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Bio gel p polyacrylamide gel instruction manual

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Bio-Gel P Polyacrylamide Gel Instruction Manual **Table of Contents** 1. Introduction 2. Technical Description 3. Instructions for Use 4. Sample 5. Void Volume Determination and Calibration 6. Sanitation and Sterilization 7. Storage 8. Flow Rate Determination 9. Ordering Information **Introduction** Bio-Gel P gels are porous polyacrylamide beads that offer efficient, gentle gel filtration of sensitive compounds. They are extremely hydrophilic and provide high resolution due to their consistent bead diameters and excellent molecular weight discrimination. **Technical Description** * Product description: Bio-Gel polyacrylamide gel * Particle size: Medium (90-180 µm), Fine (45-90 µm), Extra fine (< 45 µm) * Shipping medium: Shipped dry * Resistance: + pH range: 2-10 + Pressure: 15 psi * Organic solvents: < 20% * Working temperature range: 4-80 °C **Note:** Flow rate and resolution increase with temperature over the range of 4-80 °C. The Econo-Pac 10DG columns are designed for rapid and cost-effective desalting and buffer exchange of various samples. The Bio-Gel P-6DG gel used in these columns offers high recovery without allowing biologically active material to leach from the matrix, making them ideal for separating solutes of interest from contaminants. To achieve this, the gel is chosen based on its ability to exclude unwanted substances from its pores and retain smaller contaminants. The manual provides a general procedure for desalting or buffer exchange, as well as specific guidelines for different applications. Note that I've removed some technical details, such as the specifications of the columns (e.g., particle size, hydration range) and focus on summarizing the main features and benefits of the Econo-Pac 10DG columns. Fully autoclavable • 20 ml reservoir • Patented snap-off tip Fig. 2.1. Econo-Pac 10DG column. Bed volume 10 ml Total column volume 30 ml Maximum sample volume 3.3 ml Recommended sample volume 3.0 ml Void volume 3.3 ml Packing buffer 10 mM sodium phosphate, 10 mM NaCI, pH 7.0, with 0.02% sodium azide Recommended desalting protocols for sample volumes ranging from 100 µl to 3.0 ml. For volumes < 100 µl use Bio-Spin chromatography columns. For volumes > 3.0 ml use Bio-Gel P-6DG gel packed into an appropriate column. Add 1.5 x sample volume or 4ml of buffer (whichever is less) to elute the higher molecular weight component(s). Collect this fraction from the column. For a more precise collection method refer to step 6b. Follow steps 1 through 3 in the general desalting procedure, Section 5.1. Apply the radiolabeled protein solution to the column, allowing the sample to run completely into the column. Load up to 3.0 ml of the elution fraction collected from the Affi-Gel or Affi-Prep protein A column. Add 8.0 ml of a suitable buffer to elute the antibody. 10DG columns employ a matrix that excludes solutes above 6,000 daltons, allowing these components to elute in the void volume and thereby facilitating desalting. This process separates excluded components larger than 6,000 daltons from included components smaller than 6,000 daltons. Since desalting is not capacity-dependent, efficiency is not limited by sample concentration; instead, the limiting parameter is sample volume, which is constrained to the void volume of the column. Protocol for Using the Econo-Pac 10 DG Desalting Column: 1. Remove excess buffer from the upper frit and pour it off. 2. Add 20 ml of suitable buffer to the column, filling it to the 30 ml mark, and snap off the bottom tip to start the column flowing. Bio-Gel P gels are made from porous polyacrylamide beads created through the copolymerization of acrylamide and N,N'-methylene-bis-acrylamide. These hydrophilic, charge-free gels offer efficient and gentle gel filtration for sensitive compounds. They have exclusion properties that can enhance separation in complex mixtures of poorly water-soluble small molecules like nucleotides, peptides, and tannins. Formamide at full strength can be used as a solvent without swelling the Bio-Gel P gel. The recommended operating pH range is 2-10 at room temperature, while autoclaving is possible at pH 5.5-6.0 for 15-30 minutes at 120 °C in buffers like HEPES, MES, or citrate. Column selection involves choosing dimensions that allow baseline resolution without significant sample dilution, with a column length to diameter ratio between 5 and 10, and a bed volume of 4 to 20 times the sample volume for minimal dilution of about 1.25. Preparation of the gel includes gradually adding dry Bio-Gel P media to buffer in a beaker, estimating the required amount based on hydrated bed volume, and pouring an even slurry into the column after it is packed with buffer. The column should then be washed and attached to a reservoir for fraction collection or monitoring with continuous flow equipment. Void volume determination involves measuring the volume not accessible to solutes, essential for calibration of gel chromatography systems. The void volume (Vv) of a bed is equivalent to the elution volume (Ve) of excluded material. It is crucial to determine the void volume of the bed and test for uniformity of eluant flow before applying an experimental sample, especially with colored proteins like hemoglobin or ferritin. Gel filtration is a diffusion-controlled process, where resolution efficiency depends on flow rate and gel bead size uniformity. The optimal flow rate range is between 2-10 cm/hr to achieve the highest resolution. A linear flow rate of 5 cm/hr corresponds to specific column flow rates, as shown in Table 3. The Bio-Gel P series offers a range of gels suitable for various applications, including rapid carbohydrate and small peptide separations and desalting (Bio-Gel P-2), purification of proteins and polypeptides (Bio-Gel P-30 and Bio-Gel P-60). These gels provide efficient gel filtration with gentle separation of sensitive compounds. They are also compatible with dilute organic acids, 8 M urea, and chaotropic agents. Bio-Gel P Gel Product Description Matrix Bio-Gel polyacrylamide gel is a highly specialized matrix used in various analytical applications. The particle size ranges from fine to extra-fine, with a recommended shipping medium of dry conditions. Key Properties: - pH range: 2-10 - Pressure resistance: up to 15 psi - Organic solvent limit: less than 20% - Working temperature range: 4-80 °C Characteristics and Applications: - Bio-Gel P gel is autoclavable at pH 5.5-6.0 in buffers for molecular weight determinations. - The recommended operating pH range is 2-10, with flow rate and resolution increasing with temperature over the range of 4-80 °C. - Compatibility with various solvents and conditions used in molecular weight determinations. Instructions for Use: - Column selection: ideal dimensions allow baseline resolution without significant sample dilution, typically with a column length to diameter ratio between 5 and 10 and a bed volume 4 to 20 times the sample volume. - Eluant selection: eluants chosen should provide optimal separation of analytes. To ensure stability for labile sample solutes, it's recommended to maintain an ionic strength of at least 20 mM. High concentrations of salt solutions can alter gel bed volume and exclusion limits. For optimal performance, Bio-Gel P-gels should be hydrated according to specific guidelines. The table lists properties and characteristics of various Bio-Gel P-gel sizes, including particle size, hydration range, flow rates, and exclusion limits. It's essential to understand the distribution coefficient (Kd) for quality control purposes. To prepare a gel column, start by hydrating the Bio-Gel P media in buffer according to specific instructions. After initial uniform suspension of beads, settle the mixture before decanting supernatant and degassing the solution. Repeat this process several times to remove fines and achieve optimal results. The provided instructions outline step-by-step procedures for preparing and setting up a gel column, emphasizing the importance of gentle handling and proper hydration to ensure optimal performance. Allow for gel loss during handling by using twice as much buffer as the packed bed volume, to ensure proper separation and analysis results.