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# Subcellular Fractionation & Protein Distribution

Teacher's Guidebook

(Cat. # BE-410)



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MATERIALS INCLUDED.....	3
SPECIAL HANDLING INSTRUCTIONS.....	3
ADDITIONAL EQUIPMENT REQUIRED .....	3
TIME REQUIRED.....	3
OBJECTIVES.....	4
BACKGROUND .....	4
TEACHER'S PRE EXPERIMENT SET UP .....	5
MATERIALS FOR EACH GROUP .....	5
PROCEDURE.....	6
I. SUBCELLULAR FRACTIONATION.....	6
II. RUNNING AND STAINING PROTEIN ELECTROPHORESIS GELS.....	7
RESULTS, ANALYSIS & ASSESSMENT .....	8

## **MATERIALS INCLUDED**

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

- 1 bottle SubCell Buffer
- 6 Pestles & Tubes
- 6 Weigh Boats (3x3in)
- 6 Razor blades
- 2 vials PAGE: Sample Loading Buffer (2X)
- 60 Centrifuge Tubes (1.5ml)
- 1 bottle PAGE: LabSafe GelBlue
- 8 vials PAGEmark™ Blue PLUS Protein Marker
- 1 bottle PAGE: Electrophoresis Running Buffer (10X)

## **SPECIAL HANDLING INSTRUCTIONS**

- Store SubCell Buffer at 4°C.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

## **ADDITIONAL EQUIPMENT REQUIRED**

- Fresh liver tissue.
- Protein Electrophoresis Equipment
- 8-12% SDS polyacrylamide electrophoresis gel
- Waterbath or Beaker and thermometer
- Balance
- Low speed centrifuge for 1.5-2ml tubes

## **TIME REQUIRED**

- 3-4 hours

## OBJECTIVES

- Learn how to handle delicate biological samples for protein analysis.
- Use of centrifuge and differential centrifugation technique.
- Study subcellular fractionation of tissue samples.
- Use of protein electrophoresis for complicated analytical projects.
- Study protein diversity at a cellular level.

## BACKGROUND

Proteins within a cell are often localized to specific cellular compartments, such as the nucleus, plasma membrane, or vesicles and their specific localization can provide crucial information about the function of the protein. An important technique in identifying novel proteins and understanding their function is subcellular fractionation. This process allows cells to be fractionated into compartment enriched fractions, often utilizing differential centrifugation. In addition to separating compartment specific fractions, subcellular fractionation drastically reduces the complexity of protein samples allowing for easier protein identification.

The Subcellular Fractionation kit exposes students to how research laboratories handle delicate samples for protein analysis and it serves as an advanced training in protein electrophoresis and protein analysis. This study involves the subcellular fractionation of a tissue sample into fractions enriched with nuclear, mitochondria or cytoplasmic proteins. Students also learn about centrifugation techniques and differential centrifugation of samples for fractionation of cellular sub-compartments. Students then use electrophoresis to analyze the fractionated cellular compartments and study how protein distribution or protein fingerprints differ between the subcellular compartments. Finally, students will develop an understanding of cell organelles and cellular organization.

In this laboratory activity, subcellular fractionation is performed by grinding fresh animal tissue in an isotonic buffer, SubCell Buffer, containing an optimized concentration of sugar and buffered salt. The SubCell Buffer minimizes the damage to cell organelles. After tissue grinding, nuclei and mitochondria are fractionated by two-step differential centrifugation. The fractionated subcellular fractions are characterized by protein electrophoresis. This lab activity uses protein fingerprints for the characterization of the nuclear, mitochondria, and cytosol fractions.

## 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 electrophoresis gel containing 1% SDS (sodium dodecyl sulfate) with 10 sample loading wells (G-Biosciences Protein Electrophoresis Kit (#BE-406) is recommended). Alternatively, use a pre-made gel.
2. Obtain fresh chicken liver or other animal liver tissue locally available. Each student group needs 50-100mg fresh liver tissue. Liver tissue may be stored in cold for 12-16 hours prior to use. Do not use frozen tissue.
3. 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).
4. 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
- 1 vial PAGEmark™ Protein Marker
- 50-100mg fresh liver tissue
- 1ml SubCell Buffer
- 1 Pestle & Tube
- 1 Weigh Boat (3x3in)
- 1 Razor blade
- 1 Forceps
- 5 Centrifuge Tubes (1.5ml)
- 0.5ml Sample Loading Buffer (2X)
- 50ml LabSafe GelBlue

## PROCEDURE



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

### **I. Subcellular Fractionation**

1. Weigh about 50-100mg fresh liver tissue in a weigh boat.
2. Cut the tissue into small pieces with the razor blade. Transfer the tissue to the grinding tube using the forceps.



*WARNING: Take extreme care whilst using the razor blade. The smaller the liver is cut the easier it is to grind.*

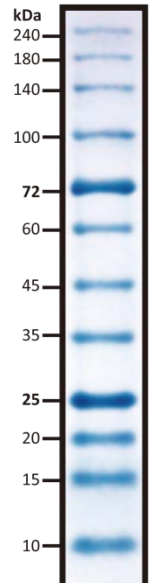
3. Add 0.5ml SubCell Buffer to the tube. Using the pestle, disaggregate the tissue with 10-20 up and down pestle strokes or until the tissue sample is completely homogenized. Do not twist the pestle. Twisting may damage the nuclei and mitochondria by shearing the membranes.
4. Let the tube stand for 2 minutes. Transfer the homogenate to a new tube and leave any large chunks of tissue fragments in the grinding tube.
5. Remove 20 l homogenate analysis later. Label this sample as "Total Lysate".
6. Centrifuge the homogenate at approximately 700xg for 5 minutes. Ask your instructor to explain how to set the centrifuge in order to generate 700xg force.
7. Transfer the supernatant to a new tube. The pellet is the Nuclei Enriched Fraction.

**Optional:** *Add 0.5ml SubCell Buffer to the nuclei pellet. Gently vortex or tap the tube to resuspend the nuclei. Centrifuge at 700xg for 5 minutes. Discard the supernatant. The pellet contains nuclei with less contaminating cellular elements.*

8. Centrifuge the supernatant at approximately 12,000xg for 10 minutes. Ask your instructor to explain how to set the centrifuge in order to generate 12,000xg force.
9. Transfer the supernatant to a new tube. This is your Cytosol Fraction containing other cell organelles, including Golgi, ribosomes, vesicles and membrane fragments. The pellet is enriched in mitochondria.

## II. Running and Staining Protein Electrophoresis Gels

1. Add 20 $\mu$ l 2X Sample Loading Buffer to the 20 $\mu$ l total lysate and mix.
2. Suspend the nuclei in 200 $\mu$ l water and mix and then add 200 $\mu$ l 2X Sample Loading Buffer. Vortex to mix the suspension.
3. Suspend the mitochondria in 100 $\mu$ l water and mix and then add 100 $\mu$ l 2X Sample Loading Buffer. Vortex to mix the suspension.
4. Transfer 20 $\mu$ l cytosol fraction to a new tube and mix with 20 $\mu$ l 2X Sample Loading Buffer. Vortex to mix the suspension.
5. Boil all the samples for 5 minutes. Vortex the tubes briefly and centrifuge 30 seconds to bring down the condensation. *Do not boil the PAGEmark™ Protein Marker.*
6. Assembly the gel according your teacher's instruction for running the samples.
7. Load 5 $\mu$ l Protein Marker into the first lane (optional). Load 10 $\mu$ l each of your 4 samples (Total lysate, Nuclei, Mitochondria, Cytosol). The PAGEmark™ 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.
8. Set the gel to run at 30mA/gel and migrate the proteins until the blue dye front is half centimeter from the bottom of the gel.
9. Disassemble the gel carefully.
10. Wash the gel twice in distilled water, five minutes each.
11. Remove all free water from the gel and add 50ml LabSafe GelBlue stain to cover the gel. Gently shake the gel for 60 minutes at room temperature.
12. 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 of the protein bands.



4-20% Tris-Glycine

## RESULTS, ANALYSIS & ASSESSMENT

1. Observe the protein bands in the electrophoresis gel carefully. Make a note of the differences among the 4 fractions – total lysate, nuclei, mitochondria, and cytosol protein lanes. Can you identify in the total lysate protein lane the nuclear, mitochondria or cytoplasmic proteins?

Q. Briefly describe the underlying methods of fractionation of cell organelles.

A. *Cell organelles were fractionated by differential centrifugation. Nuclei being the largest and the heaviest cell organelle are sediment out of the tissue extract by applying 700xg for 5 minutes. Sedimentation of mitochondria required a relatively higher centrifugation force (12,000 x g). Sedimentation of other cell organelles such as Golgi, ribosomes, vesicles, and cell membrane requires over >100,000xg force.*

Q. How can you characterize the nuclear and mitochondria fractions?

A. *Nuclear and mitochondria fractions can be verified using characteristic protein fingerprints, identifying marker proteins specific for nuclei and mitochondria, and their specific cellular activities. For example, mitochondrial fraction can be verified by testing the mitochondrial fraction for Cytochrome C activity. The nuclear fraction can be verified by testing the nuclear fraction for the presence of a nuclear marker protein, for example the nuclear structural protein lamin. This lab activity uses pre-made characteristic proteins fingerprints for the characterization of nuclear, mitochondria, and cytosol fractions.*

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## PROCEDURE



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### **I. Subcellular Fractionation**

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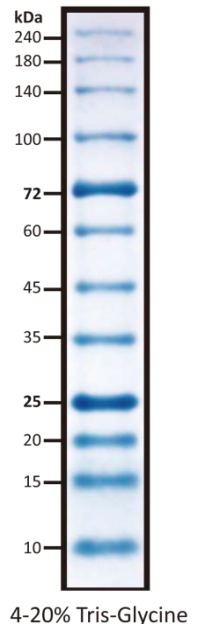
3. Add 0.5ml SubCell Buffer to the tube. Using the pestle, disaggregate the tissue with 10-20 up and down pestle strokes or until the tissue sample is completely homogenized. Do not twist the pestle. Twisting may damage the nuclei and mitochondria by shearing the membranes.
4. Let the tube stand for 2 minutes. Transfer the homogenate to a new tube and leave any large chunks of tissue fragments in the grinding tube.
5. Remove 20  $\mu$ l of the homogenate for electrophoresis analysis later. Label this sample as "Total Lysate".
6. Centrifuge the homogenate at approximately 700xg for 5 minutes. Ask your instructor to explain how to set the centrifuge in order to generate 700xg force.
7. Transfer the supernatant to a new tube. The pellet is the Nuclei Enriched Fraction.

**Optional:** *Add 0.5ml SubCell Buffer to the nuclei pellet. Gently vortex or tap the tube to resuspend the nuclei. Centrifuge at 700xg for 5 minutes. Discard the supernatant. The pellet contains nuclei with less contaminating cellular elements.*

8. Centrifuge the supernatant at approximately 12,000xg for 10 minutes. Ask your instructor to explain how to set the centrifuge in order to generate 12,000xg force.
9. Transfer the supernatant to a new tube. This is your Cytosol Fraction containing other cell organelles, including Golgi, ribosomes, vesicles and membrane fragments. The pellet is enriched in mitochondria.

## II. Running and Staining Protein Electrophoresis Gels

1. Add 20 $\mu$ l 2X Sample Loading Buffer to the 20 $\square$ l total lysate and mix.
2. Suspend the nuclei in 200 $\mu$ l water and mix and then add 200 $\mu$ l 2X Sample Loading Buffer. Vortex to mix the suspension.
3. Suspend the mitochondria in 100 $\mu$ l water and mix and then add 100 $\mu$ l 2X Sample Loading Buffer. Vortex to mix the suspension.
4. Transfer 20 $\mu$ l cytosol fraction to a new tube and mix with 20 $\mu$ l 2X Sample Loading Buffer. Vortex to mix the suspension.
5. Boil all the samples for 5 minutes. Vortex the tubes briefly and centrifuge 30 seconds to bring down the condensation. *Do not boil the PAGEmark™ Protein Marker.*
6. Assembly the gel according your teacher's instruction for running the samples.
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9. Disassemble the gel carefully.
10. Wash the gel twice in distilled water, five minutes each.
11. Remove all free water from the gel and add 50ml LabSafe GelBlue stain to cover the gel. Gently shake the gel for 60 minutes at room temperature.
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**RESULTS, ANALYSIS & ASSESSMENT**

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