

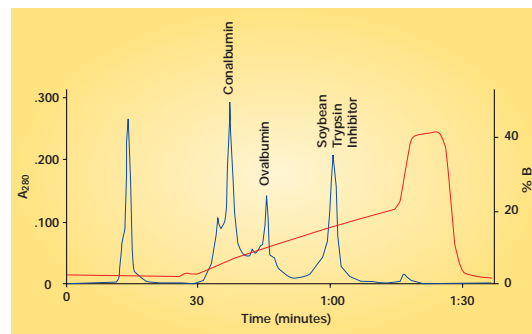
**THE HIGH RESOLUTION OF MACRO-PREP 25 RESULTS IN:**

- IMPROVED YIELD AND PURITY
- EASIER POOLING DECISIONS
- FEWER CHROMATOGRAPHIC STEPS

## New High Resolution Preparative Macro-Prep® Ion Exchange Supports

Macro-Prep 25 Q and 25 S supports are two new additions to the Macro-Prep family of preparative chromatography supports for the purification of biomolecules. These supports are 25  $\mu\text{m}$  versions of the well-established 10  $\mu\text{m}$  beads available in the pre-packed Bio-Scale S and Q columns and 50  $\mu\text{m}$  methacrylate polymer-based Macro-Prep high Q and high S bulk supports. The particle size of Macro-Prep 25 ion exchangers provides high resolution preparative separations in a cost-effective bulk format while maintaining the chemical and mechanical stability of Macro-Prep high Q and high S supports (Figure 1).

Macro-Prep 25 Q strong anion exchange supports and 25 S strong cation exchange supports offer improvements in yield and purity due to increased peak sharpness, easier pooling decisions, and fewer required chromatographic steps. An additional benefit for process scale applications is the potential for reduced validation costs due to the ability to separate a protein product from closely related variants of the protein. Figure 3 shows separation of a mixture of a Genentech® recombinant protein from a variant of the protein with oxidized surface methione residues generated by exposure to t-butyl hydroperoxide.<sup>1</sup>



**Fig. 1.** Separation of 250  $\mu\text{l}$  of a 9 mg/ml solution of conalbumin, ovalbumin, and soybean trypsin inhibitor on a 2 ml column packed with Macro-Prep 25 Q. Buffer A: 50 mM Tris, pH 8.3. Buffer B: 50 mM Tris, pH 8.3, 2 M NaCl; gradient: 0–20% B in 50 min (10 column volumes); flow rate: 0.4 ml/min.

## Properties

Macro-Prep 25 Q and 25 S supports can be regenerated and sanitized with 1 M NaOH. Changes in pH or ionic strength do not cause shrinking or swelling of the supports. The mechanical stability of the supports allows operation up to 1,000 psi. At a bed height of 12.5 cm, the narrow particle size distribution provides pressures below 100 psi (7 bar) at 500 cm/hr and below 45 psi (3 bar) at 270 cm/hr (Figure 2). The nominal 725 Å pores provide efficient transport of the biomolecule to binding sites on the support, which gives high binding capacities at elevated flow rates.

## Recommended Procedure

Macro-Prep 25 Q and 25 S supports are easy to use. To obtain the best resolution from these supports, a well-packed column is essential. Empty columns designed for use at medium pressure (up to 100 psi/7 bar) such as Bio-Scale MT columns (see ordering information) are recommended. Following column packing and equilibration, samples are loaded in low-salt buffer (e.g. 20–50 mM Tris for Macro-Prep 25 Q, or 20–50 mM phosphate for Macro-Prep 25 S) at a pH which is at least 1 pH unit above the pI of the protein in the case of Macro-Prep 25 Q, or 1 pH unit below the pI of the protein in the case of Macro-Prep 25 S. Proteins are then eluted with an increasing salt gradient (0–1 M NaCl). Elution is followed by stripping and regeneration steps of choice. For more detailed information, refer to the instruction manual.

## Macro-Prep Q and S Families

Macro-Prep Q and S ion exchange supports are also available in both 10 µm and 50 µm particle sizes. This provides unrivaled flexibility for scaling up purification protocols from lab scale to process scale. It is no longer necessary to do the development work on one type of high resolution analytical support only to be forced to transfer it to another type of preparative chromatographic support.

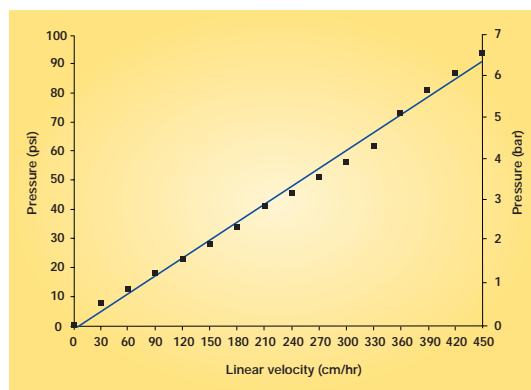


Fig. 2. Pressure vs. linear velocity for 1 M NaCl on Macro-Prep 25 S in a 1.6 cm (ID) x 12.5 cm (L) column.

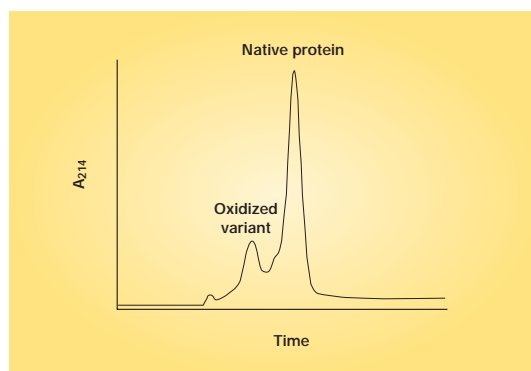


Fig. 3. Separation of a mixture of 1 mg of native protein from 0.23 mg of the protein with oxidized surface methionine residues from treatment with *t*-butyl hydroperoxide on a 1.6 cm (ID) x 12.5 cm (L) column packed with Macro-Prep 25 S. Buffer A: 50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 5.0. Buffer B: 50mM KH<sub>2</sub>PO<sub>4</sub>, 2 M KCl, pH 5.5; gradient: 50% B to 100% B in 20 min; flow rate: 2 ml/min.

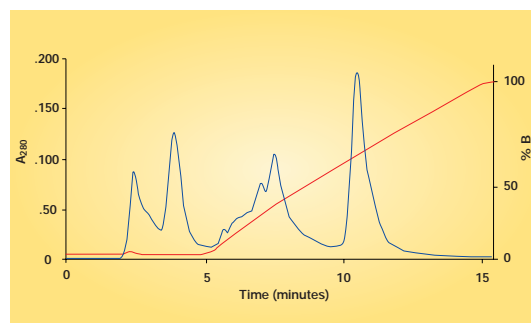


Fig. 4. Separation of 360 µl of 6xHis-tagged dihydrofolate reductase *E. coli* lysate on a 2 ml column packed with Macro-Prep 25 Q. Buffer A: 50 mM Tris, pH 8.3. Buffer B: 50 mM Tris, pH 8.3, 1 M NaCl; gradient: 0–100% B in 10 column volumes; flow rate: 2 ml/min.

### High Resolution Biomolecule Purification

Bio-Scale Q and S columns are packed with the 10  $\mu\text{m}$  Macro-Prep Q and S support. These columns can be used for rapid and reproducible high resolution separation of biomolecules with any medium or high pressure chromatography system. Four column sizes (2, 5, 10, and 20 ml) provide flexibility for economical and predictable scale-up of separation and purification protocols without sacrificing resolution due to overloading.

### Process Scale Chromatography

The 50  $\mu\text{m}$  Macro-Prep high Q and high S supports are also available in 5 and 10 liter packages for scale-up and process scale chromatography applications. The Macro-Prep supports' physical and chemical properties make them ideal for larger scale separations requiring optimal resolutions at elevated flow rates, as well as rapid equilibration between separations and fast column cleaning.

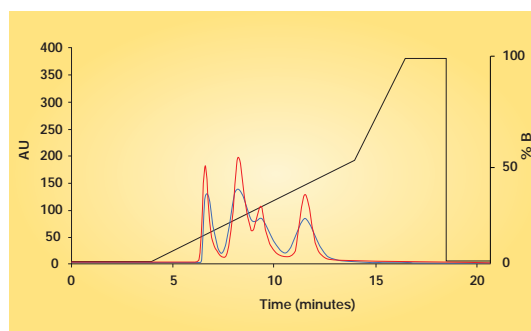
### Regulatory Support

All Macro-Prep ion exchange products have manufacturing processes registered with the United States Food and Drug Administration by submission of Type II Drug Master Files (DMF). Regulatory support files are available, on request, to companies entering into clinical trials. The Bio-Rad Life Sciences Group and its design, development, and manufacture of chemicals and analytical instruments, has been assessed and registered by National Quality Assurance Limited against the provisions of BS EN ISO:9001:1994. For more information or for technical assistance, contact your local Bio-Rad representative. In the US, call 1-800-4BIORAD (1-800-424-6723).

For more information on Bio-Rad's complete line of chromatography supports and other products for life science research and production, visit our web site at [www.discover.bio-rad.com](http://www.discover.bio-rad.com).

### Reference

1 Keck, R. G., *Anal. Biochem.*, **236**(1), 56-62 (1996).



*Fig. 5. Anion standard separation on A: Bio-Scale Q2; B: Macro-Prep 25 Q (1.0 x 2.5 cm column). Buffer A: 20 mM BTP, pH 9.5. Buffer B: A+ 1 M NaCl. Gradient volume: 20 ml; flow rate: 2 ml/min. Sample load: 2.25 mg.*



## Properties of Macro-Prep 25 Ion Exchange Supports

SUPPORT	MACRO-PREP 25 Q	MACRO-PREP 25 S
TYPE OF ION EXCHANGER	Strong anion	Strong cation
FUNCTIONAL GROUP	-N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>	-SO <sub>3</sub> <sup>-</sup>
IONIC CAPACITY (μEQ/ML)	220±40	110±30
DYNAMIC BINDING CAPACITY	>30 mg/ml BSA	>40 mg/ml IgG
SHIPPING COUNTERION	Cl <sup>-</sup>	Na <sup>+</sup>
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
MAX. LINEAR FLOW RATE	3,000 cm/hr	3,000 cm/hr
<b>CHEMICAL STABILITY</b>		
1.0 M HCl	>48 hr	>72 hr
1.0 M NaOH (20 °C)	<24 hr	Excellent
100% ETHANOL	Excellent	Excellent
CHAOTROPES (8 M UREA, 6 M GUANADINIUM HCl)	Excellent	Excellent
<b>VOLUME CHANGES</b>		
pH 4–10	<1%	<1%
0.1–1.0 M NaCl	<5%	<4%
<b>AUTOCLAVABLE</b>		
(121 °C, 30 MIN)	Yes (Do not autoclave in the OH <sup>-</sup> form)	Yes
PH STABILITY	1–14	1–14
STORAGE SOLUTION	20% Ethanol	20% Ethanol

### Ordering Information

Catalog #	Description
<b>MACRO-PREP 25 Q STRONG ANION EXCHANGE SUPPORT</b>	
153-0020	Macro-Prep 25 Q, 10 ml
153-0021	Macro-Prep 25 Q, 50 ml
153-0022	Macro-Prep 25 Q, 200 ml
153-0023	Macro-Prep 25 Q, 1
153-0024	Macro-Prep 25 Q, 5
<b>MACRO-PREP 25 S STRONG CATION EXCHANGE SUPPORT</b>	
153-0030	Macro-Prep 25 S, 10 ml
153-0031	Macro-Prep 25 S, 50 ml
153-0032	Macro-Prep 25 S, 200 ml
153-0033	Macro-Prep 25 S, 1
153-0034	Macro-Prep 25 S, 5
<b>BIO-SCALE EMPTY COLUMNS</b>	
751-0081	Bio-Scale MT2, 7 x 25 mm, 1.9–2.3 ml
751-0083	Bio-Scale MT5, 10 x 64 mm, 4.6–5.7 ml
751-0085	Bio-Scale MT10, 12 x 88 mm, 9.5–11.3 ml
751-0087	Bio-Scale MT20, 15 x 113 mm, 19.4–21.9 ml

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Hong Kong 852-2789-3300 India (91-11) 461-0103 Israel 03 951 4127 Italy 02-21609.1 Japan 03-5811-6270 Korea 82-2-3473-4460  
The Netherlands 31 318-540666 New Zealand 64-9-4152280 Singapore 65-2729877 Spain (91) 661 70 85 Sweden 46 (0)8 627 50 00  
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