

## Operating Instructions

### BioPureSPN™ SEC MIDI (10-20µL loading) & MACRO (20-40µL loading) Columns

Directions for SEC (MIDI p/n HEM P020, HNS P020-M, HEM P060, HNS P060-M, & MACRO p/n: HMM P020, HNS P020-L, HMM P060, HNS P060-L):

Bio-Gel® P polyacrylamide gels, for high-resolution gel filtration, are prepared by copolymerization of acrylamide and *N,N'*-methylenebisacrylamide. The gels are extremely hydrophilic and essentially free of charge, and provide efficient, gentle gel filtration of sensitive compounds.

Bio-Gel® P gels:

- Are supplied dry with molecular weight exclusion limits ranging from 100 to 60,000. Bio-Gel P gel is susceptible to hydrolysis of amide groups at higher or lower pH. Flow rate and resolution increase with increasing temperature in the range of 4–80°C. P-2 gels swell 3x (i.e. to 150µL MIDI or 300µL MACRO) while P-6 gels swell 6x (i.e. to ~300µL MIDI or ~600µL MACRO) their dry bed volume. The gel will expand to fill the gap between the top frit and the bed.
- Bio-Gel P gel is compatible with dilute organic acids, 8 M urea, 6 M guanidine-HCl, chaotropic agents, reducing agents such as dithiothreitol and mercaptoethanol, and detergents such as SDS, CHAPS, and Triton® X-100. Bio-Gel P gel may be used effectively with distilled water however, buffers of > 50 mM ionic strength are recommended for most protein separations.
- Miscible organic solvents may be added to the eluants used with Bio-Gel P gel. Alcohol up to 20% will not substantially alter the exclusion properties of the gel, and will in some cases enhance separation of complex mixtures of poorly water soluble small molecules such as nucleotides, peptides, and tannins. Formamide may be used at full strength, because Bio-Gel P gel is completely swelled by this solvent.
- Do not support microbial growth or leach carbohydrates (due to their synthetic composition)

Bio-Gel P-6 gel, with a molecular weight exclusion limit of 6,000, is recommended specifically for desalting and buffer exchange applications whereas Bio-Gel P-2 has a MWCO of 100 - 1200 and may exclude some small ligands. They provide rapid results with high sample recovery and minimal sample dilution. Their hydrophilic nature ensures little or no interaction between the gel and sample molecules.

- The column material is dry and must be hydrated. To hydrate the gel in a 96-Well plate completely fill the reservoir to permit the mobile phase to flow through the top frit into the bed. Let the gel swell for 1-4 hours at room temperature before subsequent aliquots of mobile phase are added to prepare the gel for your sample.
- Wash the column with 200µL of water or buffer as many times as is required for your specific application.
- Place the column in a new collecting tube/plate. Spin for 3 minutes at 110x g. A sample load of 20% of the bed volume or less will enhance the SEC separation capability.
- After centrifugation, the purified sample will be in the collecting tube and will be ready for further use.



96-Well Bio-GEL® SEC plates are available as either a 50mg (HNS P0x0-M) or 100mg (HNS P0x0-L) plateS



BioPureSPN SEC columns are available as either 50mg (HEM P0x0-M) or 100mg (HMM P0x0-L) columns, 100/pk

**Each column is designed for a one-time application only, and it is recommended that columns should not be re-used since the quality of results is affected. Cartridges are for desalting applications only. There is not enough column volume to allow true SEC fractionation.**

**FOR RESEARCH USE ONLY**

SEC 11.03.22

BioPureSPN is a trademark of The Nest Group, Inc.  
Bio-GEL is a trademark of BioRad Laboratories