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A Geno Technology, Inc. (USA) brand name

Protein Structure Analysis

Teacher's Guidebook

(Cat. # BE-407)



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MATERIALS INCLUDED WITH THE KIT

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

- 8 vials PAGEmark™ Blue PLUS Protein Marker
- 1 vial Protein-Im
- 1 vial Protein-Ov
- 1 vial Protein-Ly
- 1 vial PAGE: Non-Reducing Sample Buffer
- 1 vial PAGE: Reducing Agent (TCEP)
- 60 Centrifuge Tubes (1.5ml)
- 1 bottle PAGE: LabSafe GelBlue

SPECIAL HANDLING INSTRUCTIONS

- Store all protein samples, Protein Marker and TCEP at 4°C.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

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
P131	PAGE: Reducing Agent (TCEP)	Corrosive

ADDITIONAL EQUIPMENT REQUIRED

- Protein Electrophoresis Equipment, SDS PAGE gels and electrophoresis buffer
- 70% isopropanol
- Waterbath or Beaker and thermometer
- Sharp pins

TIME REQUIRED

- 3-4 hours

OBJECTIVES

- Study the potential of protein electrophoresis in protein research and analysis.
- Parameters affecting protein electrophoresis analysis.
- Role of denaturing and reducing agents in protein electrophoresis.
- Use of electrophoresis for characterization of proteins.
- Use of electrophoresis for analysis of proteins structure.

BACKGROUND

Proteins are the building blocks of life and are involved in every aspect of a living organism. A crucial factor of a protein, which allows the protein to function correctly, is its structure. The structure of a protein is very complex and the mechanisms of protein folding are poorly understood.

There are four levels of protein folding, primary, secondary, tertiary and quaternary, with each level adding a greater degree of complexity.

The **Primary structure** of a protein is simply the order of its amino acids. The order of amino acids is encoded for by the proteins gene. Unfortunately for scientist, although they know the genomic and therefore the protein's sequence, the primary structure tells very little about how the protein will fold.

The **Secondary structure** refers to the first stage of the three dimensional structure of the proteins and involves the folding of the protein backbone. There are two major secondary structures that the amino acid chain can adopt. The first is known as the alpha helix (Figure 1), which is a tight helix that has the protein backbone towards its center and the amino acid side chains radiating outwards.

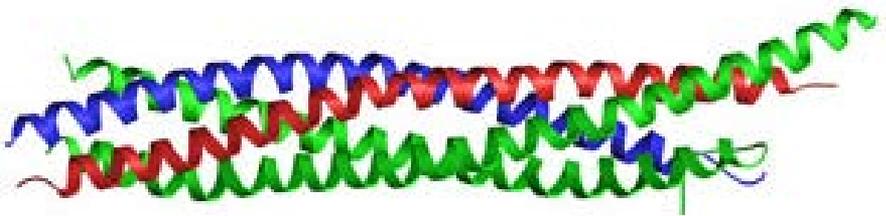


Figure 1: Four alpha helices.

The second is the beta pleated sheet that can be a parallel or an anti-parallel sheet (Figure 2).

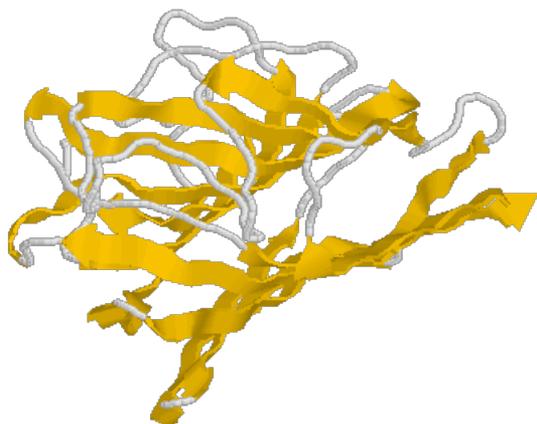


Figure 2: Two anti-parallel sheets (top and bottom):

A β -sheet is formed when hydrogen bonds are formed between two parts of the protein chain that can be far apart.

The **Tertiary structure** is basically the folding of the α -helices and β -sheets into a more complex structure by the interaction of their amino acid side chains. The rules governing the differences between secondary and tertiary structure are not too clear.

The **Quaternary structure** only occurs if more than one polypeptide chain is involved in the protein structure, as the quaternary structure is how multiple polypeptide chains come together and interact to give the protein its final structure.

This lab activity is designed to deepen the understanding of biologically active protein molecules and the potential of protein electrophoresis for determining the structure of biologically active protein molecules.

Two fundamentally different types of gel system exist, non-dissociating (non-denaturing) and dissociating (denaturing). The non-dissociating (non-denaturing) system is designed to separate native protein under conditions that preserve protein function and activity, for example enzyme activity, binding activity, and so on. In contrast, a dissociating system is designed to denature protein, either whole or partly, into their constituent's subunits or polypeptides to examine the structure as well as composition of protein molecules.

Sodium dodecyl sulfate (SDS) is commonly used for denaturing proteins into their constituent subunits or polypeptides and the method is known as sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE). In a SDS-polyacrylamide gel, the protein mixture is denatured by heating at 100°C in the presence of excess SDS.

A reducing reagent may be used to break disulfide bonds within protein molecules or between two or more polypeptide chains. By making a selective use of reducing agents it is possible to determine the structure of biologically active protein molecules or complexes.

In this lab activity, students study the role of a reducing agent in protein electrophoresis. Students learn how to study subunit composition of biologically active protein molecules and complexes. Students deepen their understanding of protein structure, from their primary structure to the more complex tertiary structures, utilizing protein electrophoresis. Complex mixtures of protein samples and characterized pure protein samples, in conjunction with electrophoresis, are utilized to study protein structure and the potential of protein electrophoresis. Using denaturing, non-reducing, reducing electrophoresis, students understand the difference between primary, secondary, tertiary and quaternary structures, the importance of disulfhydryl bridges in maintaining protein structure and electrophoresis in studying complete proteins and protein subunits.

The kit provided with reagents needed to prepare and run denaturing, non-reducing and reducing electrophoresis. An odorless reducing agent TCEP (Tris(2-carboxyethyl)phosphine) is provided with this kit. It breaks protein disulfhydryl bridges into sulfhydryl groups. Other commonly used reducing agents can also be used in the experiment, such as DTT (dithiothreitol) and β -mercaptoethanol.

TEACHER'S PRE EXPERIMENT SET UP



Acrylamide/Bis-acrylamide is toxic. Always wear gloves and protective clothing when handling the chemicals.

1. Prepare one 8-12% polyacrylamide gel containing 1% SDS (sodium dodecyl sulfate) or use premade electrophoresis gel with 7 sample lanes for each student group. G-Biosciences Protein Electrophoresis Kit is recommended for making your own gel. Note: non-denaturing gel can also be used.
2. Before opening the Protein-Im, Protein-Ov and Protein-Ly vials, centrifuge the vials for 5 minutes to bring down all pellets to the bottom of the tubes.
3. Add 150µl Non-Reducing Sample Buffer (without reducing agent, TCEP) to each vial of Protein-Im, Protein-Ov and Protein-Ly. Soak the pellets for 5 minutes with periodical vortexing to dissolve them completely.
4. Prepare electrophoresis running buffer as needed. Typical ingredients: 0.025M Tris base, 0.192M Glycine and 0.1% SDS.
5. Aliquot reagents for each student group according to the next section.

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 8-12% Polyacrylamide electrophoresis gel (containing 1% SDS), electrophoresis equipment and buffers
- 1 vial PAGEmark™ Protein Marker
- 20µl Protein-Im
- 20µl Protein-Ov
- 20µl Protein-Ly
- 30µl Reducing Agent (TCEP)
- 6 Centrifuge Tubes (1.5ml)
- 1 pin
- 50ml LabSafe GelBlue

PROCEDURE



SDS-Page gels contain Acrylamide/Bis-acrylamide that is toxic. Always wear gloves and protective clothing when handling the gels.

1. Label 6 tubes 1-6. Tubes 1-3 are for unreduced protein samples and tubes 4-6 are for reduced protein samples.
2. Transfer 10µl unreduced protein sample to each tube according to the table below.

Tube#	1	2	3	4	5	6
Protein (10µl)	Protein-Im	Protein-Ov	Protein-Ly	Protein-Im	Protein-Ov	Protein-Ly

3. Add 5µl Reducing Agent (TCEP) to tubes 4-6. Mix well by pipetting up and down a few times. Punch through the caps of tube 4-6 and protein marker with a pin. Boil only tubes 4-6 in a water bath for 5 minutes. Centrifuge briefly to bring down the condensation. Do not boil tube 1-3.

4. Assemble the gel according your teacher's instructions.

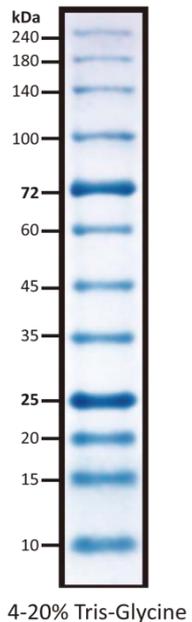
5. Using a fresh pipette tip for each sample, load 5µl Protein Marker into the first lane, all the unreduced protein samples (tubes 1-3) to lanes 2-4 and then load the reduced protein samples (tubes 4-6) to lanes 5-7. The PAGEmark™ Protein Marker consists of a mix of twelve Prestained proteins of molecular weight 240, 180, 140, 100, 72, 60, 45, 35, 25, 20, 15 and 10kDa.

6. Run the gel at 30mA/gel until the blue dye front is 0.5-2cm from the bottom.

7. Disassemble the gel carefully. Wash the gel twice in distilled water, five minutes each wash.

8. Add 50ml LabSafe GelBlue to cover the gel. Gently shake the gel for 60 minutes.

9. Decant the LabSafe GelBlue and rinse the gel with distilled water. The gel can be stored in water. Longer destaining in water will give a clearer view.



RESULTS, ANALYSIS & ASSESSMENT

Q. Observe the protein bands on the gel carefully. Describe the difference of each sample between reduced and unreduced proteins.

A. Unreduced Protein-I_m has one band representing a single molecule; reduced Protein-I_m has 2 smaller bands that are the two subunits that combine to make the single unreduced protein. Unreduced Protein-O_v has 2 bands, one dimer and one monomer. Reduced Protein-O_v has only one band, the monomer, as the reducing agent disassociates the dimer. There are no difference between reduced and unreduced Protein-L_y, as the protein is a monomer that does not form a multimeric compound.

Q. Describe the role of reducing agents in protein electrophoresis.

A. Reducing agents break disulfide bonds within protein molecules or between two or more polypeptides.

Q. Describe the role played by disulfide bonds in protein molecules.

A. Covalently links two or more polypeptides to form active protein molecule and maintains protein structure.

Q. Briefly describe non-denaturing, denaturing, reducing and non-reducing protein electrophoresis.

A. Non-denaturing electrophoresis –maintains native protein structure

Denaturing – denatures proteins, with or without reducing disulfide bonds

Non-reducing –denaturing – denatures proteins while maintaining disulfide bonds

Reducing–denaturing –denatures and reduces inter and intra molecular disulfide bonds

Q. Briefly describe primary, secondary, tertiary, and quaternary structure of protein molecules.

A. Primary: The amino acid sequence

Secondary: Folding of protein backbones into alpha helices and B-sheets. Interaction through hydrogen bonds

Tertiary: More complex folding coordinated by the interaction of amino acid side chains.

Disulfide bonds formed

Quaternary: Interaction of different polypeptide chains that come together to form the final protein.

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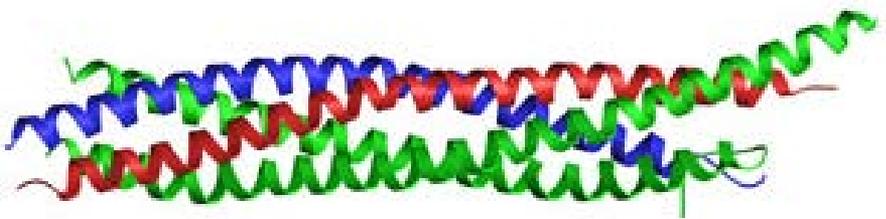


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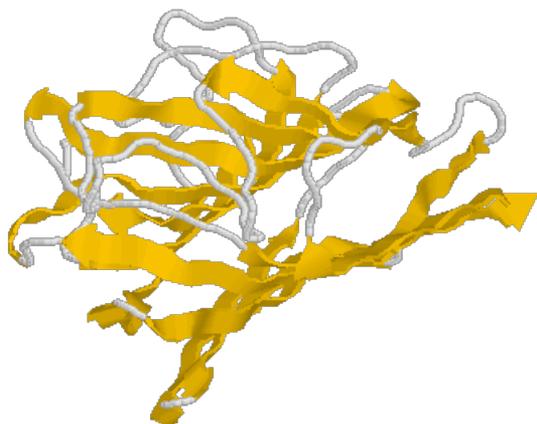


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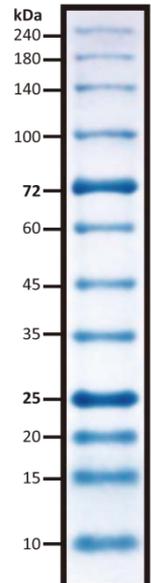


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4-20% Tris-Glycine

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