

Desalting With Bio-Gel® P-6DG Desalting Gel

Introduction

Bio-Gel P-6DG desalting gel is a spherical polyacrylamide gel that has been optimized specifically for desalting and buffer exchange. Its high gel strength, fast flow rates, low nonspecific adsorption, and economical swelling ratio make it ideal for routine desalting of macromolecules (Brown and Simoni 1984, Yang et al. 1984) in laboratory and industrial applications. Bio-Gel P-6DG gel is also convenient for buffer exchange (Persson and Wilson 1978, Gennis and Cantor 1976).

Bio-Gel P-6DG gel has a molecular weight exclusion limit of approximately 6,000 and a hydrated particle size range of 90–180 μm . Polyacrylamide is not subject to enzymatic degradation, will not shed low molecular weight sugars, and will not serve as a nutrient for microbial growth. The hydrophilic gel contains less than 0.5 μmol of carboxyl groups per ml of gel, ensuring little or no interaction between the gel and sample molecules.

Bio-Gel P-6DG gel is compatible with dilute organic acids, 8 M urea, 6 M guanidine HCl, other chaotropic agents, and detergents. Organic solvents may be added to the eluent. However, Bio-Gel P-6DG gel will not swell fully in nonpolar solvents. Alcohol, up to 20% v/v, and formamide at full strength may be used without substantially changing the exclusion properties of the gel.

Bio-Gel P-6DG gel is most stable between pH 5.5 and pH 6.5. Autoclaving and long-term storage should be carried out within this range (store in 0.02% NaN_3). The normal operating range is between pH 3 and pH 8. The gel can be used for brief periods outside this range, but hydrolysis of the amine side groups will take place, generating a negative charge on the gel. Each gram of Bio-Gel P-6DG swells to approximately 7 ml of packed gel bed.

Instructions for Use

Gel Preparation

1. Gradually add dry Bio-Gel P-6DG gel to buffer in a beaker. Each gram of dry Bio-Gel P-6DG gel will form approximately 7 ml of packed bed. Use twice as much buffer as the expected packed bed volume.
2. Allow gel to hydrate 30 min at room temperature. Let the gel settle during hydration.
3. Resuspend the gel in excess buffer. When 90–95% of the gel has settled, remove fine particles by decanting the excess buffer. Adjust to a volume of twice the expected packed bed volume.
4. Degas by aspirating at reduced pressure.

Pouring the Column

1. Fix funnel to top of column, close column outlet, and add enough buffer to fill 20% of column.
2. Smoothly pour uniform slurry into column.
3. When 2–5 cm of bed has formed, allow column to flow until fully packed.
4. Remove excess gel and equilibrate column by passing 2 bed volumes of buffer through the column at operating flow rates.

Desalting Procedure

1. Drain buffer in the level of gel bed and stop flow. Carefully layer sample onto the bed surface and drain into the bed. Follow this with buffer to wash the sample into the bed. Attach reservoir and begin run.
2. The separation can be monitored by optical density or conductivity, and the desired fractions collected. After a column has been characterized, monitoring is no longer necessary since elution volumes will be consistent. The void volume should be 30–35% of the column volume.
3. Wash the column with starting buffer until low molecular weight material has completely eluted. Two bed volumes should be sufficient.

Parameters Affecting Desalting

Sample Volume

Sample-to-gel volume ratio is the most critical parameter affecting resolution and dilution in desalting applications.

In each case in Table 1, the sample volume is 25% of the gel volume. This ensures quantitative desalting. The chromatograms in Figure 1 show separations run under the conditions listed in Table 1.

Sample volumes used in desalting are generally 25–40% of the volume of the packed gel. While the smaller sample volume ensures complete desalting, it also produces greatest dilution of the recovered material. Therefore, when total recovery of

Table 1. Recommended Desalting Conditions

Sample Volume	Gel Volume	Bed Height	Recommended Econo-Column*
1.0 ml	4 ml	5 cm	1.0 x 5 cm
1.5 ml	6 ml	7.7 cm	1.0 x 10 cm
2.0 ml	8 ml	10 cm	1.0 x 10 cm
3.0 ml	12 ml	6.8 cm	1.5 x 10 cm
5.0 ml	20 ml	11.3 cm	1.5 x 20 cm
10.0 ml	40 ml	8 cm	2.5 x 10 cm

*See the Bio-Rad catalog for ordering information.

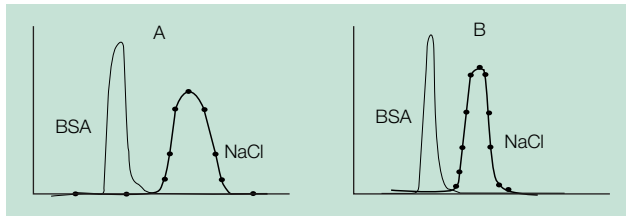


Fig. 1. Desalting of BSA on Bio-Gel P-6DG gel using conditions outlined in Table 1. A, 1.5 ml sample containing 1.25 mg/ml BSA in 2 M NaCl run on a 1.0 x 10 cm Econo-Column® chromatography column containing 6 ml Bio-Gel P-6DG at a flow rate of 39 ml/hr (gravity flow). B, 10 ml sample containing 2 mg/ml BSA in 2 M NaCl run on a 2.5 x 10 cm Econo-Column chromatography column containing 40 ml Bio-Gel P-6DG at a flow rate of 120 ml/hr (gravity flow).

sample completely free of low molecular weight contaminants is the most important factor, the sample volume should not exceed 25% of the gel volume. When minimum sample dilution is the most important factor, the largest sample size that gives adequate desalting is recommended. Figure 2 shows chromatograms with varying sample-to-gel ratios.

Flow Rate

Desalting is usually done at rapid flow rates (30–80 cm/hr). While slower flow rates give better resolution, it takes a 3–4-fold decrease in flow rate before a significant increase in resolution is noticeable. This is due to the vast molecular weight difference between the molecules being separated.

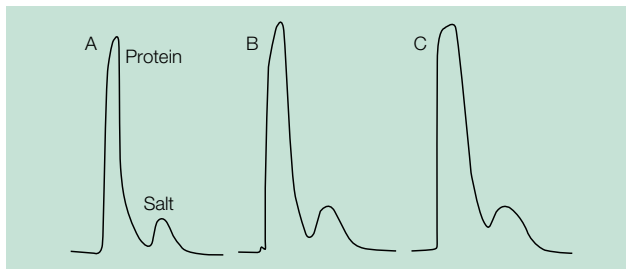


Fig. 2. Effect of sample volume on desalting. A, 1 ml sample (20% of gel volume) containing 1.3 mg/ml BSA, 2 mg/ml NaNO₃ on a 1.0 x 10 cm Econo-Column chromatography column containing 5 ml Bio-Gel P-6DG at a flow rate of 30 ml/hr. B, 1.5 ml sample (30% of gel volume); all other conditions as in A. C, 2.0 ml sample (40% of gel volume); all other conditions as in A.

Column Dimensions

In desalting, column dimensions are much less critical than in analytical fractionations. A short, wide column will give nearly the same resolution as a long, narrow column containing the same volume of gel, provided the same flow rates (ml/min) are used. Large diameter columns generally provide faster flow rates than small diameter columns and are advantageous for processing large sample volumes.

Alternatives to Desalting on Bio-Gel P-6DG Gel

Bio-Gel P-6DG desalting gel has been chosen as the optimum gel for routine desalting, but in many cases other gels in the Bio-Gel P series will perform as well. The chosen gel should have a pore size such that the macromolecule of interest is excluded and the smaller molecular weight material elutes with the included volume.

For desalting substances with molecular weights below 6,000, Bio-Gel P-2 gel or Bio-Gel P-4 gel should be selected. Any particle size material may be used, smaller particles giving better resolution, larger particles giving faster flow rates.

Desalting and Buffer Exchange Products

Bio-Rad offers a wide range of products to simplify the desalting process. The Econo-Pac® 10DG columns eliminate the tedious process of packing a column for each desalting step. With a 10 ml bed volume, the Econo-Pac 10DG columns can desalt up to 3 ml samples in <6 min. An upper frit protects the gel from disruption, assuring reproducible separations. For small-scale applications, Bio-Spin® 6 and 30 columns combine the ease of gel filtration with the speed of centrifugation. The 1 ml bed size makes them ideal for nucleic acid applications.

References

- Brown DA and Simoni RD, Biogenesis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, an integral glycoprotein of the endoplasmic reticulum, *Proc Natl Acad Sci USA* 81, 1674–1678 (1984)
- Gennis LS and Cantor CR, Double-headed protease inhibitors from black-eyed peas. I. Purification of two new protease inhibitors and the endogenous protease by affinity chromatography, *J Biol Chem* 251, 734–740 (1976)
- Persson A and Wilson IB, A new chromogenic substrate for gamma-glutamyl transpeptidase, *Anal Biochem* 89, 408–413 (1978)
- Yang H et al., Synthesis of undecagold cluster molecules as biochemical labeling reagents. 2. Bromoacetyl and maleimido undecagold clusters, *Biochemistry* 23, 3857–3862 (1984)

Ordering Information

Catalog #	Description
150-0738	Bio-Gel P-6DG Desalting Gel, 100 g
150-0739	Bio-Gel P-6DG Desalting Gel, 1 kg
732-2010	Econo-Pac 10DG Columns, 30
732-6000	Bio-Spin 6 Columns, 25
732-6004	Bio-Spin 30 Columns, 25

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