Objective:

To learn the technique of separation of biomolecules on the basis of their size by **Gel filtration chromatography**.

Principle:

Chromatography is a method of separation of biomolecules that is based on the differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the components in a mixture. A column holds the stationary phase and the mobile phase carries the sample.

Gel-filtration chromatography, also called size-exclusion or gel-permeation chromatography, separates molecules based on the differences in their size. The sample is applied on top of a column containing porous beads. As the molecules pass through the column of porous beads of crosslinked agarose, they get separated as follows:

- Large molecules cannot enter the pores and elute as the first peak in the chromatogram. They elute fast and this is called **total exclusion**.
- Intermediate molecules may enter the pores and may have an average residence time in the particles depending on their size and shape. Different molecules therefore have different total transit times through the column. This portion of a chromatogram is called the **selective permeation region**.
- Small molecules enter the pores and have the longest residence time in the column and elute together as the last peak in the chromatogram. This last peak in the chromatogram is the **total permeation limit** (Refer figure 1).

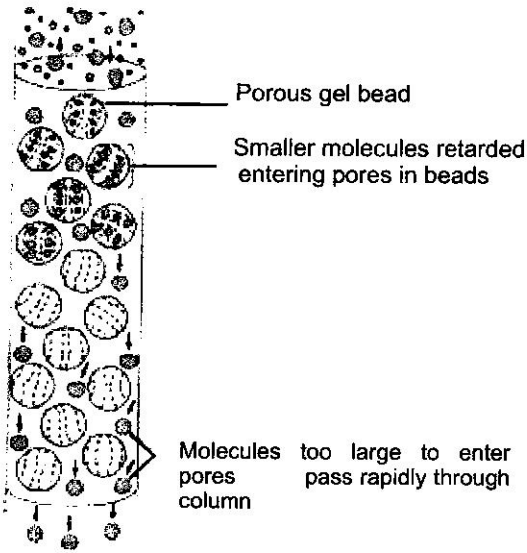

Fig 1: Schematic Diagram of a Gel Filtration Chromatography Column

Columns contain stationary phase.

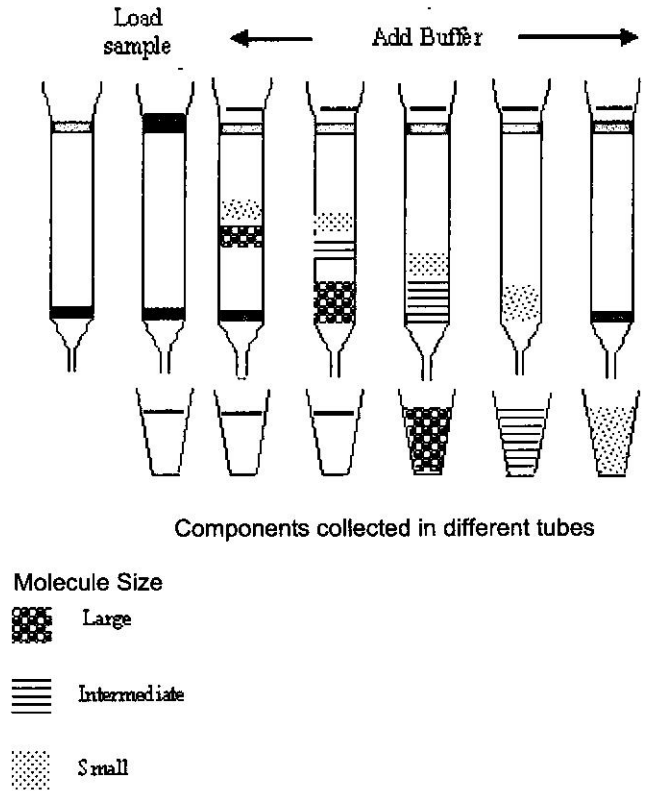

Fig 2: Schematic representation of sample separation by gel filtration chromatography

Kit Description:

In this kit, a mixture of three different biomolecules ranging in molecular size from 376 Da to 2000 kDa are supplied. These are separated on the basis of their size through a gel filtration column. The movement of the samples through the column is easily monitored as the biomolecules are colored, which also aids in collection of the resolved biomolecules.

6103900011730: The kit is designed to carry out 5 experiments.

Duration of experiment: Approximately 1 hour

Materials Provided:

The list below provides information about the materials supplied in the kit. The components should be stored as suggested.

Materials	Quantity	Store
	6103900011730 (5 Expts.)	
Gel Filtration Column (2 ml)	2 Nos.	4°C
Gel Filtration Buffer	125 ml	4°C
Sample	5 x 0.2 ml	4°C

Materials Required:

Glassware: Testtubes.
Reagent: Distilled water.
Other Requirements: Column stand, Micropipette and Tips

Note:

- Read the entire procedure carefully prior to starting the experiment.
- Allow the components to attain room temperature, 1 hour before use.
- Reconstitute one sample vial per experiment, with 0.2 ml of Gel Filtration Buffer to get a homogenous solution. Store the sample at 4°C and use within 3 months.
- Do not let the column to dry, at any time.
- Always open the upper cap first and then the lower cap to start the flow of Gel Filtration Buffer through column.
- Similarly, to stop the flow of Gel Filtration Buffer or to store the column, fix the lower cap first and then the upper cap.
- Column can be used 2-3 times only.
- Store the column at 4°C after each use.

Procedure:

1. Fix the column vertically to a stand.
2. Equilibrate the column with 4 ml of Gel Filtration Buffe Drain out the Gel Filtration Buffer completely.
3. Load 0.2 ml of the Sample onto the column, along th sides of the column.
4. Allow the Sample to sink completely and then add 0.2 ml of Gel Filtration Buffer.
5. Allow the Gel Filtration Buffer to flow out completely.
6. Keep toping the column with Gel Filtration Buffer, till a the coloured biomolecules have eluted ou (Approximately 20 ml of Gel Filtration Buffer is require experiment).
7. Collect the colored fractions in different tube (Refer fig 2).
8. Fix the lower cap and then the upper cap to stop the flo of buffer. Store at 4°C for next use.

Observation:

Note down the order in which various molecules are collected and interpret the results.

Interpretation:

- The Blue colored component is blue dextran having a molecular weight of 2,000,000 Da. These are very large molecules that exit fast from the column and are collected as first fraction.
- The Brownish red colored component collected as second fraction is hemoglobin having a molecular weight of 64500 Da. The molecules are of intermediate size, and they enter the pores, but their retention time is low, and hence take less time to exit the column.
- The Pink colored component is vitamin B12 having molecular weight of 376 Da. These are very small molecules that enter or permeate the pores of the beads and are retained in the bead for a longer time. They take a long time to exit the column and are collected as the third fraction.

Ordering Information:

Product	Size	Cat #
GeNei™ Gel Filtration Chromatography Teaching Kit (Consumables for 5 Experiments)	1 EA	6103900011730

Email:

Sales: sales@geneilabs.com

Customer Support: techsupport@geneilabs.com