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A Geno Technology, Inc. (USA) brand name

Biodiversity Study & Biomass Analysis

Teacher's Guidebook

(Cat. # BE-403)

MATERIALS INCLUDED

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

- 6 sets of Pestles & Tubes
- 1 bottle Protein Extraction & Dilution Buffer
- 1 bottle Protein Assay: CB Reagent
- 1 bottle Protein Assay: Protein Standard (2mg/ml BSA)
- 180 Centrifuge Tubes (1.5ml)
- 6 Forceps
- 6 Razors

SPECIAL HANDLING INSTRUCTIONS

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

All other reagents can be stored at room temperature.

ADDITIONAL EQUIPMENT REQUIRED

- Fresh Plant Leaves or seeds.
- Balance
- Spectrophotometer and cuvettes or Microplate reader and microplate
- Low speed centrifuge for 1.5-2ml tubes

TIME REQUIRED

- 2-3 hours

AIMS

- Extract proteins from biological samples.
- Determine protein contents of biological samples.
- Determine protein mass and biomass.
- Determine relationship between biomass to biodiversity in nature.

BACKGROUND

Biodiversity can be described as the variability among living organisms on the earth, including the variability within and between species and within and between ecosystems. Another measure of the diversity of the world around us is to study biomass, which is weight of a living material, usually expressed as dry weight per unit area. Although, we can simply walk outside and see the biodiversity around us it is often important for scientist to explore the biodiversity of similar species at a molecular level.

Proteins are described as the building blocks of life and can be used in a laboratory to visualize the biodiversity of closely related species and also their biomass.

Students collect and catalog plant leaf or seed samples from a diverse group of locally available plants. This lab activity involves determination of natural weight of each plant sample, grinding a predetermined amount of each sample, and the subsequent extraction of proteins from the samples. Students then learn to determine protein contents of each plant sample and attempt to relate the protein content with biomass. Students in this laboratory activity are challenged to think, analyze, and seek answers as to why for a given natural weight protein biomasses vary for different plants. Finally, they will relate that finding to the biodiversity of nature.

TEACHER'S PRE EXPERIMENT SET UP

1. Prepare 3 species of fresh plant leaves or seed samples, such as spinach, tree leaves or flowers or soft weed leaves.
2. 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.

- Fresh plant leaves or seeds (3 species), 0.1-0.2g each
- 1 set of Pestle & Tube
- 4ml Protein Extraction & Dilution Buffer
- CB Reagent (shared with whole class)
- 1 bottle Protein Standard (2mg/ml BSA) (shared with whole class)
- 29 Centrifuge tubes (1.5ml)
- 1 Forceps
- 1 Razor
- Marker Pen

PROCEDURE

Wear gloves throughout the whole experiment.

I. Extract Protein from the Plant Tissues

1. Cut the plant leaves or seeds in to small pieces. Weigh approximately 0.1-0.2g plant leaves and record the exact weight.

WARNING: Take extreme care whilst using the razor blade. The smaller the leaves are cut the easier they are to grind.

2. Transfer the plant samples to the Grinding Tube. Add 0.1ml Protein Extraction & Dilution Buffer and use the pestle to grind the tissue until a homogenate is achieved.
3. Slowly add 0.4ml Protein Extraction & Dilution Buffer while grinding. Continue grinding and mixing until it becomes a homogeneous suspension.
4. Transfer the entire lysate to a new tube and label it with your group number and plant name. Rinse the grinding tube and pestle in water for the next sample.
5. Prepare the remaining plant samples as above to prepare two additional plant sample extracts.
6. Centrifuge all three samples at 5,000g for 5 minutes and transfer the supernatant to new tubes. This will pellet any insoluble debris.

II. Protein Quantification Assay

Prepare Pre-diluted Protein Standards

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.

Protein Assay Protocol:

1. Label two tubes for Plant Samples-I, -II, & -III.
2. Transfer 0.03ml Protein Extraction & Dilution Buffer and 0.02ml plant samples to the appropriate tube.
3. Mix the CB Reagent by gently inverting the bottle several times.

 *To avoid foaming, DO NOT SHAKE THE BOTTLE.*

4. Add 1ml CB Reagent to the protein standards and the Plant Sample tubes. Vortex briefly to mix the content. Incubate the tubes at room temperature for 5 minutes.
5. In the meantime, turn on the spectrophotometer and allow it to warm up. Adjust the wavelength to 595nm.
6. 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.

 *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 595nm.*

If the plant protein samples are too concentrated then follow the following steps.

1. Transfer 0.02ml plant sample to a tube and add 0.18ml Protein Extraction & Dilution Buffer. Vigorously shake or vortex to mix. This will give a 1 in 10 dilution.
2. Use 0.02ml of the 1 in 10 dilution in step 2 of the Protein Assay protocol.
3. If the sample is still to concentrated, transfer 0.02ml 1 in 10 diluted sample to a tube and add 0.18ml Protein Extraction & Dilution Buffer. Vigorously shake or vortex to mix. This will give a 1 in 100 dilution.
4. Use 0.02ml of the 1 in 100 dilution in step 2 of the Protein Assay protocol.
5. If the sample is still to concentrated, transfer 0.02ml 1 in 100 diluted sample to a tube and add 0.18ml Protein Extraction & Dilution Buffer. Vigorously shake or vortex to mix. This will give a 1 in 1000 dilution.
6. Use 0.02ml of the 1 in 1000 dilution in step 2 of the Protein Assay protocol.

III. Determination of the Protein Concentration

Determine the protein concentration with a 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 Test Sample concentration from the plot using the absorbance value of the Test Sample.
4. Calculate the actual protein concentration:

$$\frac{\text{Protein concentration from the plot} \times 0.05\text{ml (BSA standard volume)}}{\text{Test fraction volume (0.02ml)}}$$

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: Biodiversity, 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 sample.
11. Calculate the actual protein concentration:

$$\frac{\text{Protein concentration from the plot } x \text{ 0.05ml (BSA standard volume)}}{\text{Test fraction volume (0.02ml)}}$$

RESULTS, ANALYSIS & ASSESSMENT

Tube #	Absorbance I (595nm)	Absorbance II (595nm)	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
Plant Sample-I				
Plant Sample-II				
Plant Sample-III				

Calculate protein content in the plant tissue

mg protein /mg tissue = Actual protein concentration (mg/ml) x 0.5ml (lysate volume) / weight of the tissue (mg)

Q. Briefly describe the biochemical nature of the solid debris.

A. The insoluble debris consists of cellulose and proteins not soluble in the extraction buffer.

Q. Why do plant leaves and seeds differ in their protein content and describe the concept of biomass and biodiversity?

A. The protein content of plant leaves and for that matter any living system is dictated that its genetic make up, role-played during the life cycle, and environmental survival considerations. With changing seasons and climate the protein content of plant leaves will change.

Biomass is total mass of a living system including water and biodiversity is the differences imposed by nature to suite survival and environmental constraints.

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