Bio-Gel® A Gels

Instruction Manual

BIO-RAD

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Section 1
Introduction

Bio-Gel A gels are a series of agarose based size exclusion gels which provide high resolving power, minimal non-specific interaction, and excellent flow properties. The porosity of the gel is controlled by the percentage of agarose incorporated into the matrix. Six fractionation ranges are available, with exclusion limits of 0.5, 1.5, 5, 15, and 50 million daltons.

Bio-Gel A gels are compatible with all commonly used aqueous buffers. The gel has excellent shrink/swell properties and exhibits no significant change in the bed volume with increasing salt concentration up to 500 mM salt. The gels can be run between pH 4–13 and can be used at temperatures between 2–30 °C.

Section 2
Technical Description

Table 1. Bio-Gel A Gel Product Description

Matrix	Bio-Gel agarose gel	
Particle size range		
Coarse	150–300 µm	50–100 mesh
Medium	75–150 µm	100–200 mesh
Fine	38–75 µm	200–400 mesh
Shipping medium	Fully hydrated in water, with 0.05% sodium azide added as a preservative.	
Resistance		
pH range	4–13	
Maximum operating pressure	15 psi	
Organic solvents	20% alcohol	
Temperature range*	2–30 °C	
Storage	4 °C to room temperature	

* Note: Above this temperature range the gel softens and freezing causes irreversible collapse of the gel structure and alteration of the exclusion limit.

Table 2. Properties of Bio-Gel A Gels

Product	Grade	Hydrated Bead Size (µm)	Typical Flow Rates* (cm/hr)	Fractionation Range/Exclusion Limit (daltons)
Bio-Gel A-0.5m gel				
151-0130	Coarse	150–300	20–25	<10,000–500,000
151-0140	Medium	75–150	15–20	<10,000–500,000
151-0150	Fine	38–75	7–13	<10,000–500,000
Bio-Gel A-1.5m gel				
151-0430	Coarse	150–300	20–25	<10,000–1,500,000
151-0440	Medium	75–150	15–20	<10,000–1,500,000
151-0450	Fine	38–75	7–13	<10,000–1,500,000
Bio-Gel A-5m gel				
151-0730	Coarse	150–300	20–25	10,000–5,000,000
151-0740	Medium	75–150	15–20	10,000–5,000,000
151-0750	Fine	38–75	7–13	10,000–5,000,000
Bio-Gel A-15m gel				
151-1030	Coarse	150–300	20–25	40,000–15,000,000
151-1040	Medium	75–150	15–20	40,000–15,000,000
151-1050	Fine	38–75	7–13	40,000–15,000,000
Bio-Gel A-50m gel				
151-1330	Coarse	15–300	20–25	100,000–50,000,000
151-1340	Fine	75–150	5–15	100,000–50,000,000

* Flow rate determined by using a 1.5 x 20 cm column and a head to bed ratio of 1:1.

Section 3
Principles of Gel Filtration

Section 3.1 Gel Filtration Basics and Sample Preparation

Gel filtration or size exclusion chromatography (SEC) separates molecules based on their size. The gel media consists of spherical beads containing pores of a specific size distribution. Separation occurs when molecules of different sizes are included or excluded from the pores within the matrix. Small molecules diffuse into the pores and their flow through the column is retarded, while large molecules do not enter the pores and are eluted in the column's void volume. Consequently, molecules separate based on their size as they pass through the column and are eluted in order of decreasing molecular weight.

Operating conditions and gel selection depend on the application and the desired resolution. Two methods used in size exclusion chromatography are group separation, including desalting and buffer exchange, and fractionation. In desalting, the molecule of interest is eluted in the void volume, while smaller molecules are retained in the gel pores. To obtain the desired separation, the gel should have an exclusion limit smaller than the molecule of interest. In fractionation, molecules of varying molecular weights are separated within the gel matrix. With this method, the molecules of interest should fall within the fractionation range of the gel. Common applications include the fractionation and molecular weight determination of proteins, nucleic acid separations, plasmid purification, and polysaccharide fractionation.

Resolution depends on the particle size, pore size, flow rate, column length and diameter, and sample volume. Generally, the highest resolution is obtained with low flow rates (2–10 cm/hr), long narrow columns, small particle size gels, small sample volumes (1–5% of the total bed volume), a 2-fold difference in molecular weight and a sample viscosity that is the same as the eluant. For desalting, the sample volume can be as much as 30–40% of the total bed volume, and shorter wider columns may be used.

Section 3.2 Column Selection

A properly executed separation will usually employ a bed with a length to diameter ratio between 5 and 10. The bed volume employed is usually 4 to 10 times the volume of the sample. The minimum anticipated dilution factor for an excluded substance approaches 1.25. Fractionation procedures generally require bed length to diameter ratios of 25 to 100 or greater and bed volumes 25 to 100 times the sample volume. If beds in excess of 1 meter long are needed, two or more short columns may be connected in series by flow adaptors.

Section 3.3 Eluant Selection

The eluant chosen should provide maximum stability for labile sample solutes. To eliminate the effect of small amounts of negatively charged groups on the gel, the ionic strength should be at least 50 mM. Using highly concentrated salt solutions greater than 500 mM may cause the gel bed to shrink, as well as alter the exclusion limit of the gel.

The recommended pH range is from pH 4.0 to pH 13.0. Prolonged operation outside these limits may lead to structural disruption of the gel beads. Strong oxidizing

agents should be avoided because they react with the gel and increase the content of charged groups on the matrix.

Bio-Gel A gels are generally not intended for use with organic solvents, but will tolerate gradual exchange with aqueous and dilute organic solutions up to 20%.

Section 4
Column Packing and Operation

Section 4.1 Column Packing

1. Bio-Gel A gel is supplied fully hydrated with sodium azide as a preservative. The gel should be washed prior to packing into a column. Pour the desired amount of gel into a Buchner funnel equipped with a filter. Apply a vacuum to remove excess water, then wash the gel with 2–3 volumes of the buffer.
2. Slurry the prepared matrix in several bed volumes of the running buffer and degas under vacuum for 5 minutes. Occasional swirling of the container will help to fully degas the slurry. A stir bar should not be used for this process.
3. Fill the column approximately 1/3 full with degassed starting buffer and remove any air bubbles that might

be trapped in the bed support or the column end piece. Close the exit stopcock.

4. Fit the column with a reservoir and filling funnel and add the gel slurry. Allow the gel to settle until it reaches a 3–5 cm bed height.
5. Open the stopcock and allow buffer to flow until a stable gel bed has packed.
6. Connect the flow adaptor tubing to the pump, fill it with buffer, and make sure it is free from air bubbles. Close the exit stopcock, remove the excess buffer from the reservoir, and attach the flow adaptor to the column. (Inserting it at a slight angle makes it easier to avoid trapping air bubbles.)
7. Open the column outlet and pump at least 5 bed volumes of run buffer through the column at a flow rate which is 10–20% higher than the flow rate at which the actual separation will be run. (For typical flow rates see Table 2.)
8. Switch off the pump and close the column outlet. Adjust the flow adaptor until it is in contact with the gel surface. During this step, you may have to disconnect the flow adaptor tubing from the pump to allow buffer to flow back through the tubing as you bring the flow adaptor in contact with the gel bed.

9. After equilibration with the starting buffer, the column is ready for sample application.

Section 4.2 Column Operation

1. Equilibrate the column you will be working with by pumping 5–10 column volumes of run buffer over the column at the flow rate at which the separation will be run. Typically slower flow rates, and taller columns, provide superior separations.
2. Apply your sample as close to the column inlet as possible.
3. Follow sample application with run buffer. Do not allow the column to run dry.
4. Monitoring your chart recorder, collect the components as desired.

Section 4.3 Void Volume Determination and Calibration

The void volume (V_o) of the bed should be determined and the bed tested for uniformity of eluant flow before applying experimental sample. To assure accurate V_o values, test substances must exhibit minimal interaction with the gel medium and should be large enough to assure elution in the true void volume. Blue

dextran is not recommended for V_o determination because it is not homogenous in size and may bind nonspecifically to the gel. Tobacco Mosaic Virus (TMV) or any large (>150 million) molecular weight marker may be used for V_o determination.

Using a standard mixture of proteins allows verification of the column packing and protein elution as well as the calibration of the column and the calculation of the molecular weight of unknown sample proteins. It also allows comparison of different columns, and different packing material, without wasting sample. Bio-Rad's Gel Filtration Standard is a mixture of five proteins with known molecular weights: thyroglobulin (M_r 670,000), bovine gamma globulin (M_r 158,000), chicken ovalbumin (M_r 44,000), equine myoglobin (M_r 17,500), and vitamin B12 (M_r 1,350). Myoglobin and vitamin B12 are visible and can be seen as they migrate through the column.

Section 5
Storage and Sterilization

Packed columns of Bio-Gel A gel may be used indefinitely if care is taken to prevent microbial growth. Sodium azide (0.02%) is an effective anti-microbial agent. For long term storage, maintain pH at neutrality.

Microbial growth is often manifested by the formation of colonies in the gel bed. A lamp held behind the column will aid in the detection of colonies. Gel beds or slurries in which microbial growth has occurred should be discarded, because complete removal of organisms from the gel matrix is impossible.

Sterilization procedures must take into account the physical and chemical properties of the gels, as well as the resistance of the organisms to be destroyed. With Bio-Gel A gel, cold sterilization procedures must be employed because the agarose begins to dissolve above 40 °C. Slurries or packed columns may be cold-sterilized with a solution of diethyl-carbonate (0.01%). Bio Gel A gels are not stable in solutions with a pH >7 for extended periods (greater then 12 hr).

Section 6
Product Information

Catalog Number	Product Description
151-0130	**Bio-Gel A-0.5m Gel, coarse**, 500 ml
151-0140	**Bio-Gel A-0.5m Gel, medium**, 500 ml
151-0150	**Bio-Gel A-0.5m Gel, fine**, 500 ml
151-0430	**Bio-Gel A-1.5m Gel, coarse**, 500 ml
151-0440	**Bio-Gel A-1.5m Gel, medium**, 500 ml
151-0450	**Bio-Gel A-1.5m Gel, fine**, 500 ml
151-0730	**Bio-Gel A-5m Gel, coarse**, 500 ml
151-0740	**Bio-Gel A-5m Gel, medium**, 500 ml
151-0750	**Bio-Gel A-5m Gel, fine**, 500 ml
151-1030	**Bio-Gel A-15m Gel, coarse**, 500 ml
151-1040	**Bio-Gel A-15m Gel, medium**, 500 ml
151-1050	**Bio-Gel A-15m Gel, fine**, 500 ml
151-1330	**Bio-Gel A-50m Gel, coarse**, 500 ml
151-1340	**Bio-Gel A-50m Gel, fine**, 500 ml
151-1630	**Bio-Gel A-150m Gel, coarse**, 500 ml
151-1640	**Bio-Gel A-150m Gel, fine**, 500 ml
151-1901	**Gel Filtration Standard**