Sephadex G25

Cat #: C-BETB002

Size:

1. Resin: 10 mL, 50 mL, 100 mL, 250 mL, 500 mL, 1 L;
2. Prepacked Column: 5mL-Column, 500mL-Column

Storage: 20% ethanol or 0.01 M NaOH, 4-30°C

Product Overview

Sephadex G25 is a dextran chromatography medium. In Sephadex G25, the degree of cross-linking of the dextran determines the extent to which macromolecules can permeate the beads. Large molecules are excluded while smaller sized molecules enter the beads to varying extents according to their different sizes. Large molecules thus leave the column first, followed by smaller molecules.

Depending on the different particle size, Sephadex G25 is available in four grades (Coarse, Medium, Fine, and Superfine).

Product Specifications

	Sephadex G25 M
Dry particle diameter (μm)	50-150
Fractionation Range (Da)	$1\times10^3 – 5\times10^3$
Recommended operational flow rate (cm/h)	50-250
pH Range	2-13
Recommended pressure (MPa)	< 0.2
Chemical stability	Stable in water, salt solution, organic solvent, alkali and weak acid solvent. Bur it will be degradated in strong acids, so it is usually only exposed to dilute strong acids,

	such as 0.02 M HCl.
Storage temperature	4℃ to 30℃, 20% ethanol or 0.01 M NaOH

Application Example

Column: XK16/30 Desalting Column, bed height was 25 cm (Sephadex G25 M).

Equilibration buffer: 20 mM PB + 150 mM NaCl, pH 7.0, filtered through a 0.45 μm filter.

Flow rate: 2 mL/min.

Column equilibration: Connect the chromatography column to a purification instrument and wash the desalting column with 2-3 column volume (CV) of equilibration buffer.

Sample loading: Add 1 mL sample, to maximize the lifespan of the chromatography column, the sample should be clarified before loading, such as centrifugation at 10,000 g for 10 minutes or filtration through a 0.45 μm filter.

Elution: Wash the desalting column with equilibration buffer for at least 2 CVs, using a flow rate that does not exceed the recommended maximum flow rate.

The chromatographic diagram is as follows:

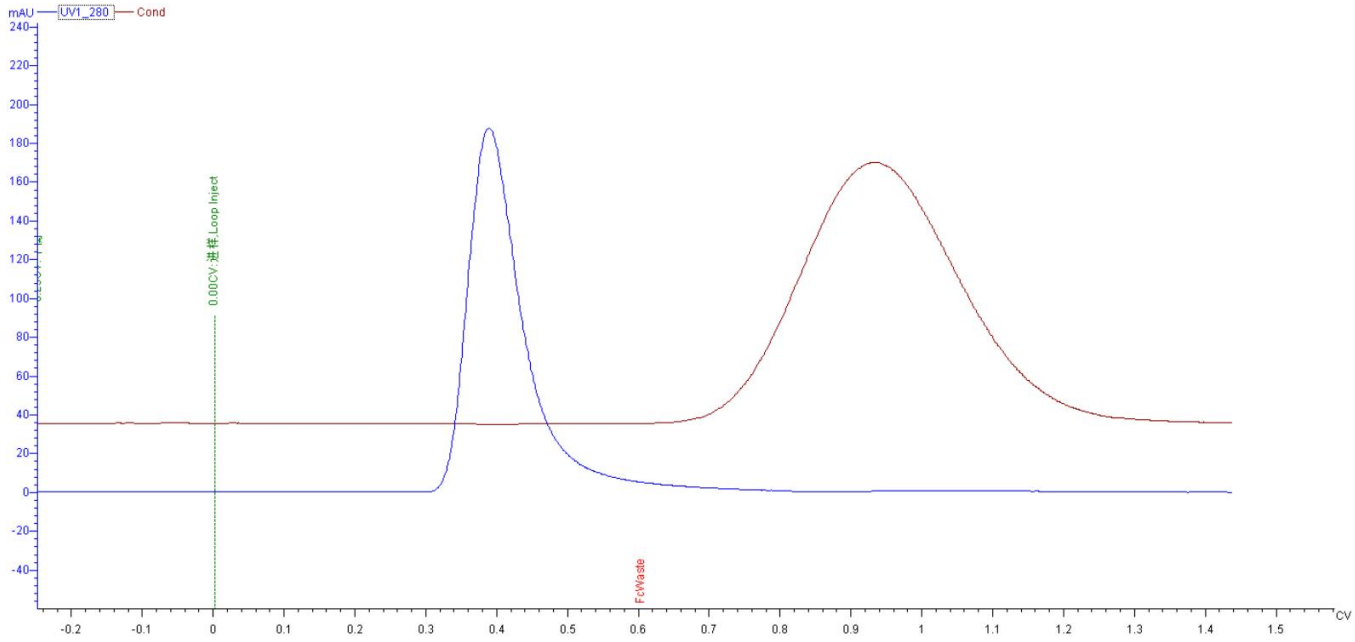

Fig.1 Desalting chromatogram on 5mL-Column

Usage Method

Sephadex G25 is commonly used for desalting, buffer exchange, and separation of proteins with different molecular weights. The sample volume varies depending on the specific application, and for buffer exchange or desalting, it can be up to 20% of the column volume.

Swelling

The Sephadex G25 media need to be swelled for the first time. Place in excess deionized water and swell, for example, add 100-150 mL water to swell 10 g media and allow it to swell at least 3 h. Swelling can also be accelerated by using a water bath (e.g., at 90°C for 1 h), but vigorous stirring should be avoided to prevent gel breakage.

Column Packing

1. Preparation of Gel Suspension

Calculate the required amount of swollen media (approximately 1.15 kg of settled gel per 1 L of column bed volume). For example, for a 16/20 column with a bed height of 10 cm, the required amount of swollen media would be $\pi \times (1.6/2)^2 \times 10 \times 1.15 = 23.11$ g. Weigh 23.11 g of media in a clean beaker and add a certain amount (e.g., 12 mL) of packing solution. Stir to form a homogeneous gel suspension.

2. Column Packing

2.1 Inspect the chromatography column to ensure all components are intact and clean. Install the bottom frit, tighten the O-ring, and vertically fix the column on an iron stand. Use a spirit level to check and adjust the column to be level.

2.2 Add an appropriate amount of packing solution to the column to remove air. Screw on the bottom plug, leaving 1-2 cm of packing solution in the column.

2.3 Use a glass rod to guide the gel suspension along the inner wall of the column and pour it continuously to minimize the generation of bubbles. Allow the media to settle naturally until the volume no longer changes, and at this point, the media and solution will form distinct layers, with the upper layer being completely clear.

2.4 Connect the top frit to a low-pressure chromatography system and start the pump at a certain flow rate to remove any remaining air in the tubing. Pause the system, install the top frit onto the column, with the bottom end of the frit about 1 cm above the gel surface. Tighten the O-ring and pump the column at a flow rate of 300-500 cm/h until the bed

volume no longer changes. Continue packing for 5-10 minutes and mark the position of the gel surface with a marker pen.

2.5 Pause the system, screw on the bottom plug to disconnect the column from the pump, and rotate the adjustment rod downward to stop at 0-0.5 cm below the marked position. Screw on the top plug to complete the column packing.

3. Cleaning and Regeneration

Cleaning-in-Place is performed to remove denatured proteins, lipoproteins, or lipids. The common used cleaning solutions include 0.2 M NaOH or non-ionic detergents, and the column should be washed with 2-3 CV.

4. Storage Conditions

Store at room temperature in 20% ethanol or 0.01 M NaOH.

Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.