Macro-Prep®
Ion Exchange Supports

Instruction Manual

BIO-RAD

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Section 1
Introduction

The high quality Macro-Prep ion exchange supports are tailored to meet the demands of modern protein chromatography. These rigid, macroporous, hydrophilic supports provide high resolution and high protein capacity, and the physical structure permits high linear flow rates in most column configurations. The new Macro-Prep supports offer many advantages in both laboratory and process scale applications; they are easy to use, they pack well, fast and consistently, and can be used a long time.

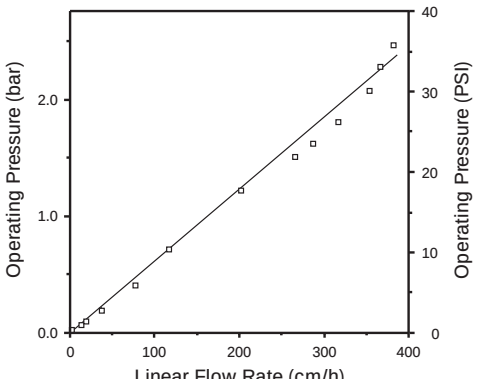
The Macro-Prep ion exchange supports allow efficient and reproducible chromatography. Their high capacity and excellent flow properties create dynamic solutions to common separation problems.

Section 2
Technical Description

The Macro-Prep ion exchange supports are rigid methacrylate supports with little shrinkage and swelling,

thus making them suitable for both low and medium pressure chromatography (see Figure 1). The macroporous nature of the matrix allows both small and large molecules to access the exchange sites located throughout the matrix. This results in high resolution separations with analytical or preparative sample loads.

For maximum flexibility we provide the Macro-Prep high Q strong anion exchange support with high binding capacity, and the Macro-Prep Q support, with lower binding capacity, permitting binding and elution under very mild conditions, and the Macro-Prep DEAE weak anion exchange support. Also available are the Macro-Prep high S strong cation exchange support, the lower capacity Macro-Prep S support, and the Macro-Prep CM weak cation exchange support.



Support:	Macro-Prep high Q support
Column:	Amicon Moduline® glass column, 14 cm inner diameter, with 1/4" plumbing.
Bed height:	17.4 cm
Pump:	Cole-Parmer MasterFlex® pump, size 18 Norprene tubing, Easy-Load™ pump head.

Fig. 1. Linear flow rate vs. operating pressure. The 50 µm Macro-Prep bead allows high flow rates at low to moderate operating pressures.

Table 1. Properties of Macro-Prep Ion Exchange Supports

	Macro-Prep DEAE Support	Macro-Prep High Q Support	Macro-Prep Q Support	Macro-Prep High S Support	Macro-Prep S Support	Macro-Prep CM Support
Type of support	Weak anion	Strong anion	Strong anion	Strong cation	Strong cation	Weak cation
Functional ligand	$-N^+(C_2H_5)_2$	$-N^+(CH_3)_3$	$-N^+(CH_3)_3$	$-SO_3^-$	$-SO_3^-$	$-COO^-$
Ionic capacity	175±75 µeq/ml	400±75 µeq/ml	190±40 µeq/ml	160±40 µeq/ml	160±40 µeq/ml	210±40 µeq/ml
Dynamic binding capacity	>30 mg BSA/ml	>25 mg BSA/ml	>15 mg BSA/ml	>55 mg IgG*/ml	>35 mg IgG*/ml	>20 mg BSA/ml
Counterion	Cl^-	Cl^-	Cl^-	Na^+	Na^+	Na^+
Nominal particle size	50 µm	50 µm	50 µm	50 µm	50 µm	50 µm
Nominal pore size	1,000 Å	1,000 Å	1,000 Å	1,000 Å	1,000 Å	1,000 Å
Recommended maximum linear flow rate	3,000 cm/hour	3,000 cm/hour	3,000 cm/hour	3,000 cm/hour	3,000 cm/hour	3,000 cm/hour
Chemical stability						
1.0 M HCl	>72 h	>48 h	>48 h	>72 h	>72 h	>72 h
1.0 M NaOH (20 °C)	excellent	<24 h	<24 h	excellent	excellent	excellent
Volume changes, pH 4–10	<1%	<1%	<1%	<3%	<3%	<1%
0.1–1.0 M NaCl	<5%	<5%	<5%	<9%	<9%	<4%
Autoclavable (121 °C, 30 min)	Yes	Yes	Yes	Yes	Yes	Yes
pH stability	1–14	1–14	1–14	1–14	1–14	1–14
Antimicrobial agent	20% ethanol	20% ethanol	20% ethanol	20% ethanol	20% ethanol	20% ethanol
Regeneration	70% ethanol	70% ethanol	70% ethanol	70% ethanol	70% ethanol	70% ethanol
Sanitization	1 M NaOH	1 M NaOH	1 M NaOH	1 M NaOH	1 M NaOH	1 M NaOH
Data sheet	A-400	A-100	N/A	A-200	N/A	A-300

* Human IgG

Section 3
Instructions For Use

3.1 Preparation

Macro-Prep supports are supplied fully hydrated in 20% (v/v) ethanol. If buffer or sample precipitation occurs in the presence of the 20% ethanol shipping buffer, wash the ethanol solution from the matrix with deionized water before the column is packed.

Small volumes of the Macro-Prep supports are easily washed in a Buchner funnel with 4-5 volumes of starting buffer. For large volumes, it is more convenient to pour the desired amount of matrix into a suitable container, allow the matrix to settle, and decant the ethanol solution. Add one volume of deionized water, resuspend the matrix, allow it to settle, and decant the supernatant. Repeat this procedure with buffer 4-5 times and then pack the column (see Column Packing).

If precipitation is not an issue, washing the 20% ethanol from the matrix before packing the column is not necessary. The support may be prepared by decanting the excess ethanol solution and resuspending the matrix in the application buffer prior to column packing (Column Packing, Step 1).

Due to the high buffering capacity of its functional groups, the Macro-Prep CM support may be slow to equilibrate. The following procedure for the CM supports is suggested before column packing:

1. Pour an appropriate amount of support in a suitable container, allow the matrix to settle, and decant the ethanol solution. Slurry with a volume of application buffer that is 4-5 times the volume of the matrix.
2. Allow the matrix to equilibrate for at least 30 minutes. Adjust to the operating pH with acid or base and re-equilibrate. Repeat pH adjustment until the pH is stable.
3. When the pH is stable, decant the buffer and repeat steps 1 and 2 using fresh buffer. Repeat until no pH change is noted after the addition of fresh buffer. This may take several buffer changes.
4. The CM weak cation support is now ready for column packing.

3.2 Column Packing

1. Slurry, approximately 1:1 (v/v), the prepared matrix in the application buffer and degas.
2. Close the outlet of the column. Fill 10% of the column with degassed starting buffer. Remove any air bubbles that might be trapped in the bed support or the column end piece.
3. Add an appropriate amount of the matrix in an even slurry to the column.
4. Fill the remainder of the column with buffer.
5. Connect the flow adaptor to the pump, fill it with buffer, and make sure it is free from air bubbles. Attach the flow adaptor 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 bed volumes through the column. Always pack the column at the highest flow rate your chromatography system permits. A dense, well-packed bed gives better chromatography.
7. Switch off the pump and close the column outlet. Remove the inlet tubing from the buffer reservoir, release the pump pressure plate, and adjust the flow adaptor until it is in contact with the matrix surface. During this step, buffer will back-flow through the flow adaptor.
8. Re-insert the inlet tubing in the buffer reservoir (remove any air trapped in the tubing, if necessary), tighten the pump pressure plate, open the column outlet, and run 4-5 more column volumes of starting buffer, then repeat steps 7 and 8 for a final adjustment of the flow adaptor.
9. After equilibration in the starting buffer, the column is ready for sample application.

Section 4
Operating Conditions

All buffers commonly used for anion or cation exchange chromatography can be used with the appropriate ion exchange supports (see Table 3). The chemical stability and broad operating pH range of these ion exchangers allow the use of a variety of buffers. It is best to use buffering ions which have the same charge as the functional group on the ion exchanger, e.g. phosphate (-) with a cation

exchanger, and Tris (+) with an anion exchanger. The purification may be optimized by changing the pH, changing the ionic strength of the elution buffer, modifying the gradient profile, or experimenting with different buffers.

Note: Due to rigidity of the Macro-Prep support it requires some special consideration for use. When using flow rates in excess of 200 cm/h the flow should be increased or decreased steps, i.e. from 200 to 100 cm/h before stopping. If the flow is abruptly turned on or off, the pressure pulse may cause a small flexing of the column. This flexing of the hardware may cause the support to separate from the walls or the end plates (depending on the column's weak spot), resulting in cracking or channelling of the packed resin bed.

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

Type of Ion Exchanger	Buffer	Buffering Range
Cation	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
Anion	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	7.3-8.0
	Tris	7.5-8.0

Section 5
Chemical Stability

The Macro-Prep ion exchangers can withstand treatment in acid, base, chaotropic agents, and detergents, while retaining high ionic and protein capacities. These conditions are commonly employed for cleaning, regeneration, or elution. Table 4 illustrates the different conditions tested and the corresponding minimal effects of exposure.

Table 4. Chemical Stability of Macro-Prep Ion Exchange Supports (percentage of original protein binding capacity)

	Macro-Prep		
Exposure	**Q[1]**	**S[2]**	**CM[3]**
1% SDS, 24 hours	97	104	99
8 M Guanidine-HCl, 24 hours	98	106	90
1 M HCl, > 48 hours	91	104	99
1 M NaOH, > 48 hours	97*	98	98

The proteins tested were 1. ferritin, 2. human IgG, and 3. human hemoglobin.

* Macro-Prep Q and high Q support shows a shift in elution pattern towards lower ionic strength after long term exposure to 1 M NaOH. We recommended routine cleaning with 0.1 M NaOH.

Section 6
Thermal Stability

The Macro-Prep supports can be autoclaved at 121 °C for up to 30 minutes in deionized water as a slurry or a moist cake. Table 5 demonstrates the retention of protein binding capacity after 30 minutes of autoclaving at 121 °C.

Table 5. Thermal Stability of Macro-Prep Ion Exchange Supports (percentage of original protein binding capacity)

	Q[1]	**S[2]**	**CM[3]**
Binding Capacity	97	102	94

The proteins tested were 1. ferritin, 2. human IgG, and 3. human hemoglobin.

Section 7
Regeneration

After each run the packed bed should be washed with 2-4 bed volumes of 1.0 M NaCl to remove reversibly bound material. Samples may then be loaded onto the column after re-equilibration in starting buffer. When a column no longer yields reproducible results, the matrix may require thorough cleaning and sanitation to remove strongly bound contaminants. The methacrylate ion exchange supports are most efficiently regenerated in a column with the recommended cleaning in place (CIP) procedures given below.

7.1 Cleaning in Place (CIP) for Macro-Prep Ion Exchange Supports

1. Wash the support in the column with 2-4 bed volumes of 1.0 M NaOH at the operating flow rate. Follow the NaOH wash of the anion exchange supports with 2 column volumes of 1 M NaCl, to replace the OH^- with Cl^-.
2. Follow with deionized H_2O to pH < 10 if precipitation of salts from your buffers may occur.

3. Equilibrate with at least 4-5 bed volumes of starting buffer.
4. Check the conductivity and pH of the effluent to verify that the column is equilibrated in the starting buffer before loading the sample.
5. If contaminants are tightly bound to the matrix, wash with a 20% ethanol solution and proceed to step 2.
6. The support may also be reegenerated with a mixture of 30% acetic acid: 10% isopropanol in water. The acid cleaning procedure should only be performed after the sodium hydroxide cleaning described in step 1 has been performed. It is essential for the integrity of the packed bed that the column is rinsed with 4 bed volumes of water between the sodium hydroxide and the acid step.

Section 8
Storage

When not in use, store the Macro-Prep ion exchange supports in 0.05% NaN_3 or in a 20% v/v ethanol solution as a bacteriostat. The ion exchange supports may also be

autoclaved at 121 °C, 2 bar, for up to 30 minutes and stored in a bacteriostatic solution.

Section 9
Shelf Life

The Macro-Prep ion exchange supports are stable for at least 3 years when stored sealed in the original container at room temperature.

Section 10
Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative, or in the USA, call 1-800-4BIORAD.

Section 11
References

1. Harris, E. L. V. and Angal, S., *Protein Purification Methods, a Practical Approach,* IRL Press, Oxford, 1989.
2. Scopes, R. K., *Protein Purification, Principles and Practice,* (Second Edition), Springer-Verlag, New York, 1987.
3. Snyder, L. R. and Kirkland, J. J., *Introduction to Modern Liquid Chromatography,* (Second Edition), John Wiley & Sons, Inc., New York, 1979.

Section 12
Ordering Information

Catalog Number	Product Description
Ion Exchange Chromatography	
156-0030	**Macro-Prep DEAE Support,** 100 ml
156-0031	**Macro-Prep DEAE Support,** 500 ml
156-0032	**Macro-Prep DEAE Support,** 5 liters
156-0033	**Macro-Prep DEAE Support,** 10 liters

Catalog Number	Product Description
156-0040	**Macro-Prep High Q Support,** 100 ml
156-0041	**Macro-Prep High Q Support,** 500 ml
156-0042	**Macro-Prep High Q Support,** 5 liters
156-0043	**Macro-Prep High Q Support,** 10 liters
732-0021	**Econo-Pac Q Cartridge,** 1
156-0050	**Macro-Prep Q Support,** 100 ml
156-0051	**Macro-Prep Q Support,** 500 ml
156-0052	**Macro-Prep Q Support,** 5 liters
156-0053	**Macro-Prep Q Support,** 10 liters
156-0030	**Macro-Prep High S Support,** 100 ml
156-0031	**Macro-Prep High S Support,** 500 ml
156-0032	**Macro-Prep High S Support,** 5 liters
156-0033	**Macro-Prep High S Support,** 10 liters
732-0061	**Econo-Pac S Cartridge,** 1
156-0060	**Macro-Prep S Support,** 100 ml
156-0061	**Macro-Prep S Support,** 500 ml
156-0062	**Macro-Prep S Support,** 5 liters
156-0063	**Macro-Prep S Support,** 10 liters

Catalog Number	Product Description
732-0001	**Econo-Pac CM Cartridge,** 1
156-0070	**Macro-Prep CM Support,** 100 ml
156-0071	**Macro-Prep CM Support,** 500 ml
156-0072	**Macro-Prep CM Support,** 5 liters
156-0073	**Macro-Prep CM Support,** 10 liters