PROCEDURE FOR USE

SEPADEXTRAN™ 25 & SEPADEXTRAN™ 50 Medium, Fine & Superfine

DESCRIPTION

Gel Filtration Chromatography allows biomolecule separations depending on size and with no significant dilution. Smaller molecules pass significantly slower through the column than medium molecules. And large molecules are totally excluded. So larger molecules leave the column first followed by medium and smaller ones in the order of their decreasing size. Sepadextran™ is a beaded gel filtration medium prepared by cross-linking dextran and is supplied in dry form.

ABT offers two types of Sepadextran™ (Sepadextran™-25 & Sepadextran™-50) that differ in their degree of cross-linking and hence in their degree of swelling and molecular fractionation range. Both types are available in three different particles sizes (Medium, Fine & Superfine). Medium grade is suitable for separations at high flow rates and low operating pressures and Fine & Superfine grades are for preparative separations and routine laboratory work.

Sepadextran™-25 can be used for protein and nucleic acid purifications with the exclusion limit of 5kD for proteins and 10 bases for nucleic acids. Desalting (before IEX and after HIC or Affinity Chromatography) & buffer exchange (between different chromatography steps) are other uses.

Sepadextran™-50 can be used for protein and nucleic acid purifications with the exclusion limit of 25kD for proteins and 20 bases for nucleic acids.

Detailed below are some recommendations to consider during hydration, column packaging and equilibration as well as in the subsequent sample application.

INSTRUCTIONS

HYDRATION AND COLUMN PACKAGING

Sepadextran™ can be hydrated in aqueous media (working buffer of choice containing no more than 20% alcohol) and allowed to swell for at least 3 hours at room temperature or 1hour at 90ºC.

The procedure for the correct packaging of the column is described below:

1. Filling a column requires that the slurry be not too thick as to retain air bubbles. An optimal relation of settled gel volume to buffer degassed volume is 3:1.

   Note: It is advisable to previously de-gas all the solutions before adding them to the column to avoid formation of bubbles.

   Manually shake the bottle to obtain a homogenous suspension and place a funnel in the head of the column and slowly run suspension down the walls of the column.

   Note: it is advisable to make the addition slowly to avoid formation of bubbles.
2. Repeat previous steps until the desired column height is obtained.
3. Insert the adapter gently in the column head until it begins to displace the liquid and make sure no air is trapped under the net.
4. Connect the pump to the column and watch that the column height remains the same as the flow of working buffer is passing through. Use as high a flow rate as possible without deforming the beads. Sepadextran™ can be pressurized up to 3 bar.
5. For regeneration and later reuse the gel should be washed with 2 column volumes of 0.2M NaOH or a solution of non-ionic detergent, rinsed with water, and re-equilibrated with 2-3 column volumes of buffer. For storage, antimicrobial agents should be added to the suspension to prevent contamination (0.02% sodium azide or 20% ethanol are acceptable). When necessary, the gel can be removed from the column and autoclaved.

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