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A Geno Technology, Inc. (USA) brand name

Protein Folding Study

Teacher's Guidebook

(Cat. # BE-411)



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MATERIALS INCLUDED

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

- 1 bottle PAGE: PAGE Mix (19:1 Acrylamide/Bisacrylamide)
- 1 vial PAGE: APS (Ammonium Persulfate)
- 1 bottle Detergent Solution (10% SDS)
- 1 bottle PAGE: PAGE Stacking Buffer (1M Tris-HCl, pH 6.8)
- 1 bottle PAGE: PAGE Separating Buffer (1M Tris-HCl, pH 8.8)
- 1 vial PAGE: TEMED
- 1 vial PAGE: Sample Loading Buffer (2X)
- 1 bottle PAGE: Electrophoresis Running Buffer (10X)
- 8 vials PAGEmark™ Blue PLUS Protein Marker
- 1 vial Protein: GB Protein Lysate
- 1 vial Sterile Water
- 30 Centrifuge Tubes (1.5ml)

SPECIAL HANDLING INSTRUCTIONS

- Store GB Protein Lysate and Protein Marker at -20°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
P151	PAGE: TEMED	Toxic
P011	PAGE: APS (Amm. Persulfate)	Oxidizing
P061	PAGE: PAGE Mix (19:1 Acrylamide/Bisacrylamide)	Very Toxic

ADDITIONAL EQUIPMENT REQUIRED

- Protein Electrophoresis Equipment
- 70% isopropanol
- Waterbath or beaker and thermometer
- UV Light box/ Transilluminator

TIME REQUIRED

- 3-4 hours

OBJECTIVES

- Study denaturation and renaturation of proteins.
- Study protein activation and folding.
- Understand dependency of protein function on its structure.

BACKGROUND

Proteins are crucial to every reaction and function of a living organism and as a result their expression and degradation has to be closely regulated. Basically, several processes exist that control the life cycle of a protein molecule. A protein's life cycle begins once its gene is turned on and its mRNA is transcribed and translated to produce the polypeptide strand.

This is the first crucial stage of the life cycle of a protein, the correct folding of a protein. As previously described in the "Protein Structure Analysis" experiment a newly synthesized protein is folded into secondary, tertiary and sometimes quaternary structures. Correct folding is essential for a protein to be functional, whereas incorrect folding can have severe detrimental effects. For example, several known diseases are attributed to misfolded proteins, including bovine spongiform encephalopathy (BSE) and its human equivalent Creutzfeldt-Jakob disease (CJD), Alzheimer's disease, Parkinson's disease, type II (non-insulin dependent) diabetes and some types of cancer. The symptoms of Mad Cow Diseases (BSE) and Alzheimer's are a result of misfolded proteins aggregating and forming insoluble protein deposits in the brain.

As the protein is translated by a ribosome, the polypeptide chain lengthens and specialized proteins, known as chaperones, bind to the nascent polypeptide chain to prevent misfolding, by association of hydrophobic amino acids. On completion of translation, the chaperone proteins are released, a step requiring ATP, and in most cases the polypeptide chain folds into its correct structure. In some cases, an additional protein complex, known as a chaperonin, is required. The nascent protein is bound inside the chaperonin and in the presence of ATP folds correctly and is then ejected from the chaperonin. An example of a protein using chaperonin is actin.

This experiment is designed to demonstrate that protein folding is crucial to a protein's function. Using an electrophoretic technique and a strong anionic detergent, students will denature and refold a protein. The correctly folded protein emits light under ultra violet light, if incorrectly folded the protein fails to emit light.

TEACHER'S PRE EXPERIMENT SET UP



Acrylamide/Bisacrylamide is toxic. Always wear gloves and protective clothing when handling the chemicals.

1. A precast SDS polyacrylamide gel in the range of 7-12.5% can be used as a replacement to pouring your own gels.
2. Dissolve 8g PAGE Mix (19:1 Acrylamide/Bisacrylamide) in distilled water to a final volume of 20ml. The concentration of Acrylamide/Bisacrylamide will be 40%. The solution can be stored in cold for two months.
3. Dissolve 0.15g APS (Ammonium Persulfate) in 1.5ml distilled water to make a 10% solution. This solution is stable for one week in a cold refrigerator.
4. Reconstitute the GB Protein Lysate by adding 150 μ l Sterile Water, allow to soak for 5 minutes and then vortex the tube. Briefly centrifuge at 5,000xg for 5 minutes to remove any insoluble particles.
5. Dilute 10X Electrophoresis Running Buffer to 1X with distilled water (mix 100ml buffer with 900ml distilled water). The amount of Electrophoresis Running Buffer needed depends on the device used. You may need to make more running buffer if necessary (0.025M Tris base, 0.194M Glycine, 0.1% SDS).
6. 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 bottle 40% Acrylamide/Bisacrylamide solution (shared with whole class)
- 1 vial 10% APS (Ammonium Persulfate) (shared with whole class)
- 1 bottle 10ml Detergent Solution (10% SDS) (shared with whole class)
- 1 bottle 10ml Stacking Buffer (shared with whole class)
- 1 bottle 10ml Separating Buffer (shared with whole class)
- 1 vial 100 μ l TEMED (shared with whole class)
- 1 vial PAGEmark™ Protein Marker
- 25 μ l Sample Loading Buffer (2X)
- 25 μ l GB Protein Lysate
- 2 Centrifuge Tube

PROCEDURE



Acrylamide/Bisacrylamide is toxic. Always wear gloves and protective clothing when handling the chemicals.

I. Preparation of a Polyacrylamide Gel (Stacking and Resolving Gels).

1. The data provided in the following table is for making two 10% 8x10cm mini polyacrylamide gel. Different percentage resolving gels or multiple gels can be prepared. Calculate the reagents needed accordingly and fill in the blank columns.

Gel Concentration	10% Separating Gel	4% Stacking Gel
Solution volume	5ml	5ml
40% Acrylamide/Bis-acrylamide solution	1.25ml	0.5ml
1M Separating Buffer	2ml	X
1M Stacking Buffer	X	0.6ml
10% SDS	0.5ml	0.5ml
10% APS	50 μ l	50 μ l
Distilled water	To 5ml	To 5ml
TEMED	5 μ l	5 μ l

2. Clean the gel plates, spacers and stand as per manufacturer's instruction. Assemble the gel plates and spacers on the gel stand for casting the gel. Make sure the gel plates and spacers are aligned at the bottom.
3. Label two 15ml tubes for "Separating Gel" and "Stacking Gel". Add all the reagents, except TEMED, to the tube according to the table above. Mix the solution thoroughly. Do not add TEMED until you are ready to cast the gel.



DO NOT ADD TEMED until ready to cast gel or gel will polymerize in the tube.

4. When ready to cast the gel, add TEMED to *only* the "Separating Gel" solution and mix well. Using a 1ml pipette swiftly add the running gel solution until the gel solution is 2.5-3.0cm from the top of the small plate.
5. Slowly add 1ml 70% isopropanol to prevent evaporation and create an even surface on top of the gel. Let the gel polymerize for 30-40 minutes.

6. Pour off the isopropanol and rinse the top layer of the resolving gel with 0.5ml distilled water.
7. Add TEMED to the “stacking gel” solution and mix well. Slowly fill up the top of the gel.
8. Carefully put the comb between the two glass plates without introducing air bubbles. Let the stacking gel polymerize for 30minutes. Now the gel is ready.



If necessary, this is a convenient stopping point. The gels can be wrapped in plastic wrap and stored at 4°C for up to a week.

II. Protein Denaturation

1. To see the active protein, place your tube of GB protein lysate onto a UV box. Note the color of the sample under UV light.



Ensure UV safety goggles are worn when UV lamp is turned on.

2. Label a tube with “Folded” and add 10 μ l GB protein lysate to the tube.
3. Label a second tube with “Denatured” and add 10 μ l GB protein lysate to the tube.
4. Add 10 μ l Sample Loading Buffer to the “Denatured” tube, mix by gently pipetting up and down. Place **ONLY** the “Denatured” tube in a boiling waterbath for 5 minutes.
5. After boiling, briefly centrifuge to spin down the condensation.
6. Add 10 μ l Sample Loading Buffer to the “Folded” tube, mix by gently pipetting up and down.



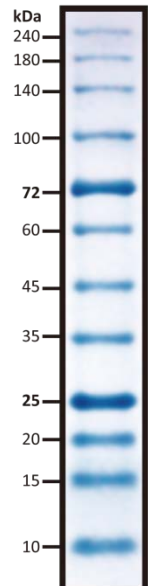
DO NOT BOIL.

7. Compare tubes, “Folded” and “Denatured” under UV light and note the color.



View the tubes under UV light as soon as possible, the SDS in the loading buffer will eventually denature the protein if left too long.

8. Set up the electrophoresis gel to load your samples as per your teacher’s instructions.
9. Load 5 μ l PAGEmark™ Protein Marker to the first lane, followed by 20 μ l “Folded” and then “Denatured” protein samples. 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.
10. Run the gel at 120 volts for 20-40 minutes or until the blue dye front is over half way down the gel.
11. Disassemble the gel carefully and *immediately* view on a UV box. Note the differences between the two samples.



4-20% Tris-Glycine

12. Place the gel in water and place on a shaker for 20 minutes. View again on a UV box. Repeat the wash with fresh water for a further 20 minutes. View gel after wash. Repeat until green color can be seen in the denatured lane.

The above procedure provides the optimal results, however if the washing steps extend past the session length then it is recommend that the teacher/supervisor continues the washes until the protein refolds. As soon as the green color appears dehydrate the gel with ethanol, by discarding the water and adding 100ml ethanol to the gel tray. The dehydrated gel can be viewed the next day on a UV light box to demonstrate to students the refolded protein.

RESULTS, ANALYSIS & ASSESSMENT

Compare and describe the color seen under UV light before and after boiling and after electrophoresis and after washing.

The protein solutions glow green under UV light and maintain that property until boiled. The unboiled "Folded" sample glowed green in the gel after electrophoresis, unlike the "Denatured" sample. The "Denatured" sample gradually started glowing after each wash step.

Explain your results below.

The heat of the boiling waterbath destroyed the structure of the protein therefore destroying its function. The SDS detergent prevented the protein refolding during electrophoresis, but the SDS was removed with extensive washing allowing the protein to refold.

Q. Describe the nature of the SDS interaction

A. *The SDS coats the entire length of the protein with negatively charged S*

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5. After boiling, briefly centrifuge to spin down the condensation.
6. Add 10 μ l Sample Loading Buffer to the “Folded” tube, mix by gently pipetting up and down.



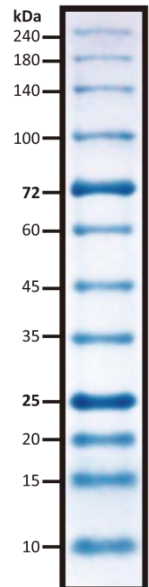
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Explain your results below.

Describe the nature of the SDS interaction

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