Macro-Prep® SE 100/40 and 1000/40 Gels

Instruction Manual

BIO-RAD

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4006121 Rev A

Introduction

The Macro-Prep SE 100/40 and SE 1000/40 gels are new, high performance, size exclusion gels based on a 40 µm, spherical agarose bead. The beads are produced from highly purified agarose utilizing a proprietary cross-linking process which results in a mechanically and chemically stable gel ideally suited for the separation of proteins.

The gels are available in the following fractionation ranges:

SE 100/40 for bio-molecules from 5,000–100,000 Daltons

SE 1000/40 for bio-molecules from 10,000–1,000,000 Daltons

Both gels exhibit steep selectivity curves (see Figures 1 and 3) which, combined with the 40 µm particle size and narrow particle size distribution, provide high resolution separations at high flow rates and low back pressures. See Figures 2 and 4.

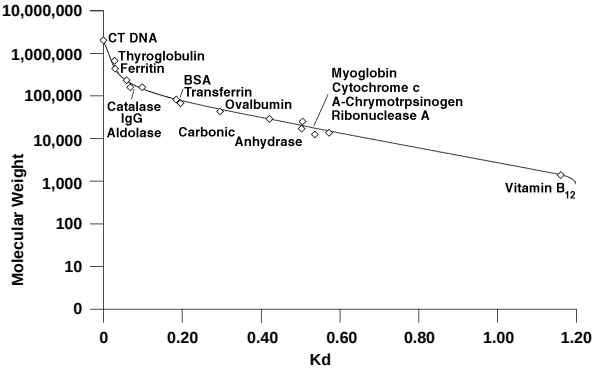

Fig. 1. Kd Macro-Prep SE 100/40 gel.

This manual provides the means to insure optimum use and service of your Macro-Prep SE gel. To fully utilize the performance of the Macro-Prep SE gel, it should be packed in a suitable column. An extensive range of columns and packing reservoirs is available from Bio-Rad Laboratories.

Note: Bio-Prep SE pre-packed columns (Bio-Prep SE-100/17 and SE-1000/17 8 x 300 mm columns) are based on a 17 µm agarose bead and are available for analytical and methods development work. Separations developed and optimized on the Bio-Prep SE columns are easily transferred and scaled-up to the bulk 40 µm material with little or no modification.

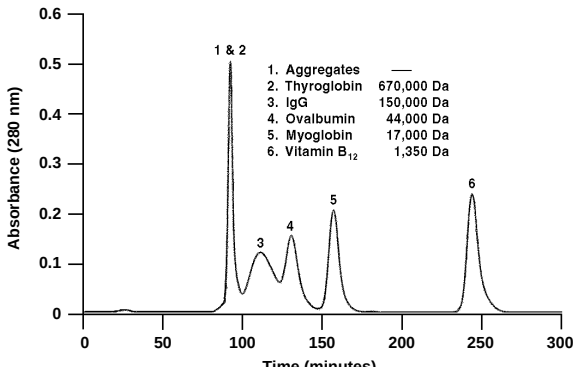

Fig. 2. Separation of a gel filtration standard using the SE 100/40 material packed in a 2.5 x 50 cm column.

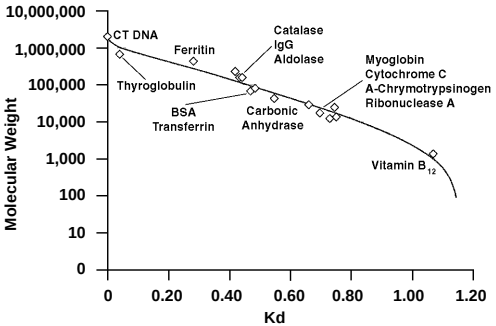

Fig. 3. Kd Macro-Prep SE 1000/40 gel.

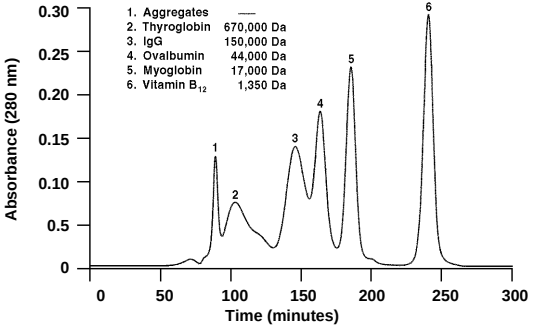

Fig. 4. Separation of a gel filtration standard using the SE 1000/40 material packed in a 2.5 x 50 cm column.

Unpacking and Inspection

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to your local supplier, sales representative, or directly to Bio-Rad Laboratories.

Preparation of Macro-Prep SE Gel

The gel is supplied pre-swollen in a 22% ethanol solution. Before packing, the ethanol solution must be removed and replaced with deionized water or some other suitable buffer. Preparation of a 50% slurry is recommended and an additional 5–10% of the required

bed volume should be prepared as the gel bed may compress during the initial packing and equilibration.

Gel and Slurry Preparation

1. Dispense the required volume of gel plus an additional 5–10%.
2. Wash the gel with five to ten bed volumes of DI or WFI quality water to remove the ethanol storage buffer. This operation can take place in any suitably sized vessel such as a flask, beaker, or buchner funnel. Use of a buchner funnel is reccommended.
3. After washing, allow the gel bed to run dry. Suction may be used to speed up this process.

4. Place the washed gel in a suitable size container and prepare a 50% slurry by adding DI, WFI, or other degassed buffer to the gel bed. Avoid any type of vigorous mechnaical stirring which could damage the beads.
5. The slurry is now ready for packing.

Column Packing

Note: Size exclusion columns are typically long and thin. This configuration affords optimal resolution but tends to dilute the sample when compared to short columns. Typical bed heights range from 30 cm to 1 meter.

1. If the slurry was prepared in a vessel other than the column to be used, add buffer to the column until it is approximately one third full. Open the column outlet to purge air bubbles and wet the column parts. If the slurry was prepared in the column, proceed to step 4.
2. Close the column outlet. Add additional buffer if required to maintain the buffer volume at approximately one third of the column capacity.

3. Add the gel slurry to the column. Drain buffer as needed to accommodate the gel. Do not allow the gel bed to run dry.
4. When the material is in the column, insert the column adaptor and begin pumping buffer through the system packing the column under flow. Packing the material under flow is done to insure a uniformly packed bed which will provide optimum separations. See Table 1 for additional guidelines on optimal packing flow rates to be used with various size columns.
5. Pack the column at a flow rate in excess of that to be used during the actual chromatographic separation. As the bed compresses, carefully monitor the back pressure and adjust the flow rate accordingly so as to insure the column is not overpressurized.
6. When the bed has compressed and appears stable, switch off the pump and adjust the flow adaptor so that it is contact with the gel bed. Repeat this step as necessary to insure contact between the gel bed and flow adaptor.
7. The packed column is now ready for operation.

Table 1. Guidelines for column packing flow rates

Column diameter (cm)	X-sectional area (cm^2)	Flow rate (ml/min)	Linear Flow Rate (cm/hr)
0.5	0.20	0.25	75
0.7	0.39	0.5	75
1.0	0.79	1.0	75
1.5	1.77	2.2	75
2.5	4.91	6.1	75
5.0	19.63	24.5	75

Note: These flow rates should serve as guides by which a column can be packed. Column packing procedures should be developed which meet the demands placed upon the materials and which reflect the operational extremes the material might encounter.

Column Operation

1. Connect the column to your chromatography system and equilibrate the column. Typically one to five bed volumes are run through the system at the desired flow rate. Readjust the flow adaptor if necessary.

2. The operational flow rate is dependent on the composition and the purity of the sample. Slower flow rates typically provide higher resolution separations.
3. Apply the sample to the column as close to the column inlet as possible. For optimal separation, the sample size should not exceed 2.5% of the bed volume. Sample volumes of up to 5% of the bed volume are possible, however, the resolution may be compromised.

Column Calibration

Following the steps outlined under column operation, the Bio-Rad Size Exclusion Standards, catalog number 151-1901, may be used to evaluate your columns performance. One vial contains 18 mg of a lyophilized mixture of thyroglobulin (M_r 670,000), bovine gamma-globulin (M_r 158,000), chicken ovalbumin (M_r 44,000), equine myoglobin (M_r 17,000), vitamin B-12 (M_r 1,350). Kd data obtained with these standards can be correlated to the kd data found in Figures 1 and 3. If the results you obtain are within 20% of those given, then the column is well packed.

Note: Do not use Blue Dextran as a void volume marker. Use calf thymus DNA instead.

Maintenance of the Column

Sample composition will determine how often the column must be cleaned. Samples containing large amounts of lipids or insoluble material may clog the top of the column, requiring a small portion of the fouled bed to be replaced with fresh gel.

A typical cleaning procedure recommended for use after every 25 cycles is given below.

1. Wash the column with 5 column volumes of 1.0 M NaCl at a standard operational flow rate.
2. Follow this with 1 to 5 column volumes of 0.5 M NaOH.
3. Remove the 0.5 M NaOH by running 5 to 10 column volumes of 1.0 M NaCl over the column. Check the column effluent pH to ensure all the NaOH has been removed.
4. Store in 20% ethanol or re-equilibrate for continued use.

4. If the column will be going directly back into operation, re-equilibrate the column with appropriate equilibration buffer. If the column is going to be stored pump storage buffer over the column.

Storage

To prevent bacterial growth correct storage is critical.

1. Short term storage (12–24 hr). Use fresh buffer and store at 4 °C.
2. Long term storage (24+ hr). Wash the column with distilled water followed by a storage solution of ~22% ethanol or 0.02% sodium azide. Cap the column inlet and outlet securely.

   Never allow the column or gel to freeze.

Sanitization

Do not autoclave your packed column. If sterilization is needed, it must be performed chemically.

1. Wash the column with 0.5 M NaOH following the instructions under Maintenance. or
2. Wash the column with 70% ethanol. **Note:** Ethanol will increase the pressure drop over the column.

Product Specifications

Particle size	32–60 µm, d50 ~40 µm
Fractionation range	
Macro-Prep SE 100/40	5,000–100,000 daltons
Macro-Prep SE 1000/40	10,000–1,000,000 daltons
Exclusion limit	
Macro-Prep SE 100/40	~200,000 daltons
Macro-Prep SE 1000/40	~1,500,000 daltons
pH range	1–14
Operating temperature	4 °C to 40 °C
Stability	> 250 cycles when tested by running a sample of plasma and washing with 1.0 M sodium hydroxide (one column volume) between each cycle.
Chemical stability	Methanol, ethanol, 8 M urea, 6 M guanidinium hydrochloride, 30% acetonitrile, 70% formic acid, 1.0 M sodium hydroxide, 5% sodium dodecyl sulphate, 5% 2-mercaptoethanol, 30% acetic acid. Use of strong oxidizing agents should be avoided.

Product Information

Catalog Number	Product Description
160-0001	**Macro-Prep SE 100/40 Gel**, 50 ml
160-0002	**Macro-Prep SE 100/40 Gel**, 300 ml
160-0003	**Macro-Prep SE 100/40 Gel**, 1 liter
160-0010	**Macro-Prep SE 1000/40 Gel**, 50 ml
160-0011	**Macro-Prep SE 1000/40 Gel**, 300 ml
160-0012	**Macro-Prep SE 1000/40 Gel**, 1 liter