Macro-Prep® 25 Ion Exchange Supports

Instruction Manual

BIO-RAD

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Section 1
Introduction

Macro-Prep 25 S strong cation exchange support and Macro-Prep 25 Q strong anion exchange support are designed to provide high resolution and high capacity for both analytical and preparative separations at high flow rates. Both supports possess the same rigid, macroporous structure of 50 µm Macro-Prep High Q and High S supports, but in a 25 µm bead that offers higher resolution. They are easy to use, allow quick and reliable packing, and can be used for multiple cycles.

Bio-Rad's chromatography supports are manufactured in an ISO 9001 registered manufacturing facility. Manufacturing processes are audited and registered by National Quality Assurance Limited under the provisions of ANSI/ISO/ASQ 9001:2000. Bio-Rad Laboratories guarantees quality of product in unopened containers for 1 year from date of shipment.

Section 2
Intended Use

Macro-Prep 25 ion exchange supports are appropriate for capture, intermediate, and polishing purification steps that remove contaminants or impurities from partially purified targets. Due to their rigidity and unique surface chemistry, these supports are particularly suited for flow-through operations requiring high throughput and high recovery of target molecules. The slightly hydrophobic base bead also provides selectivity for removal of trace contaminants such as residual host cell proteins, endotoxins, and viruses.

All Macro-Prep ion-exchange supports contain carboxyl groups. Depending on the support, pH conditions, and samples, the supports may act in mixed mode. With proper planning, this property can be exploited to give unique selectivity, which may increase yield and purity.

Section 3
Technical Specifications and Characteristics

Macro-Prep 25 ion exchange supports are rigid methacrylate supports, which exhibit little shrinkage and swelling, making them suitable for medium pressure applications with a peristaltic or higher pressure pump (see Figure 1). The Macro-Prep 25 ion exchange supports are not suitable for gravity flow. The macroporous nature of the support allows both small and large molecules to access the exchange sites located throughout the support. This results in high resolution separations with analytical or preparative sample loads.

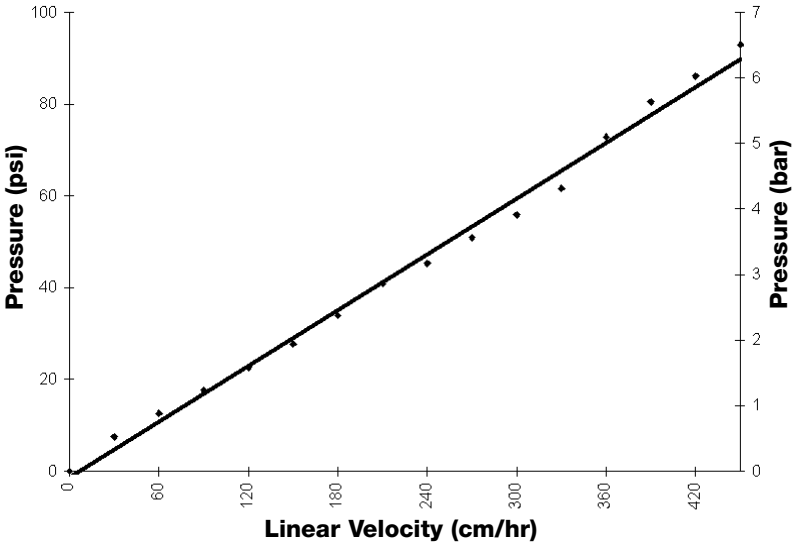

Fig. 1. Linear flow rate vs. operating pressure. The Macro-Prep 25 S support was evaluated for linear flow rate vs. operating pressure by measuring the pressure at a series of flow rates using 1 M NaCl on a 1.6 cm (ID) x 12.5 cm (H) column. The pressure plotted was determined by subtracting the pressure generated by the column when empty from the pressure generated from the column when packed.

The Macro-Prep 25 ion exchange supports are available as the Macro-Prep 25 S strong cation exchanger and the Macro-Prep 25 Q strong anion exchanger. Table 1 lists the properties of these supports.

Table 1. Properties of Macro-Prep Ion Exchange Supports.

	Macro-Prep 25 Q Support	Macro-Prep 25 S Support
Type of ion exchanger	Strong anion	Strong cation
Functional group	$-N^+(CH_3)_3$	$-SO_3^-$
Ionic capacity	220 ± 40 µeq/m	110 ± 30 µeq/ml
Dynamic binding capacity	>30 mg/ml BSA	>40 mg/ml BSA
Shipping counterion	Cl^-	Na^+
Nominal particle size	25 µm	25 µm
Nominal pore size	725 Å	725 Å
Recommended linear flow rate range	50–300 cm/hr	50–300 cm/hr
Maximum linear flow rate	3,000 cm/hr	3,000 cm/hr
Volume changes pH 4–10 0.1–1.0 M NaCl	 <1% <5%	 <1% <5%
Autoclavable (121°C, 30 min)	Yes*	Yes
pH stability	1–10	1–12
Storage condition	20% ethanol	20% ethanol

*Do not autoclave in the OH^- form

Chemical Stability

Macro-Prep 25 ion exchange resins are stable in most aqueous buffer solutions commonly used in purification of biomolecules. These ion exchangers also withstand treatment in solutions of acid, chaotropic agents, detergents, and pH <10, while retaining full functional performance. We do not recommend routine cleaning or operation of Macro-Prep 25 Q above pH 10. Macro-Prep 25 ion exchange supports should not be sanitized or stored in NaOH.

Thermal Stability

The Macro-Prep 25 supports can be autoclaved at 121°C for up to 30 min in deionized water as a slurry or a moist cake. Do not autoclave Macro-Prep 25 Q in OH^- form.

Section 4
Preparation for Use

Macro-Prep 25 supports are supplied fully hydrated in 20% (v/v) ethanol. Wash the ethanol storage solution from the support with deionized water before packing the column. Small volumes of the Macro-Prep 25 supports are easily washed in a Büchner funnel with 4–5 volumes of deionized water. For large volumes (for example, >1 liter), it is more convenient to pour the desired amount of support into a suitable container, allow the support to settle, and decant the ethanol solution. Add one volume of deionized water, resuspend the support, allow it to settle, and decant the supernatant. Repeat this procedure with deionized water (approximately 4–5 times, or until the ethanol is removed) and then pack the column (see Section 5 Column Packing). The support may be prepared by decanting the ethanol solution and resuspending it in the application buffer prior to column packing.

Section 5
Column Packing

The Macro-Prep 25 S and Macro-Prep 25 Q ion exchange supports have a nominal particle size of 25 µm, thus affording high resolution separations at medium pressures. In order to obtain the most resolution from these supports, a well packed column is essential. Empty columns designed for use at medium pressures (up to 100 PSI/7 bar) such as the Bio-Scale™ MT columns (see Section 9 Ordering Information) from Bio-Rad Laboratories are recommended. These columns must be used with bed supports with 10 µm or smaller pore sizes to prevent high backpressure due to bed support plugging.

Note: NaCl solution or buffers containing 0.5–1.0 M NaCl is recommended for optimum column packing.

1. Slurry, approximately 1:1 (v/v), the prepared matrix in 0.5–1.0 M NaCl and degas.
2. Close the outlet of column. Fill 10% of the column with degassed 0.5–1.0 M NaCl. Remove any air bubbles trapped in the bed support or the column end piece.
3. Add an appropriate amount of the support in a homogeneous slurry to the column.
4. Fill the remainder of the column with 0.5–1.0 M NaCl.
5. Connect the flow adapter to the pump, fill it with 0.5–1.0 M NaCl, and make sure it is free from air bubbles. Attach the flow adapter to the column. Inserting it at a slight angle makes it easier to avoid trapping air bubbles.
6. Open the column outlet and pump 4–5 column volumes (CV) of 0.5–1.0 M NaCl through the column at the highest flow rate your pump/column system permits. A dense, well-packed bed gives better chromatography.

7. Switch off the pump and close the column outlet. Disconnect the flow adapter tubing from the pump and position the flow adapter in contact with the support. During this step, the packing solution will backflow through the adapter.

8. Reconnect the flow adapter tubing to the pump, open the column outlet, and run 4–5 more CV of 0.5–1.0 M NaCl at the highest flow rate your pump/column system will permit, then repeat steps 7 and 8 for a final adjustment of the flow adaptor.

9. After equilibration in the start buffer, the column is ready for sample application.

Section 6
Operation and Maintenance

Macro-Prep 25 ion exchange columns may be operated in two different modes. In bind-elute mode, the protein of interest (target molecule) binds to the support during loading. It is subsequently eluted by a change of conductivity and/or pH. This allows removal of contaminants that do not bind to the support and contaminants that may bind more weakly or more strongly than the protein of interest. In flow-through mode, the protein of interest does not bind during loading, while contaminants that may bind to the support are removed. This approach can simplify chromatography conditions, conserve buffer, and increase recovery of the target molecule.

For flow-through mode, a linear flow rate of 300 cm/hr in a 20 cm bed is practical. If operating in a bind-elute mode, flow rates of 100–200 cm/hr are recommended. Do not exceed 70–75% of the maximum pressure attained during the initial column packing. If you do not have a pressure indicator, do not exceed flow rates in excess of 70–75% of the maximum flow rate used during packing.

All buffers used for anion or cation exchange chromatography can be used with the appropriate ion exchange supports (see Table 2). It is best to use buffering ions that have the same charge as the functional group on the ion exchanger, for example, phosphate (–) with the Macro-Prep 25 S cation exchanger and Tris (+) with the Macro-Prep 25 Q anion exchanger. Buffers should be chosen so operating pH is within 0.5 pH unit from pK_a of buffer substance.

The purification may be optimized by changing the pH, ionic strength of elution buffer, gradient profile, or buffer. Typical chromatographic conditions are use of buffer concentrations in the range of 20–50 mM, at least one pH unit below the pI of the protein for cation exchange, and one pH unit above the pI of the protein for anion exchange. Gradients are then run from 0 to 1 M NaCl over 10–20 CV.

Proper adjustment of the sample pH and ionic strength is critical for consistent and reproducible chromatography. For best results, the sample should be exchanged into the start buffer or diluted to the concentration of the start buffer.

Buffer exchange can be accomplished using Bio-Spin® 6 or Bio-Spin 30 columns, Econo-Pac® 10 DG desalting columns, Bio-Gel® P-6 DG size exclusion gel, or the Econo-Pac P6 cartridge. The choice of product depends on the sample volume.

Table 2. Common Buffers for Ion Exchange Chromatography.[1, 2, 3]

Type of Ion Exchanger	Buffer	Buffering Range
Macro-Prep 25 S	Acetic acid	4.8–5.2
	Citric acid	4.2–5.2
	HEPES	7.6–8.2
	Lactic acid	3.6–4.3
	MES	5.5–6.7
	MOPS	6.5–7.9
	Phosphate	6.7–7.6
	PIPES	6.1–7.5
	Pivalic acid	4.7–5.4
	TES	7.2–7.8
	Tricine	7.8–8.9
Macro-Prep 25 Q	Bicine	7.6–9.0
	Bis-Tris	5.8–7.2
	Diethanolamine	8.4–8.8
	Diethylamine	9.5–11.5
	L-histidine	5.5–6.0
	Imidazole	6.6–7.1
	Pyridine	4.9–5.6
	Tricine	7.4–8.8
	Triethanolamine	7.3–8.3
	Tris	7.5–8.0

Buffer Preparation

When preparing buffers for ion exchange chromatography, it is important that excess conductivity is not produced during buffer pH adjustment. For example, back-titration of Tris-HCl with NaOH elevates conductivity, which will lower binding capacity. To prepare Tris buffer, begin with Tris base and titrate with Tris-HCl to the target pH. Apply the same principle with other buffers.

Column Equilibration

To equilibrate the column, wash with 1–2 CV of buffer containing 0.5–1.0 M of the buffer salt used in the start buffer, then follow with 3 CV of the start buffer or until pH and conductivity are stable.

Sample Preparation

Adjust salt concentration and pH as necessary to achieve desired selectivity for binding of target or contaminants. If sample conductivity exceeds conductivity of the column equilibration buffer, sample capacity may be reduced or the target molecule may not bind. Adjustment of pH and conductivity of sample can be done by dilution or buffer exchange.

Sample Load and Adsorption

The sample load is determined empirically by loading and evaluating breakthrough of the molecule of interest. Sample volume is not a critical factor. Large volumes of dilute feed such as cell culture supernatant and clarified lysates may be loaded onto the supports without prior concentration.

Wash-Through

When operating in a bind-elute mode, after loading of the sample onto the column, follow with 2–5 CV of the equilibration buffer. This will wash out unbound contaminants. In flow-through mode, after sample is completely through the column, regenerate the resin as described under “Regeneration” below.

Elution

Elute target molecules with a step or a linear gradient. The salt concentration at which the desired product elutes is predetermined at small scale. With this knowledge, the pH and salt concentration used in wash-through are adjusted to eliminate the maximum amount of contamination before starting elution of the target.

Regeneration

After each run, the packed bed should be washed with 2–4 CV of a high salt buffer (0.5–2.0 M) to remove remaining bound material. Samples may then be loaded onto the column after reequilibration in starting buffer. If column no longer yields reproducible results, the supports may require more thorough cleaning and sanitization.

Cleaning-in-Place (CIP)

Acceptable CIP agents include 0.4 M NaCl in 1% acetic acid/1% phosphoric acid, up to 30% acetic acid, 1% Triton X-100, up to 70% ethanol or up to 30% isopropyl alcohol, 8 M urea, and 6 M guanidine-HCl. Any of these agents can be combined in an appropriate cleaning protocol, which is often developed empirically. As a general guide, we recommend the following:

1. Use high salt buffer for regeneration, as above.
2. Remove additional contaminants with 0.4 M NaCl in 1% acetic acid/1% phosphoric acid (3–5 CV) at 100 cm/hr.
3. For aggregated or precipitated proteins, or when dirty feedstock (for example, crude lysate) has been used, wash with 3–5 CV of 6 M guanidine-HCl or 8 M urea at 100 cm/hr.
4. For lipids or hydrophobically bound contaminants, wash with 0.1% Triton X-100, or 20–70% ethanol or 20–30% isopropyl alcohol, or 10–30% acetic acid. Use 3–5 CV at 100 cm/hr.
5. Finally, wash with 2 column volumes of deionized water and 4–5 CV of starting buffer. Check the conductivity and pH of the effluent to verify that the column is equilibrated in the starting buffer before loading the sample.

Sanitization and Storage

To sanitize and store between campaigns, wash the column with 3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5. Store the column at 4–40°C. When not in use, store the Macro-Prep 25 ion exchange supports in either 1% acetic acid/1% phosphoric acid (pH 1.5) or in 20% (v/v) ethanol solution or in 2% benzyl alcohol. The Macro-Prep 25 ion exchange supports may also be autoclaved at 121°C, 2 bar, in a neutral pH slurry, for up to 30 min and stored in one of the above solutions.

Shelf Life

The Macro-Prep ion exchange supports are stable for at least one year when stored sealed in the original container at room temperature.

Section 7
Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative. In the USA, call 1-800-4BIORAD. For online product information, visit our chromatography web site at **www.bio-rad.com**

Section 8
References

1. Harris ELV and Angal S (eds), Protein Purification Methods: A Practical Approach, IRL Press, Oxford (1989)
2. Scopes RK, Protein Purification: Principles and Practice, 2nd edn, Springer-Verlag, New York (1987)
3. Snyder LR and Kirkland JJ, Introduction to Modern Liquid Chromatography, 2nd edn, Wiley, New York (1979)

Section 9
Ordering Information

Catalog #	Description
Macro-Prep 25 Q Support	
153-0020	Macro-Prep 25 Q Support, 10 ml
153-0021	Macro-Prep 25 Q Support, 50 ml
153-0022	Macro-Prep 25 Q Support, 200 ml
153-0023	Macro-Prep 25 Q Support, 1 L
153-0024	Macro-Prep 25 Q Support, 5 L
Macro-Prep 25 S Support	
153-0030	Macro-Prep 25 S Support, 10 ml
153-0031	Macro-Prep 25 S Support, 50 ml
153-0032	Macro-Prep 25 S Support, 200 ml
153-0033	Macro-Prep 25 S Support, 1 L
153-0034	Macro-Prep 25 S Support, 5 L
Bio-Scale MT Columns	
751-0081	Bio-Scale MT2 Column, 7 x 52 mm, 1.9–2.3 ml
751-0083	Bio-Scale MT5 Column, 10 x 64 mm, 4.6–5.7 ml
751-0085	Bio-Scale MT10 Column, 12 x 88 mm, 9.5–11.3 ml
751-0087	Bio-Scale MT20 Column, 15 x 113 mm, 19.4–21.9 ml

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