 for the cuvette labelled as sample.

CONCLUSION

We have successfully determined the serum protein quantitatively by the Bradford method.

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Quantitative Determination of Blood Glucose by glucose oxidase method.

Aim:-

To determine blood glucose by glucose oxidase method.

Materials Required :-

1. Blood sample collected in EDTA bottle.
2. Glucose oxidase Reagent.
3. 2 N sodium hydroxide (NaOH)
4. Sodium sulphate - zinc
5. 200mg of standard glucose solution.
6. Distilled Water
7. Test tubes
8. Micropipettes
9. Eppendhof tube
10. 5 ml. glass pipette.
11. Pipette pump.

Theory :-

Glucose is a major carbohydrate present in blood and is produced from dietary sources. The maintainance of glucose in our body is mainly controlled by hormones insulin and glucagon. A defect in insulin secretion, insulin action or both results initially on impaired glucose tolerance and cause hyperglycemia which further leads to diabetes mellitus. So, a simple, rapid and economical method of determing the blood sugar level is obvious, especially in the management of diabetes mellitus. The calorimetric method, combined with an enzymatic reaction is most widely used for the determination of glucose in human serum.

Estimation of glucose by the enzyme glucose oxidase gives the true glucose conc due to its high specificity and sensivity Glucose oxidase catalyzes the oxidation of β-D glucose to D-glucono-δ-lactone and hydrogen peroxide.

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PROCEDURE:

1) First 900 µl. of sodium sulphate-zinc sulphate was transferred to 1.5 ml microfuge tube, which as labelled as test.
2) Then, 50µl of 2N NaOH and 50µl. of blood sample was transferred into the same microfuge tube.
3) Then, the sample was centrifuged at 3000 rpm for 5 min.
4) After 5 min, the centrifuge tube was taken out and 600 µl of supernatant was transferred carefully from the centrifuge tube to a test tube labelled as "TEST".
5) Then, 125 µl of glucose standard solution was transferred to a test tube labelled as 50 mg/dl.
6) Similarly, 250 µl of glucose standard solution was added to the second tube labeled as 100 mg/dl.
7) Then, 375 µl, of glucose standard was added to the third tube labeled as 150 mg/dl. and 500 µl. of glucose standard was added to the fourth test tube labelled as 200 mg/dl
8) Then a fresh tip was inserted to the micropipette and 500 µl. of distilled water was transferred to a test tube labeled as "blank"
9) In the same way, 375 µl. of distilled water to the first test tube, 250 µl of distilled water to the second test tube and 125 µl. to the third tube was added.
10) Then, the glass pipette was connected to the pipette pump and 5 µl of glucose oxidase reagents was added to each test tube.
11) The test tubes were covered with aluminium foil and placed in the water bath at 37°C for 1 hour.
12) After 1 hour, the test tubes were taken out from the water bath and the blank solution was transferred to the cuvette.

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13) The cuvette was inserted into the slot of colorimeter and the value was set at zero.

14) Then, 50 mg/dl. solution was transferred in the first test tube and the cuvette was inserted into the slot of colorimeter and readings were noted.

15) Similarly the reading for all the test tube were taken and plotted in a graph.

OBSERVATION

① The reading for the first test tube for 50 mg/dl was found to be 0.37 (absorbance).

CONCLUSION

From this experiment, we have successfully determined blood glucose by glucose oxidase method.

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