G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Biuret Protein Assay

Teachers Handbook

(Cat. # BE-402B)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

- 4 bottles Protein Assay: Protein Standard (2mg/ml BSA)
- 160 Centrifuge Tubes (1.5ml)
- 1 bottle Protein Assay: Biuret Reagent-I
- 1 bottle Protein Assay: Biuret Reagent-II

SPECIAL HANDLING INSTRUCTIONS

- Protein Standard can be stored at room temperature up to 6 weeks
- For long-term storage, store at 4°C.
- All other reagents can be stored at room temperature.

The majority of reagents and components supplied in the *BioScience Excellence*™ kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
P381	Protein Assay: Biuret Reagent-I	Toxic

ADDITIONAL EQUIPMENT REQUIRED

- DI Water
- Spectrophotometer and cuvettes or microplate reader and microplate

TIME REQUIRED

- 2-4 hours

OBJECTIVES

- Learn the principles of protein assays.
- Explore the methods used for quantification of proteins.
- Determine protein concentrations using three separate methods.
- Study the effect of common laboratory reagents on the methods of for protein quantification.

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 protein estimation and three of the most widely used methods in protein estimation. The first two are the alkaline copper solution methods and the third is the dye binding protein assays, they are: Biuret Protein Assay, Lowry Protein Assay, and the CB Protein Assay.

The "Assays for Protein Quantification" kit provides all the reagents required to perform all three protein assays in a single lab activity. An often underestimated factor in quantifying protein is the presence of non-protein interfering agents, such as salts and detergents. This kit teaches students about common laboratory agents that affect the protein assays, the reasoning behind their interferences and how to overcome the interference. Students also learn how to select a protein assay for different applications.

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 assay (CB Protein Assay) is 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 referred 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 Biuret protein assay methods.

TEACHER'S PRE EXPERIMENT SET UP

1. Mix equal volume of Biuret Reagent-I and Biuret Reagent-II to make Working Biuret Reagent just before the start of the experiment. Each student group needs 20ml Working Biuret Reagent.

2. To prepare the unknown standard, label six tubes as Unknown Protein and add 0.35ml Protein Standard and 1.75ml water to each tube. Shake to mix.

3. Aliquot reagents for each student group according to the next section.

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.

- 15ml Working Biuret Reagent
- 3ml Protein Standard (2mg/ml BSA)
- 1.2ml Unknown Protein
- DI Water
- 14 Centrifuge Tubes (2ml)

PROCEDURE

Always wear gloves and protective clothing throughout the whole experiment.

I. Biuret Protein Assay

1. Label two sets of tubes with the numbers 1, 2, 3, 4, 5, and 6 for preparation of a standard curve. Label two tubes for the Unknown Protein. Pipette distilled (DI) water and Protein Standards solution into the tubes as indicated in Table 1 below. Pipette 0.5ml Unknown Protein solution to each labeled tube.

Table 1

Tube# (In Duplicate)	*Distilled (DI) Water (ml)*	*Protein Standard (2mg/ml) (ml)*	*Unknown Proteins*	*Protein Conc. (mg/ml)*
1 (Blank)	*0.5*	*0*	*--*	*0.0*
2	*0.4*	*0.1*	*--*	*0.4*
3	*0.3*	*0.2*	*--*	*0.8*
4	*0.2*	*0.3*	*--*	*1.2*
5	*0.1*	*0.4*	*--*	*1.6*
6	*0*	*0.5*	*--*	*2.0*
Unknown Protein	*0*	*--*	*0.5*	

2. Add 1ml Working Biuret Reagent to each tube. Tighten the caps and vortex briefly to ensure thorough mixing of the protein and the Biuret Reagent.

3. Incubate the tubes for 20 minute at room temperature.

4. In the meantime, turn on the spectrophotometer to allow it to warm up. Adjust the wavelength to 540nm.

5. Add 1ml distilled water to a cuvette to zero the absorbance of the spectrophotometer. Measure the absorbance of each tube and record the value 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 520-570nm.

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: Biuret 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

Biuret Protein Assay (Section I)

Tube #	Absorbance I (540nm)	Absorbance II (540nm)	Average	Protein Conc. (mg/ml)
1 (Blank)				0.0
2				0.4
3				0.8
4				1.2
5				1.6
6				2.0
Unknown Protein				0.58

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.

A. Unknown protein concentration is approximately 0.6mg/ml. The range is 0.04mg–1mg BSA protein used the linear part of the curve.

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

A: Biuret Protein Assay- based on binding of copper ions to peptide bonds under alkaline condition which produces purple color. Weakness – not very sensitive and requires large amounts of protein (0.04mg – 1 mg BSA protein per reaction volume).

Last saved: 4/26/2018 SM

www.GBiosciences.com

G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Biuret Protein Assay

Student's Handbook

(Cat. # BE-402B)

OBJECTIVES

- Learn the principles of protein assays.
- Determine protein concentrations using the Biuret 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 Biuret 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 referred 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 Biuret 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.

- 15ml Working Biuret Reagent
- 3ml Protein Standard (2mg/ml BSA)
- DI Water
- Unknown Protein
- 14 Centrifuge Tubes (2ml)

PROCEDURE

Always wear gloves and protective clothing throughout the whole experiment.

I. Biuret Protein Assay

1. Label two sets of tubes with the numbers 1, 2, 3, 4, 5, and 6 for preparation of a standard curve. Label two tubes for the Unknown Protein. Pipette distilled (DI) water and Protein Standards solution into the tubes as indicated in Table 1 below. Pipette 0.5ml Unknown Protein solution to each labeled tube.

Table 1

Tube# (In Duplicate)	*Distilled (DI) Water (ml)*	*Protein Standard (2mg/ml) (ml)*	*Unknown Proteins*	*Protein Conc. (mg/ml)*
1 (Blank)	*0.5*	*0*	*--*	*0.0*
2	*0.4*	*0.1*	*--*	*0.4*
3	*0.3*	*0.2*	*--*	*0.8*
4	*0.2*	*0.3*	*--*	*1.2*
5	*0.1*	*0.4*	*--*	*1.6*
6	*0*	*0.5*	*--*	*2.0*
Unknown Protein	*0*	*--*	*0.5*	

2. Add 1ml Working Biuret Reagent to each tube. Tighten the caps and vortex briefly to ensure thorough mixing of the protein and the Biuret Reagent.

3. Incubate the tubes for 20 minute at room temperature.

4. In the meantime, turn on the spectrophotometer to allow it to warm up. Adjust the wavelength to 540nm.

5. Add 1ml distilled water to a cuvette to zero the absorbance of the spectrophotometer. Measure the absorbance of each tube and record the value 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 520-570nm.*

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: Biuret 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

Biuret Protein Assay (Section I)

Tube #	Absorbance I (540nm)	Absorbance II (540nm)	Average	Protein Conc. (mg/ml)
1 (Blank)				0.0
2				0.4
3				0.8
4				1.2
5				1.6
6				2.0
Unknown Protein				

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.

_____
_____
_____
_____

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

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Last saved: 4/26/2018 SM

www.GBiosciences.com