

Protein estimation in biological and non-biological fluids

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Method Article

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Abstract

Among the various methods of protein estimations, Lowry's modified method is the most efficient. It can be used to measure proteins as low as 1 microgram per ml.

Introduction

The method is Lowry's method of protein estimation. The copper protein complex reduces the mixture of phosphomolybdate-phosphotungstate present in Folin reagent. This gives blue color complex. The intensity of blue color depends on the concentration of protein.

Reagents

Reagent A: 2% sodium carbonate, 0.1 N NaOH. Reagent B: 0.5% Copper Sulphate pentahydrate, 1% sodium potassium tartrate. Reagent C: Mix 50 ml of Reagent A and 1 ml of reagent C. (Always use fresh solution) Reagent D: it is commercially available as 2N solution. For use in this test is to be diluted 1:1 (Just before use) Protein Standard solution: Bovine Serum Albumin (BSA) 1mg per ml.

Equipment

1. Visible Spectrophotometer. 2. Quartz cuvetts. 3. Test Tubes.

Procedure

Prepare Standard Solution: 1. prepare working standard BSA solution of 200ug per ml from the standard BSA solution (1mg per ml) 2. Take 200 ul, 400 ul, 600 ul, 800 ul and 1000 ul of standard solution. 3. Take 100 ul of test protein solution. 4. Make up the volume to 1 ml in all the tubes. 5. Make up the volume upto 1 ml in all the tubes. 6. Take 1 ml of diluent as a blank. Test Proper: 1. Add 5 ml of Reagent C to all tubes including blank and mix vigorously. 2. Mix well and allow to stand for 10 minutes. 3. Add 0.5 ml of reagent D (freshly diluted 1:1) and mix all the tubes vigorously. 4. Incubate the tubes in dark for 30 minutes. 5. Read the absorbance at 660 nm. 6. Calculate the concentration of protein in the test solution by making a standard graph.

Timing

Around 1 hour.

Troubleshooting

The Protein solution mixed with SDS, Tris, EDTA, thiol reagent, potassium, magnesium, etc. do not reliable results in this test. These compounds are to be removed by precipitation of protein with 10%

trichloroacetic acid. And finally redissolve the protein pellet in 2N NaOH solution. The folin reagent must be stored in 4 deg C. A good quality reagent is straw yellow in colour.

Anticipated Results

The colour of protein solution at the end of test is blue in color. If the color is not blue the test should be repeated.

References

Folin, O and Ciocalteu, C. 1927. Journal of Biological Chemistry. 73: 627. Lowry, OH, Rosebrough NJ, and Randall, RJ. 1951. Journal of Biological Chemistry. 193:265.