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Coomassie Protein Assay

Student's Handbook

(Cat. # BE-402L)

OBJECTIVES 3
BACKGROUND 3
MATERIALS FOR EACH GROUP 4
PROCEDURE 4
I. LOWRYPROTEIN ASSAY 4
II. DETERMINATION OF THE PROTEIN CONCENTRATION OF THE UNKNOWN PROTEIN 5
RESULTS, ANALYSIS & ASSESSMENT 6

OBJECTIVES

- Learn the principles of protein assays.
- Determine protein concentrations using the Lowry Protein Assay.

BACKGROUND

The determination of protein concentration is an essential technique in all aspects of protein studies and proteomics. This lab activity is designed to teach students the principles behind a common protein estimation assay known as the Lowry Protein Assay.

Although there are a wide variety of protein assays available none of the assays can be used without first considering their suitability for the application. Each method has its own advantages and limitations and often it is necessary to obtain more than one type of protein assay for research applications. Protein assays based on these methods are divided into two categories: dye binding protein assays and protein assays based on alkaline copper.

The dye binding protein assays are based on the binding of protein molecules to Coomassie dye under acidic conditions. The binding of protein to the dye results in a spectral shift, the color of Coomassie solution changes from brown (absorbance maximum 465nm) to blue (absorbance maximum 610nm). The change in color density is read at 595nm and is proportional to the protein concentration.

In the copper ion based protein assays, protein solutions are mixed with an alkaline solution of copper salt, cupric ions (Cu^{2+}). The protein assay is based on the interaction of cupric ions with protein in an alkaline solution and is commonly referred to as the Biuret assay. The interaction of cupric ions (Cu^{2+}) with protein results in a purple color that can be read at 545nm. The amount of color produced is proportional to protein concentration.

Under alkaline conditions cupric ions (Cu^{2+}) chelate with the peptide bonds resulting in reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}). The Cuprous ions can also be detected with Folin Ciocalteu Reagent (phosphomolybdic/phosphotungstic acid); this method is commonly refereed to as the Lowry method. Cuprous ions (Cu^{+}) reduction of Folin Ciocalteu Reagent produces a blue color that can be read at 650-750nm. The amount of color produced is proportional to the amount of peptide bonds, i.e. size as well as the amount of protein/peptide.

In this lab activity students will study perform the Lowry protein assay methods.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components are shared by the whole class and should be kept on a communal table.

- 1.5ml Copper Solution
- 15ml Folin Ciocalteu Reagent
- 0.7ml Protein Standard (2mg/ml BSA)
- DI Water
- 350μl Unknown Protein
- 14 Centrifuge Tubes (2ml)

PROCEDURE

Wear gloves throughout the whole procedure.

I. LowryProtein Assay

Prepare Prediluted Protein Standards (BSA)

1. Add 0.7ml DI water to the tube with the Protein Standard (2mg/ml BSA) to dilute to make a 1mg/ml BSA solution.

2. For a standard curve, prepare a range of protein standards with the diluted BSA (1mg/ml) from step 1, as indicated in Table 1.

Table 1

BSA Concentration (mg/ml)	0.0	0.1	0.3	0.5	0.8	1.0
Diluted BSA (ml)	0.00	0.05	0.15	0.25	0.40	0.50
DI Water (ml)	0.50	0.45	0.35	0.25	0.10	0.00

3. Label two sets of tubes with 0, 0.1, 0.3, 0.5, 0.8 and 1.0 and transfer 0.05ml of the appropriate standard to the tubes.

4. Label two tubes for the Unknown Protein and add 0.05ml of each sample to the appropriate tube.

5. Add 0.1ml Copper Solution to each tube. Gently mix by vortexing.

6. Add 1ml Folin Ciocalteu Reagent and incubate at room temperature for 30 minutes.

7. In the meantime, turn on the spectrophotometer to allow it to warm up. Adjust the wavelength to 650-700nm.

8. Add 1ml distilled water to a cuvette to zero the absorbance of the spectrophotometer. Measure the absorbance of each tube and record the values in

the results section. See Section IV to determine the concentration of Unknown Protein.

The absorbance can be measured with a microplate reader instead of using a spectrophotometer. Transfer 250µl from each assay tube to a microtiter plate well. Add 250µl distilled water to a well as reference blank. Read the absorbance at 650-700nm.

II. Determination of the Protein Concentration of the Unknown Protein

Determine the protein concentration with traditional paper plot.

1. Draw the points with protein concentrations as *x* values and the average absorbance as *y* values on a grid or graph paper.

2. Draw a straight line through the points.

3. Lookup the Unknown Protein concentration from the plot using the absorbance value of the Unknown Protein.

Determine the protein concentration using Microsoft Excel

1. Input the BSA standard concentration in a column and the absorbance value in a second column.
   Highlight the data in the two columns
   From the menu bar, choose **Insert** then **Chart.**
   A "Chart Wizard" Menu will appear

2. Under the "Standard Types" tab:
   For "Chart type" select: XY (Scatter)
   For "Chart sub-type" select the top chart
   (The one without any lines connecting the points) Click *Next*>

3. Under series in select columns
   Click *Next*>

4. Under the "Titles" tab: Type in appropriate titles (names) for the graph (chart) and the x and y-axes
   (e.g., Chart title: Lowry Assay, x-axis: Absorbance, y-axis: Protein Concentration (mg/ml))
   Under the "Legend" tab: Click on the checkmark next to "Show legend"
   (The checkmark should disappear), Click *Next*>

5. Click on circle to left of "As object in" (a dot should appear in the circle)
   Click *Finish.*
   Your graph should appear on the sheet where your data is.

6. Highlight the chart by clicking inside the chart area.
   From the menu bar, choose **Chart** then **Add Trendline**
   The "Add Trendline" window will appear

7. Under the "Type" tab: For "Trend/Regression type" select: Linear

8. Under the "Options" tab:
   Click on box to left of "Display equation on chart" (a checkmark appears)
   Click on box to left of "Display R-squared on chart" (a checkmark appears), Click *OK.*

9. Move the equation and R-squared value to a suitable location on the graph. Save and print the data sheet.

10. Use the equation on the chart to calculate the concentration of your unknown protein.

RESULTS, ANALYSIS & ASSESSMENT

Lowry Protein Assay

Tube #	Absorbance I (650nm)	Absorbance II (650nm)	Average	Protein Conc. (mg/ml)
0				0
0.1				0.1
0.3				0.3
0.5				0.5
0.8				0.8
1				1
Unknown Protein-I				

Q. Determine the concentration of the "Unknown Protein" and the range of total amounts of protein used in each assay reaction for the three assays and comment on your findings.

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Q. Briefly describe the principles behind the protein assay and their weakness and strengths.

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