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

A Geno Technology, Inc. (USA) brand name

Hydrophobic & Hydrophilic Proteins

Teacher's Guidebook

(Cat. # BE-404)

MATERIALS INCLUDED

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

- 6 vials Protein: Tissue Extract & Pestle
- 6 vials Protein: Hemoglobin
- 1 bottle Hydrophilic Protein Extraction Buffer
- 1 bottle Hydrophobic Protein Extraction Buffer
- 6 BlueBalls
- Lowry Protein Assay Kit
    - 1 bottle Protein Assay: Folin Ciocalteu Reagent
    - 1 bottle Protein Assay: Copper Solution
    - 1 bottle Protein Assay: Protein Standard (2mg/ml BSA)
- 1 bottle Protein Extraction & Dilution Buffer
- 180 Centrifuge Tubes (1.5ml)

SPECIAL HANDLING INSTRUCTIONS

- Protein Standard can be stored at room temperature for 6 weeks. Store at 4°C for long-term storage.
- All other reagents can be stored at room temperature.

ADDITIONAL EQUIPMENT REQUIRED

- Low speed centrifuge for 1.5-2ml tubes
- Incubator
- Spectrophotometer and cuvettes or microplate reader and microplate

TIME REQUIRED

- 3-4 hours

OBJECTIVES

- Learn to extract different types of proteins from animal tissues.
- Class proteins based on their solubility.
- Learn the characteristics of hydrophobic and hydrophilic molecules in aqueous solution.
- Learn fractionation of hydrophilic and hydrophobic proteins from biological samples.
- Study the role of detergents on protein solubility.
- Study the role of detergents in protein research and analysis.

BACKGROUND

Recent proteomic studies have led scientist to estimate that there are almost a million *different* proteins in a single human cell. The functions and properties of these proteins are highly distinct, ranging from structural proteins involved in cell integrity, including hydrophobic cell membrane proteins, to soluble signal proteins that are responsible for passing cellular messages from the cell membrane to the nucleus. A major property of proteins that determines their function and location is their solubility within a cell. There are two major classes; the hydrophobic (water-"scared") and hydrophilic (water-"friendly") proteins.

This lab activity is designed to demonstrate the different classes of protein molecules and their classification based on the solubility of protein molecules. Students learn fractionation of soluble, insoluble membrane proteins, and cytoskeletal proteins from a tissue sample. The insoluble protein fraction is further fractionated in to hydrophilic and hydrophobic membrane proteins. Cell membrane structure and the role of hydrophobic membrane proteins are considered. This lab activity also provides an opportunity to understand characterization of detergents and the role of detergents in the solubilization of hydrophobic membrane proteins.

This lab activity begins with the demonstration of the properties of hydrophobic and hydrophilic molecules, followed by the extraction of proteins from a tissue sample in to soluble and insoluble protein fractions. The insoluble protein fraction is further fractionated based on their hydrophobicity (hydrophobic and hydrophilic proteins) using temperature induced phase transition of a detergent preparation.

This kit is supplied with two extraction buffers: Hydrophilic Protein Extraction Buffer consists of a detergent free buffered salt solution optimized for the extraction of soluble proteins from tissue samples. The Hydrophobic Protein Extraction Buffer is a buffered salt solution with an optimized mixture of non-ionic detergents that have phase transition properties, i.e., the detergent separates from the buffer at room temperature. The Blue Balls are glass balls coated with a hydrophobic dye, specifically developed by G Biosciences, for use with detergents for teaching and research applications. This kit is also provided with the hydrophilic protein hemoglobin to study

the behavior of hydrophilic proteins. Students will test the behavior of hydrophobic and hydrophilic agents and apply their findings to the extraction of hydrophobic and hydrophilic proteins from a test tissue sample.

TEACHER'S PRE EXPERIMENT SET UP

1. The kit provides 6 vials of animal Tissue Extract for your convenience. If you prefer, provide each student group with 0.05gm fresh chicken liver or other tissue. If fresh tissue is provided, empty the supplied Tissue Extract tube of the content and wash the tube with lab water. Supply each group with the pestle & tube for grinding fresh tissue.

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

3. Chill the Hydrophobic Protein Extraction Buffer on ice for 30 minutes and invert 3-4 times to mix the content before aliquoting.

MATERIALS FOR EACH GROUP

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

- 1 vial Tissue Extract & Pestle or 50mg fresh liver tissue & 1 grinding tube (tissue extract tube) & Pestle
- 1.5ml Hydrophilic Protein Extraction Buffer
- 1ml Hydrophobic Protein Extraction Buffer (Must be kept on ice until used)
- 1 BlueBall
- 1 vial Hemoglobin
- 1 bottle Folin Ciocalteu Reagent (shared with whole class)
- 1 bottle Copper Solution (shared with whole class)
- 1 bottle Protein Standard (2mg/ml BSA) (shared with whole class)
- 4ml Protein Extraction & Dilution Buffer
- 27 Centrifuge Tubes (1.5ml)
- Marker Pen

PROCEDURE

Wear gloves throughout the whole experiment.

Note: *Chill Hydrophilic Protein Extraction Buffer and Hydrophobic Protein Extraction Buffer on ice for 5-10 minutes before starting the experiments.*

I. Extraction of hydrophobic dye from the BlueBall

1. Place the BlueBall in a tube and add 0.2ml Hydrophilic Protein Extraction Buffer to the tube.

2. Incubate for 5 minutes, tap the tube and examine the BlueBall for any loss of blue dye. Record your observations.

3. Gently invert the Hydrophobic Protein Extraction Buffer a few times to mix. Add 0.2ml ice-cold Hydrophobic Protein Extraction Buffer to the tube containing the BlueBall.

4. Mix the buffers by gently tapping the tube. Incubate for 20 minutes on ice, periodically mixing the content by tapping the tube. Examine the BlueBall and record your observations.

5. Incubate for 15 minutes in an incubator or water bath set at 37°C.

6. Centrifuge the tube for 5 minutes, and then examine the tube carefully. Two phases, the top aqueous detergent poor phase and the bottom detergent rich phase, will be visualized. Record the distribution of the hydrophobic blue dye.

7. Save the contents of the tube for later use.

II. Extraction of hemoglobin

1. Add 0.2ml Hydrophilic Protein Extraction Buffer to the tube containing hemoglobin. Incubate for 5 minutes and tap the tube to see if the hemoglobin dissolves. Record your observations.

2. Gently invert the Hydrophobic Protein Extraction Buffer a few times to mix. Add 0.2ml ice-cold Hydrophobic Protein Extraction Buffer to the tube containing the hemoglobin.

3. Mix by gently tapping the tube. Incubate for 5 minutes and periodically mix the content by tapping the tube.

4. Incubate for 15 minutes in an incubator or water bath set at 37°C.

5. Centrifuge the tube for 5 minutes then examine the tube carefully. Two phases, the top aqueous detergent poor phase and the bottom detergent rich phase, will be visualized. Record the distribution of the hemoglobin.

6. Transfer the contents of the tube to the tube containing the BlueBall (Section I).

7. Mix the contents by tapping the tube. Incubate for 10 minutes in an incubator or water bath set at 37°C. And then centrifuge for 5 minutes. Record your observations.

III. Extraction of hydrophobic and hydrophilic proteins from a tissue sample

1. Add 0.2ml Hydrophilic Protein Extraction Buffer to the tube containing the tissue extract or fresh tissue (skip step 2 if using fresh tissue).

2. Incubate for 5 minutes at room temperature.

3. Grind the tissue using the pestle and slowly add 0.3ml Hydrophilic Protein Extraction Buffer and continue to grind the tissue until a uniform homogenate forms.

4. Centrifuge the tube for 5 minutes at maximum speed 5,000xg and transfer the supernatant to a new tube. This is your soluble hydrophilic proteins. Record the volume of your protein solution.

5. Add 0.5ml Hydrophilic Protein Extraction Buffer to the pellet and break the pellet with the pestle.

6. Gently invert the ice-cold Hydrophobic Protein Extraction Buffer a few times to mix. Add 0.5ml ice-cold Hydrophobic Protein Extraction Buffer to the tissue homogenate. Vortex the tube to mix the content. Incubate for 10 minutes on ice with periodic vortexing the tube.

7. Transfer the tube to a 37°C incubator or water bath. Incubate for 10 minutes. Vortex the tube at 5 minutes and at the end of the incubation.

8. Centrifuge the tube for 5 minutes at maximum speed 5,000xg. Examine the tube carefully. Two phases, the top aqueous detergent poor phase containing mild hydrophobic proteins and the bottom detergent rich phase containing hydrophobic proteins, will be observed. The pellet in the tube is insoluble material. This insoluble cellular material consists of proteins not soluble in the detergent mixture used in this lab activity and also contains insoluble cellular.

9. Carefully transfer the top phase to a new tube without disturbing the bottom phase. Slowly transfer the bottom phase to a new tube and discard the insoluble pellet. Measure the volume of both phases and record them.

 *Note: The hydrophobic protein solution is very viscous. You need to pipette it very slowly.*

IV. Protein assay

Prepare Pre-diluted Protein Standard containing 1mg BSA/ml, as follows:

1. Add 0.7ml BSA protein standard (2mg/ml) to a tube and dilute with 0.7ml Protein Extraction & Dilution Buffer to make 1mg/ml 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
Protein Buffer (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. Save the remaining protein solutions for the Bradford Protein Assay (Section III).

Lowry Protein Assay

1. Label two tubes for each of your hydrophobic, mild hydrophobic and hydrophilic protein samples.

2. Pipette Protein Extraction & Dilution Buffer and test samples into the tubes as indicated below.

Tube# (In Duplicate)	**Protein Extraction & Dilution Buffer (ml)**	**Test Samples (ml)**
Hydrophobic	0.03	0.02
Mild Hydrophobic	0.04	0.01
Hydrophilic	0.045	0.005

3. Add 0.1ml Copper Solution to each tube. Gently mix by vortexing.
4. Add 1ml Folin Ciocalteu Reagent and incubate at room temperature for 15 minutes.

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

6. Add 1ml distilled water to a cuvette and use to zero the absorbance of the spectrophotometer. Measure the absorbance of each tube and record the values the results section. See Section V to determine the unknown protein concentration.

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.

V. Determination of the Protein Concentration

Determine the protein concentration with traditional paper plot.

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

2. Draw a straight line through the points.

3. Lookup the hydrophobic and hydrophilic protein concentrations from the plot using the absorbance value of the Test Sample.

4. Calculate the actual protein concentration by the concentration obtained in above step 3 multiplied by the dilution factor.

Dilution factor of hydrophobic protein: 0.05ml/0.02ml = 2.5

Dilution factor of hydrophilic protein: 0.05ml/0.005ml = 10

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 the hydrophobic and hydrophilic protein samples.

11. Calculate the actual protein concentration by the concentration obtained in above step10 multiplied by the dilution factor.
    Dilution factor of hydrophobic protein: 0.05ml/0.02ml = 2.5
    Dilution factor of mild hydrophobic protein: 0.05ml/0.01ml = 5
    Dilution factor of hydrophilic protein: 0.05ml/0.005ml = 10

RESULTS, ANALYSIS & ASSESSMENT

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
Hydrophobic				
Mild Hydrophobic				
Hydrophilic				

Q. What is the content of hydrophilic and hydrophobic proteins in the tissue extract?

A. _____*mg hydrophobic proteins* _____*mg hydrophilic proteins.*

Q. What are the percentages of hydrophilic and hydrophobic proteins in the tissue extract?

A. _____*% of the total protein is hydrophobic proteins (note: the % of hydrophobic protein is less than 10% for the samples supplied with this kit).*

Q. Describe in 2-3 words the characteristics of hydrophobic and hydrophilic molecules

A: Hydrophobic Molecules -- water-"scared", move away from water

Hydrophilic molecules-----water –"friendly", move into water

Q. Why did the blue dye not dissolve in the Hydrophilic Protein Extraction Buffer but did dissolve when the Hydrophobic Protein Extraction Buffer is added?

A. Because the blue dye is hydrophobic, the Hydrophobic Protein Extraction Buffer contains detergents and favors solubilization of hydrophobic molecules.

Q. Describe the separation of the blue dye and hemoglobin in the mixture of Hydrophilic Protein Extraction Buffer and Hydrophobic Protein Extraction, after centrifugation.

A. Hemoglobin being hydrophilic and charged molecules migrated in to the detergent poor phase and the blue dye being a hydrophobic molecule moved in to the detergent rich phase.

Q. Describe the nature of proteins not soluble in an aqueous medium and how to solubilize such proteins.

A: *Proteins that are generally not soluble in the absence of detergents are often rich in hydrophobic regions and requires detergents for solubilization.*

Q. Describe how hydrophobic proteins solubilize in detergent solutions.

A. Detergents in aqueous medium form micelles, the inner core of micelles is hydrophobic. In the presence of detergent the hydrophobic proteins solubilize in the micellar environment of detergents.

Q. Describe the nature of the insoluble pellet after detergent extraction.

A. This insoluble cellular material consists of proteins not soluble in the detergent mixture used in this lab activity and also contains insoluble cellular debris.

Q. List the amino acids that generally contribute to protein hydrophobic regions.

A. The hydrophobic regions of proteins are generally rich in tyrosine, tryptophan, and phenylalanine, and to a lesser extent leucine, isoleucine, and valine.

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OBJECTIVES

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- Learn the characteristics of hydrophobic and hydrophilic molecules in aqueous solution.
- Learn fractionation of hydrophilic and hydrophobic proteins from biological samples.
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- Study the role of detergents in protein research and analysis.

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This lab activity begins with the demonstration of the properties of hydrophobic and hydrophilic molecules, followed by the extraction of proteins from a tissue sample in to soluble and insoluble protein fractions. The insoluble protein fraction is further fractionated based on their hydrophobicity (hydrophobic and hydrophilic proteins) using temperature induced phase transition of a detergent preparation.

This kit is supplied with two extraction buffers: Hydrophilic Protein Extraction Buffer consists of a detergent free buffered salt solution optimized for the extraction of soluble proteins from tissue samples. The Hydrophobic Protein Extraction Buffer is a buffered salt solution with an optimized mixture of non-ionic detergents that have phase transition properties, i.e., the detergent separates from the buffer at room temperature. The Blue Balls are glass balls coated with a hydrophobic dye, specifically developed by G Biosciences, for use with detergents for teaching and research

applications. This kit is also provided with the hydrophilic protein hemoglobin to study the behavior of hydrophilic proteins. Students will test the behavior of hydrophobic and hydrophilic agents and apply their findings to the extraction of hydrophobic and hydrophilic proteins from a test tissue sample.

MATERIALS FOR EACH GROUP

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

- 1 vial Tissue Extract & Pestle or 50mg fresh liver tissue & 1 grinding tube (tissue extract tube) & Pestle
- 1.5ml Hydrophilic Protein Extraction Buffer
- 1ml Hydrophobic Protein Extraction Buffer (Must be kept on ice until used)
- 1 BlueBall
- 1 vial Hemoglobin
- 1 bottle Folin Ciocalteu Reagent (shared with whole class)
- 1 bottle Copper Solution (shared with whole class)
- 1 bottle Protein Standard (2mg/ml BSA) (shared with whole class)
- 4ml Protein Extraction & Dilution Buffer
- 27 Centrifuge Tubes (1.5ml)
- Marker Pen

PROCEDURE

Wear gloves throughout the whole experiment.

Note: *Chill Hydrophilic Protein Extraction Buffer and Hydrophobic Protein Extraction Buffer on ice for 5-10 minutes before starting the experiments.*

I. Extraction of hydrophobic dye from the BlueBall

1. Place the BlueBall in a tube and add 0.2ml Hydrophilic Protein Extraction Buffer to the tube.

2. Incubate for 5 minutes, tap the tube and examine the BlueBall for any loss of blue dye. Record your observations.

3. Gently invert the Hydrophobic Protein Extraction Buffer a few times to mix. Add 0.2ml ice-cold Hydrophobic Protein Extraction Buffer to the tube containing the BlueBall.

4. Mix the buffers by gently tapping the tube. Incubate for 20 minutes on ice, periodically mixing the content by tapping the tube. Examine the BlueBall and record your observations.

5. Incubate for 15 minutes in an incubator or water bath set at 37°C.

6. Centrifuge the tube for 5 minutes, and then examine the tube carefully. Two phases, the top aqueous detergent poor phase and the bottom detergent rich phase, will be visualized. Record the distribution of the hydrophobic blue dye.

7. Save the contents of the tube for later use.

II. Extraction of hemoglobin

1. Add 0.2ml Hydrophilic Protein Extraction Buffer to the tube containing hemoglobin. Incubate for 5 minutes and tap the tube to see if the hemoglobin dissolves. Record your observations.

2. Gently invert the Hydrophobic Protein Extraction Buffer a few times to mix. Add 0.2ml ice-cold Hydrophobic Protein Extraction Buffer to the tube containing the hemoglobin.

3. Mix by gently tapping the tube. Incubate for 5 minutes and periodically mix the content by tapping the tube.

4. Incubate for 15 minutes in an incubator or water bath set at 37°C.

5. Centrifuge the tube for 5 minutes then examine the tube carefully. Two phases, the top aqueous detergent poor phase and the bottom detergent rich phase, will be visualized. Record the distribution of the hemoglobin.

6. Transfer the contents of the tube to the tube containing the BlueBall (Section I).

7. Mix the contents by tapping the tube. Incubate for 10 minutes in an incubator or water bath set at 37°C. And then centrifuge for 5 minutes. Record your observations.

III. Extraction of hydrophobic and hydrophilic proteins from a tissue sample

1. Add 0.2ml Hydrophilic Protein Extraction Buffer to the tube containing the tissue extract or fresh tissue (skip step 2 if using fresh tissue).

2. Incubate for 5 minutes at room temperature.

3. Grind the tissue using the pestle and slowly add 0.3ml Hydrophilic Protein Extraction Buffer and continue to grind the tissue until a uniform homogenate forms.

4. Centrifuge the tube for 5 minutes at maximum speed 5,000xg and transfer the supernatant to a new tube. This is your soluble hydrophilic proteins. Record the volume of your protein solution.

5. Add 0.5ml Hydrophilic Protein Extraction Buffer to the pellet and break the pellet with the pestle.

6. Gently invert the ice-cold Hydrophobic Protein Extraction Buffer a few times to mix. Add 0.5ml ice-cold Hydrophobic Protein Extraction Buffer to the tissue homogenate. Vortex the tube to mix the content. Incubate for 10 minutes on ice with periodic vortexing the tube.

7. Transfer the tube to a 37°C incubator or water bath. Incubate for 10 minutes. Vortex the tube at 5 minutes and at the end of the incubation.

8. Centrifuge the tube for 5 minutes at maximum speed 5,000xg. Examine the tube carefully. Two phases, the top aqueous detergent poor phase containing mild hydrophobic proteins and the bottom detergent rich phase containing hydrophobic proteins, will be observed. The pellet in the tube is insoluble material. This insoluble cellular material consists of proteins not soluble in the detergent mixture used in this lab activity and also contains insoluble cellular.

9. Carefully transfer the top phase to a new tube without disturbing the bottom phase. Slowly transfer the bottom phase to a new tube and discard the insoluble pellet. Measure the volume of both phases and record them.

 *Note: The hydrophobic protein solution is very viscous. You need to pipette it very slowly.*

IV. Protein assay

Prepare Pre-diluted Protein Standard containing 1mg BSA/ml, as follows:

1. Add 0.7ml BSA protein standard (2mg/ml) to a tube and dilute with 0.7ml Protein Extraction & Dilution Buffer to make 1mg/ml 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
Protein Buffer (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. Save the remaining protein solutions for the Bradford Protein Assay (Section III).

Lowry Protein Assay

1. Label two tubes for each of your hydrophobic, mild hydrophobic and hydrophilic protein samples.

2. Pipette Protein Extraction & Dilution Buffer and test samples into the tubes as indicated below.

Tube# (In Duplicate)	Protein Extraction & Dilution Buffer (ml)	Test Samples (ml)
Hydrophobic	0.03	0.02
Mild Hydrophobic	0.04	0.01
Hydrophilic	0.045	0.005

3. Add 0.1ml Copper Solution to each tube. Gently mix by vortexing.
4. Add 1ml Folin Ciocalteu Reagent and incubate at room temperature for 15 minutes.

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

6. Add 1ml distilled water to a cuvette and use to zero the absorbance of the spectrophotometer. Measure the absorbance of each tube and record the values the results section. See Section V to determine the unknown protein concentration.

 *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.*

V. Determination of the Protein Concentration

Determine the protein concentration with traditional paper plot.

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

2. Draw a straight line through the points.

3. Lookup the hydrophobic and hydrophilic protein concentrations from the plot using the absorbance value of the Test Sample.

4. Calculate the actual protein concentration by the concentration obtained in above step 3 multiplied by the dilution factor.

Dilution factor of hydrophobic protein: 0.05ml/0.02ml = 2.5

Dilution factor of hydrophilic protein: 0.05ml/0.005ml = 10

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 the hydrophobic and hydrophilic protein samples.

11. Calculate the actual protein concentration by the concentration obtained in above step10 multiplied by the dilution factor.
    Dilution factor of hydrophobic protein: 0.05ml/0.02ml = 2.5
    Dilution factor of mild hydrophobic protein: 0.05ml/0.01ml = 5
    Dilution factor of hydrophilic protein: 0.05ml/0.005ml = 10

RESULTS, ANALYSIS & ASSESSMENT

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
Hydrophobic				
Mild Hydrophobic				
Hydrophilic				

Q. What is the content of hydrophilic and hydrophobic proteins in the tissue extract?

_____

_____

_____

Q. What are the percentages of hydrophilic and hydrophobic proteins in the tissue extract?

_____

_____

_____

Q. Describe in 2-3 words the characteristics of hydrophobic and hydrophilic molecules

_____

_____

_____

Q. Why did the blue dye not dissolve in the Hydrophilic Protein Extraction Buffer but did dissolve when the Hydrophobic Protein Extraction Buffer is added?

_____

_____

_____

Q. Describe the separation of the blue dye and hemoglobin in the mixture of Hydrophilic Protein Extraction Buffer and Hydrophobic Protein Extraction, after centrifugation.

_____

_____

_____

Q. Describe the nature of proteins not soluble in an aqueous medium and how to solubilize such proteins.

_____

_____

_____

Q. Describe how hydrophobic proteins solubilize in detergent solutions.

_____

_____

_____

Q. Describe the nature of the insoluble pellet after detergent extraction.

_____

_____

_____

Q. List the amino acids that generally contribute to protein hydrophobic regions.

_____

_____

_____

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