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

A Geno Technology, Inc. (USA) brand name

Size Exclusion Chromatography

Teacher's Guidebook

(Cat. # BE-414)

MATERIALS INCLUDED WITH THE KIT

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

- 6 Column: Size Exclusion Columns
- 1 vial Protein: Protein Extract
- 1 vial Vitamin-B
- 1 vial Alk. Buffer (0.3N)
- 1 bottle Gel Filtration Buffer
- 60 Centrifuge Tubes (1.5ml)

SPECIAL HANDLING INSTRUCTIONS

- Store Size Exclusion Columns and Protein Extract at 4°C.
- All other reagents can be stored at room temperature.

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 Safety Data Sheets (SDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
A031	Alk. Buffer (0.3N)	Corrosive

ADDITIONAL EQUIPMENT REQUIRED

- Stands and clamps

TIME REQUIRED

- 1-2 hours

OBJECTIVES

- Learn the principles of gel filtration or size exclusion chromatography.
- Understand the factors affecting gel filtration chromatography.
- A hands-on size exclusion chromatography lab activity.
- Importance of size exclusion strategies in protein purification.

BACKGROUND

A key step in proteomics, the study of proteins function and structure, is the purification of proteins. The ability to isolate and purify specific proteins is an essential feature of modern biochemistry as it allows scientist to study proteins in isolation from other proteins, which greatly aids the understanding of a particular protein's function.

Unfortunately there is no single ideal protein purification procedure and often the purification of a protein involves several techniques. The main idea behind protein purification is to select the best techniques to isolate a protein of interest, based on differences in its physical properties from other "unwanted proteins. One general separating technique, with many different approaches, is chromatography. The "Protein Chromatography" kits will aim to cover some of the chromatography techniques routinely used in protein purification.

Size exclusion chromatography (SEC), also called gel filtration chromatography or gel-permeation chromatography (GPC) uses porous particles to separate molecules of different sizes. It is generally used to separate biological molecules and to determine molecular weights and molecular weight distributions of polymers, such as proteins. Proteins enter the gel bed and the smaller proteins enter the porous beads, which slows their progress through the gel, where as larger proteins pass around the beads and rapidly flow through the column.

Size exclusion column bed has three functional components: the pore volume, the void volume, and the matrix volume (bed volume). The pore volume refers to the pore-lumen space within the particles. The void volume refers to the excluded volume i.e., the space between the particles. And the matrix volume refers to the solid component of the particles that fills the column bed.

The pore diameter defines the exclusion limit of the gel. Proteins too large to enter the pores are excluded and have access to only the void volume. Proteins larger than the exclusion limit elute together in a single peak at the beginning of the chromatography: the void volume. Proteins molecules that are smaller than the pore size enter the particles and their separation is determined by the pore size distribution within the pore volume. Among these proteins, the larger proteins are eluted earlier than the smaller protein molecules giving rise to the fractionation of protein molecules based on their size.

The protein molecules to be fractionated must have an opportunity to diffuse in and out of the pores and therefore the flow rate of the sample entering the column is critical. The importance of having a suitable diffusion time makes size exclusion chromatography is the slowest of the fractionation techniques.

The Size Exclusion Chromatography kit teaches gel filtration or size exclusion chromatography and the use of this method in the purification of proteins from biological samples. The kit is provided with Size Exclusion Columns filled with a bead matrix prepared by cross-linking dextran with epichlorohydrin. The size exclusion column (GT 600) provided in this lab activity has exclusion limit of 6,000 Daltons, meaning that molecules with molecular weights less than 6,000 Daltons will enter the gel and separate according to their molecular size. Molecules whose molecular weights are larger than 6,000 Daltons will be excluded and pass through the gel quickly.

This lab activity is provided with tissue extract consisting of a wide variety of protein including hemoglobin, a red protein that is a tetramer of four identical 16,000 Daltons subunits, and vitamin B9 (folic acid), a yellow molecule with a molecular weight of 440 Daltons. This lab activity teaches students the use of size exclusion chromatography for the separation of vitamin B9 from hemoglobin.

TEACHER'S PRE EXPERIMENT SET UP

1. Add 0.4ml Gel Filtration Buffer to the protein extract vial. Periodically vortex the tube until the protein is completely dissolved.

2. Add 0.4ml Alk. Buffer (0.3N) to the Vitamin-B vial. Periodically vortex the tube until the Vitamin-B is completely dissolved.

3. Add the protein extract to the vial with the Vitamin-B solution. This is the Gel Filtration Sample. Aliquot 100µl to each student group.

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 Size Exclusion Column
- 100µl Gel Filtration Sample
- 4ml Gel Filtration Buffer
- 10 Centrifuge Tubes (1.5ml)

PROCEDURE

Always wear gloves and protective clothing throughout the whole experiment.

1. Clamp the Size Exclusion Column in an upright position to the stand.

2. Open the top cap first and then the bottom cap of the column to prevent air entering the resin. Allow the buffer to drain out of the column, under gravity, to a waste container. Ensure that the resin settles evenly in the column.

3. ***Equilibrate the column*****:** Apply 100µl Gel Filtration Buffer to the top of the column and allow it to drain out freely into a waste container. Repeat this step 9 more times, so that a total of 1ml Gel Filtration Buffer has been added.

4. Carefully load 50µl Gel Filtration Sample to the column without disturbing the column surface. Allow the sample to enter the column.

5. ***Elute the sample*****:** Apply 100µl Gel Filtration Buffer to the top of the column and allow it to drain freely into a waste container.

6. Repeat step 5 until sample begins to elute from the column. When the red hemoglobin starts to elute from the column, position a centrifuge tube under the column and collect 0.2-0.3ml elution into the tube. Collect a few fractions until all red hemoglobin has been eluted from the column.

7. Once the elution of hemoglobin is complete, monitor the column and when the elution of the yellow Vitamin B begins, collect 0.2-0.3ml Vitamin B fractions into separate 1.5ml tubes.

RESULTS, ANALYSIS & ASSESSMENT

Q. Describe the meaning of size exclusion limit and void volume, the terminologies commonly used in size exclusion chromatography.

A. The pore size (diameter) of the separation particles defines the exclusion limit of the gel. The void volume refers to the excluded volume i.e., the space between the particles.

Q. Briefly describe the order of elution of hemoglobin and vitamin B.

A. Hemoglobin is eluted first because the size of the hemoglobin is larger than the exclusion limit (6000 molecular weight) of the size exclusion column (GT 600).

Vitamin B is eluted later because its small molecular weight means it easily enters into the pores of the size exclusion column beads and hence passage through the column is delayed.

Q. Describe the term used to define the elution volume for hemoglobin.

A. Hemoglobin is eluted in a volume defined as void volume for the size exclusion chromatography column.

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