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

A Geno Technology, Inc. (USA) brand name

Protein Fingerprinting

Teacher's Guidebook

(Cat. # BE-408)

MATERIALS INCLUDED

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

- 1 vial Protein: Mouse Brain Protein
- 1 vial Protein: Mouse Heart Protein
- 1 vial Protein: Mouse Liver Protein
- 1 vial Protein: Mouse Lung Protein
- 1 vial PAGE: Sample Loading Buffer (2X)
- 30 1.5ml Tubes
- 1 bottle LabSafe GelBlue

SPECIAL HANDLING INSTRUCTIONS

- All protein samples can be stored at room temperature for 4 weeks. Store at 4°C for long-term storage.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

ADDITIONAL EQUIPMENT REQUIRED

- Protein Electrophoresis Equipment, SDS PAGE gels and electrophoresis buffer
- Sharp pin

TIME REQUIRED

- 3-4 hours

OBJECTIVES

- Learn manipulation of protein samples.
- Learn the concept of protein sequence.
- Learn time course dependent experiments.
- Use of protein electrophoresis for problem solving tasks.
- Identification of sample by protein fingerprinting.

BACKGROUND

The organs of each organism perform specialized and very different functions from each other, for example the heart is responsible for moving blood around the body, where as the kidneys remove toxic wastes from the blood. The differences between organs can be clearly demonstrated by exploring their differences at a subcellular level.

The building blocks for organs are the proteins that are responsible for both the structural and functional differences between organs, as a result the examination of the protein distribution between different organs will represent their structural and functional differences.

The Protein Fingerprinting kit provides students with four mouse organ samples (brain, heart, liver and lung) that contain all the proteins from the respective organ. Students prepare the samples for protein electrophoresis, a technique to separate the large number of proteins based on their molecular weight.

Following separation of the proteins by electrophoresis, the resulting protein gel is stained to visualize the proteins and the resulting distribution of proteins, known as the protein fingerprint, can be compared to the fingerprints of the other organs.

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 or use a premade gel for each student group. G-Biosciences Protein Electrophoresis Kit is recommended for making your own gel. One 10-lane gel is suitable for two groups.

2. Before you open the vials centrifuge all protein samples vials for 5 minutes to bring down all pellets to the bottom of the tubes.

3. Add 50µl Sample Loading Buffer and 50µl distilled (Lab pure water) water to each vial of mouse proteins. Soak the protein samples for 5 minutes with periodical vortexing to dissolve them completely.

4. Prepare your own electrophoresis running buffer (24mM Tris base, 192mM Glycine and 0.1% SDS).

5. OPTIONAL: Protein markers (not supplied) may be provided to each group. Provide 10µl protein standard maker for each group and instruct students to load the protein markers in the first lane.

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 8-12% Polyacrylamide gel (two groups can share one 10-lane gel)
- 50ml LabSafe GelBlue
- 15µl Mouse Brain Protein
- 15µl Mouse Heart Protein
- 15µl Mouse Liver Protein
- 15µl Mouse Lung Protein
- 1X Electrophoresis running buffer (shared with whole class)
- 1 pin

PROCEDURE

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

1. Using the pin punch a small hole into the caps of the four protein samples and then place in a boiling waterbath for 5 minutes.

2. Vortex the tubes briefly and centrifuge for 1 minute.

3. For running the samples, assemble the gel according your teacher's instructions.

4. Load the entire samples in separate wells.

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

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

7. Add 50ml LabSafe GelBlue to cover the gel. Gently shake the gel for 60 minutes at room temperature.

8. 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

Compare the protein fingerprints and discuss the major differences and similarities between the four samples:

Q. Explain why the protein fingerprints are so different?

A. *The differences are due to the organs having different structural and functional roles and therefore require different proteins.*

Q. Which of the four protein fingerprints would have the most similarities to a protein fingerprint from skeletal muscle?

A. *The heart fingerprinting is expected to have the greatest similarity as the heart consists of cardiac muscle, which has a similar composition to skeletal muscle.*

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