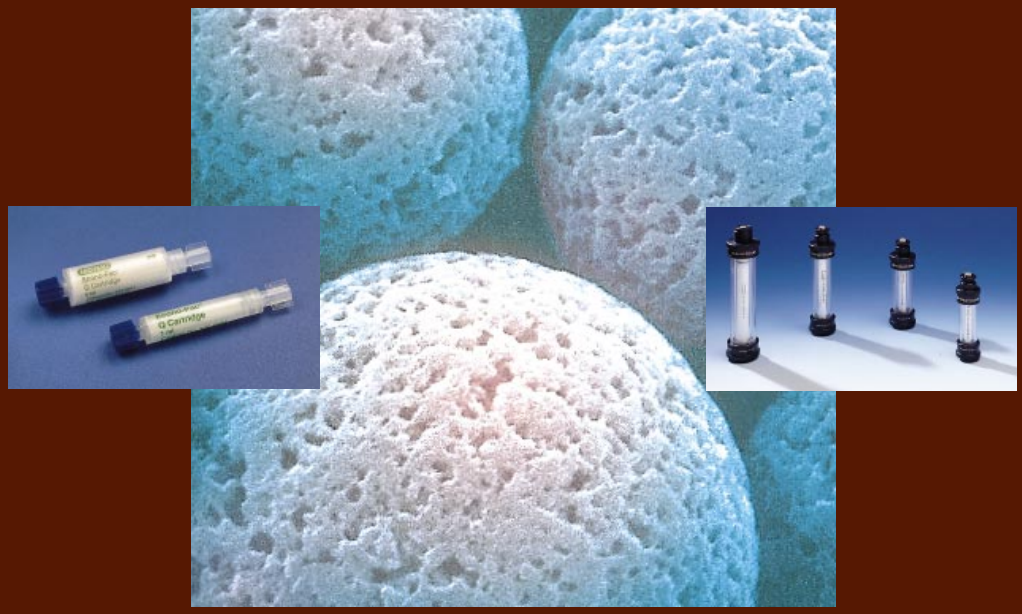
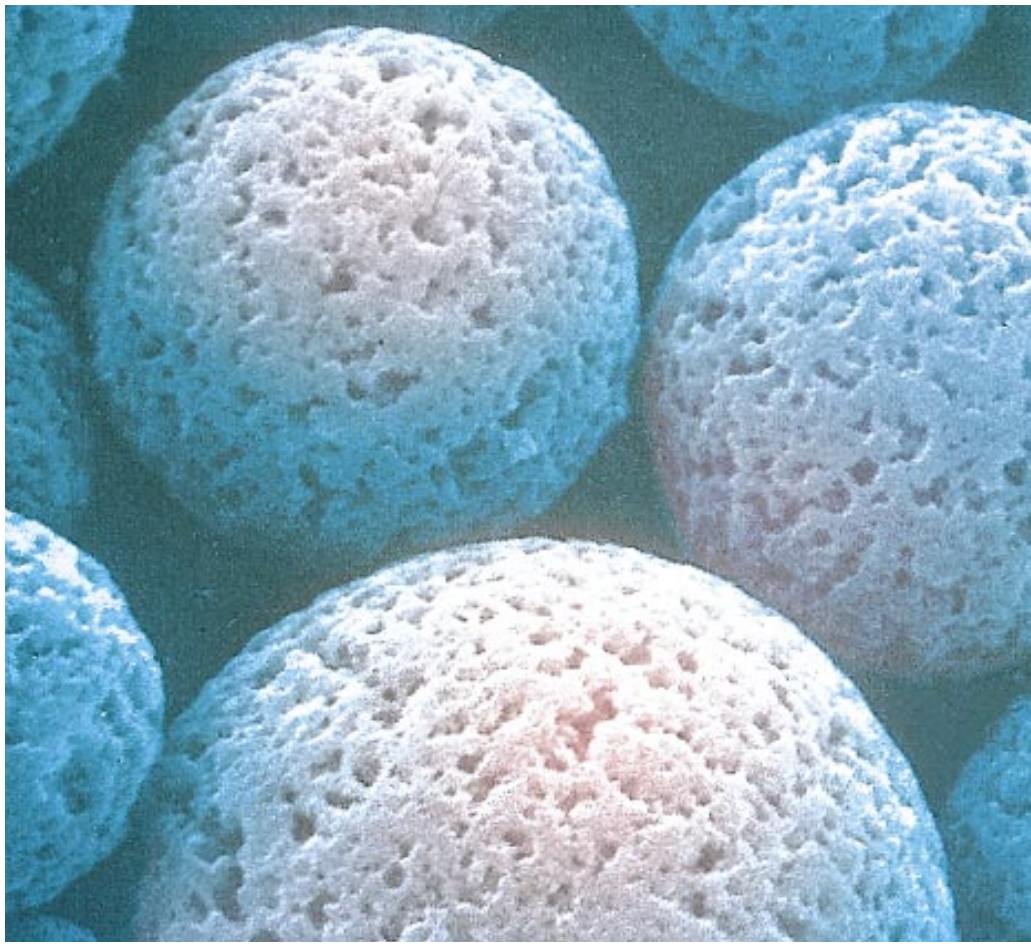
Macro-Prep®
Chromatography Supports



Strong Anion and Cation Exchange

BIO-RAD

The Macro-Prep Q and S Families



Ion exchange chromatography is one of the most widely used techniques for protein purification. Two of the most commonly used ion exchange supports are strong anion and cation exchangers. The Macro-Prep Q strong anion exchange support, with quaternary amine functional groups, is ideal for purifying acidic and neutral proteins and peptides. The Macro-Prep S strong cation exchanger, with sulfonate functional groups, is ideal for purifying basic and neutral proteins and peptides. The Macro-Prep Q and S supports are excellent choices for the wide range of separation and purification applications required in today's biotechnology industry.

High Resolution

The rigid nature and the narrow particle size distribution of the methacrylate based Macro-Prep bead provides excellent pressure/flow characteristics and insures optimal resolution across the range of particle sizes, from the 10 µm support in the Bio-Scale columns, to the 50 µm support available pre-packed in Econo-Pac® cartridges and in bulk volumes. For low pressure liquid chromatography applications on any scale, the 50 µm Macro-Prep high Q and high S supports can be used at high flow rates without loss of resolution.

High Recovery Of Proteins

The hydrophilic Macro-Prep supports demonstrate extremely low non-specific binding of biomolecules and typically give high recovery of biological activity.

High Dynamic Capacity

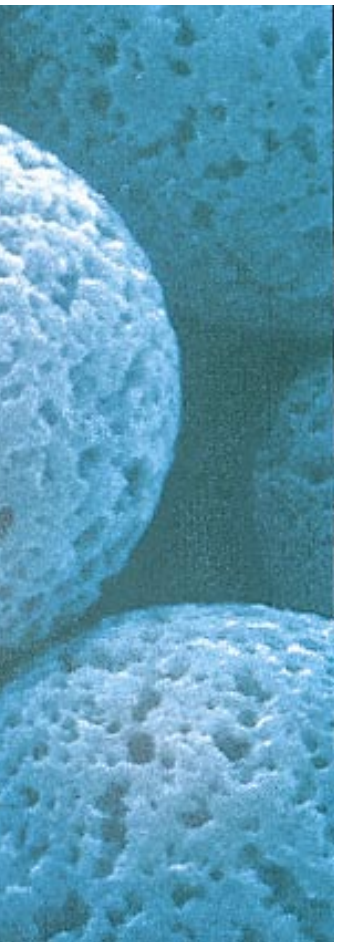
The Macro-Prep supports combine high resolution and high dynamic protein capacity over a very broad linear flow rate range.

High Chemical Stability

Macro-Prep supports are excellent choices for long term use requiring cleaning and sanitization. Stable from pH 1–13, Macro-Prep ion exchange supports can withstand repeated cleaning and sanitization with 1 M NaOH with negligible changes in performance. The supports are also compatible with high concentrations of acids, various organic solvents, and detergents. Macro-Prep supports can be sterilized by autoclaving.

Low Operating Pressure

The 50 µm Macro-Prep bead is small enough to give good resolution in low pressure applications, but is not so small that it necessitates special pressure stable equipment. Separations can be run at high flow rates with low operating pressures.



Available In Several Formats And Particle Sizes

The Macro-Prep Q and S supports are available in two particle sizes (10 and 50 µm) and in a variety of package sizes. This provides total flexibility for

- High resolution lab scale protein purification
- Rapid methods development, without changing over to different support materials
- Low pressure liquid chromatography
- Process scale chromatography and industrial production of biomolecules

High Resolution Protein Purification

The Bio-Scale Q and S columns are packed with the 10 µm Macro-Prep Q and S support. These columns can be used for rapid and reproducible high resolution separation of biomolecules with any HPLC or FPLC® system. Four different column sizes (2, 5, 10, and 20 ml) provide unrivaled flexibility for economical and predictable scale-up of separation and purification protocols without sacrificing resolution due to overloading. Other features of the Bio-Scale columns include

- Biocompatible materials to preserve protein integrity
- Fingertight fittings eliminate the need for tools
- Adjustable bed support to minimize column void volumes
- Top-off resin kit (optional) to extend column life

Low Pressure Liquid Chromatography

The 50 µm Macro-Prep supports are available in 100 and 500 ml bottles for packing low pressure columns of any size. The supports pack well and form stable columns which will provide long, trouble free use.

For maximum ease and convenience, the 50 µm Macro-Prep high Q and high S supports are available in convenient 1 ml and 5 ml pre-packed Econo-Pac cartridges. The Econo-Pac cartridges simplify chromatography because there is no gel to prepare or column to pack. The cartridges are designed to be used not only with Bio-Rad's Econo System and other low pressure LC systems and pumps, but are also easily adapted for use with FPLC and HPLC systems. Up to three Econo-Pac cartridges can be connected in series for instant scale-up with no loss of resolution. For simple step elution, the cartridges can be used with a syringe.

Process Scale Chromatography

The 50 µm 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 ideally suited for larger scale separations requiring optimal resolution at elevated flow rates, as well as rapid equilibration between separations and fast column cleaning. All Macro-Prep 50 µm supports are manufactured under cGMP and have Drug Master Files.

Scaling Up with the Macro-Prep Q Support

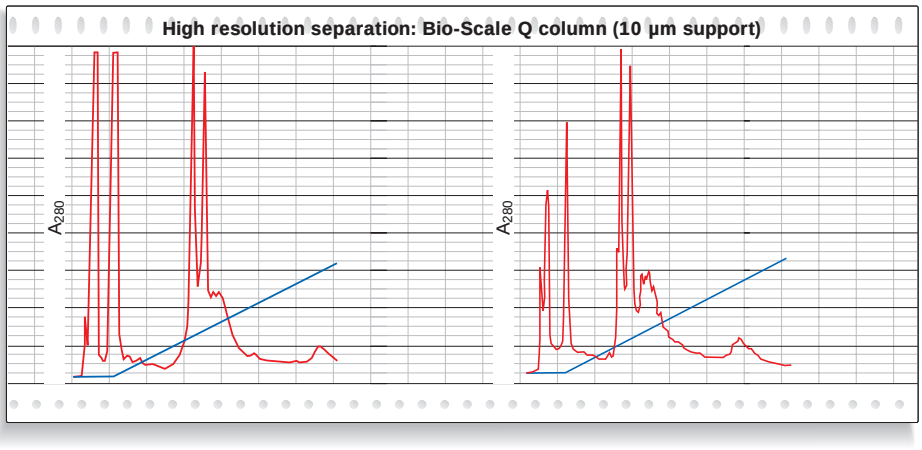


Fig. 1. Bio-Scale Q2 2 ml column. Sample :4 mg . Flow rate: 0.5 ml/min (80 cm/h).

Fig. 2. Bio-Scale Q5 5 ml column. Sample: 20 mg. Flow rate: 1.0 ml/min (80 cm/h).

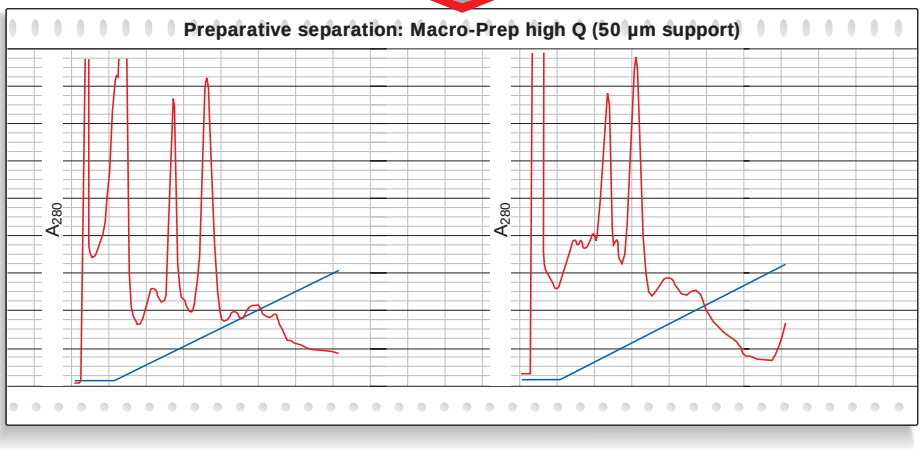


Fig. 3. Macro-Prep high Q 20 ml column. Sample: 105 mg. Flow rate: 2.3 ml/min (80 cm/h).

Fig. 4. Macro-Prep high Q 60 ml column. Sample: 1 gram. Flow rate: 6.4 ml/min (80 cm/h).

Scaling Up

The Macro-Prep supports are available in both 10 µm and 50 µm particles 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.

The scale-up applications show the excellent resolution obtained from crude protein extracts using different formats of the Macro-Prep Q and S supports with simple linear salt gradients.

Fractionation of Solubilized Proteins from Yeast

Genetically engineered varieties of yeast are commonly used as expression systems for recombinant proteins. The advantages of yeast over *E. coli* expression systems include the post-translational modifications of proteins.

These applications show how a separation is scaled up from 4 mg of yeast enzyme extract on a 2 ml Bio-Scale column, to 1 gram of sample on a 60 ml Macro-Prep column using a simple linear salt gradient.

Scaling Up with the Macro-Prep S Family

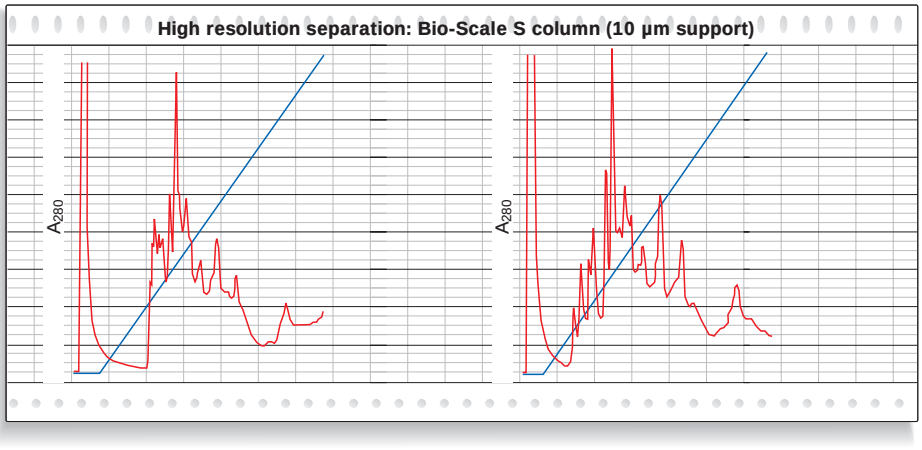


Fig. 5. Bio-Scale S2 2 ml column. Sample: 4 mg. Flow rate: 0.5 ml/min (80 cm/h).

Fig. 6. Bio-Scale S5 5 ml column. Sample: 20 mg. Flow rate: 1 ml/min (80 cm/h).

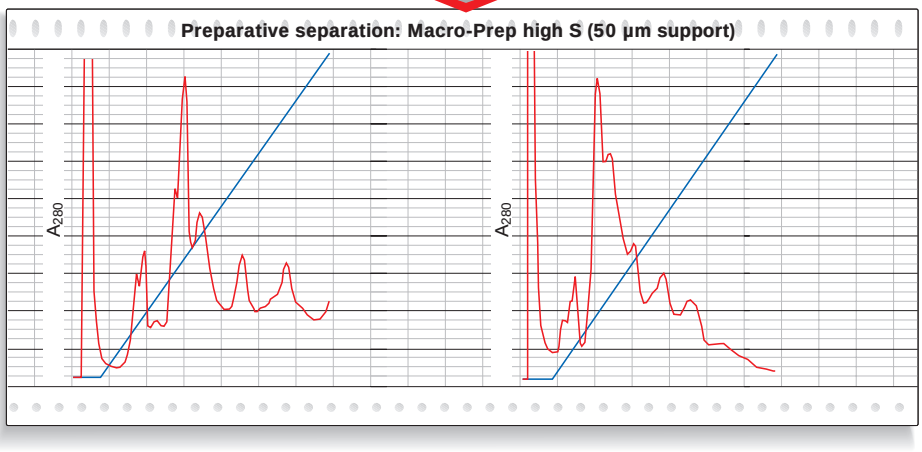


Fig. 7. Macro-Prep high S 20 ml column. Sample: 105 mg. Flow rate: 2.3 ml/min (80 cm/h).

Fig. 8. Macro-Prep high S 60 ml column. Sample: 1 gram. Flow rate: 25 ml/min (80 cm/h).

Fractionation of Snake Venom (Crotalus Atrox)

Snake venom is a rich source of pharmacologically-active substances, including proteins and peptides. Cation exchange chromatography is an excellent first step in a separation scheme due to its high capacity and resolution. These applications show the scale-up separations of venom from the Western Diamondback rattlesnake using different formats of the Macro-Prep S family.

Performance and Properties

Table 1. Bio-Scale Q Column

	Q2	Q5	Q10	Q20
Column volume (ml)	2	5	10	20
Recommended max. protein loading (mg)	20	50	100	200
Recommended flow-rates (ml/min)	0.5 to 3.0	0.5 to 5.0	0.5 to 7.0	0.5 to 10.0
Dynamic protein binding capacity (mg BSA)	40	100	200	400
Ionic capacity (µmol/ml)	115 ± 25	115 ± 25	115 ± 25	115 ± 25
Average particle size (µm)	10 ± 3	10 ± 3	10 ± 3	10 ± 3
Column dimensions (mm)	7 x 52	10 x 64	12 x 88	15 x 113
Maximum operating pressure (psi/bar)	1,000/70	750/50	600/40	500/34
Back-pressure at maximum recommended flow-rate (psi/bar)	360/24	320/21	290/19	320/21

Table 2. Bio-Scale S Column

	S2	S5	S10	S20
Column volume (ml)	2	5	10	20
Recommended max. protein loading (mg)	20	50	100	200
Recommended flow-rates (ml/min)	0.5 to 3.0	0.5 to 5.0	0.5 to 7.0	0.5 to 10.0
Dynamic protein binding capacity (mg HIgG)	100	250	500	1000
Ionic capacity (µmol/ml)	127 ± 25	127 ± 25	127 ± 25	127 ± 25
Average particle size (µm)	10 ± 3	10 ± 3	10 ± 3	10 ± 3
Column Dimensions (mm)	7 x 52	10 x 64	12 x 88	15 x 113
Maximum operating pressure (psi/bar)	1,000/70	750/50	600/40	500/34
Back-pressure at maximum recommended flow-rate (psi/bar)	360/24	320/21	290/19	320/21

Table 3. Econo-Pac Cartridge

	High Q Cartridge		High S Cartridge	
Cartridge volume (ml)	1	5	1	5
Recommended flow-rates (ml/min)	0.5 to 1.0	1.0 to 3.0	0.5 to 1.0	1.0 to 3.0
Dynamic protein binding capacity (mg BSA)	>40 BSA	>170 BSA	>55 hIgG	>230 hIgG
Average particle size (µm)	50 ± 10	50 ± 10	50 ± 10	50 ± 10
Maximum operating pressure (psi/bar)	50/3	50/3	50/3	50/3
Back-pressure at maximum recommended flow-rate	<10 psi	<10 psi	<10 psi	<10 psi

Table 4. Macro-Prep Support

	High Q	High S
Functional ligand	$-N^{+}(CH_3)_3$	$-SO_3^-$
Ionic capacity (µmol/ml)	400±75	160 ±40
Dynamic protein binding capacity (mg BSA)	>25 mg	>55 mg hIgG/ml
Nominal particle size	50 µm	50 µm
Nominal pore size	1000Å	1000Å
Recommended max. linear flow rate	3,000 cm/hour	3,000 cm/hour
Chemical stability 1.0 M HCl	>72 hours	>72 hours
1.0 M NaOH (20 °C)	excellent	excellent
pH stability	1-14	1-14
Antimicrobial agent	20% ethanol	20% ethanol
Regeneration	2M NaCl	2M NaCl
Sanitization	1 M NaOH	1 M NaOH

Note: Many chromatography support manufacturers choose to publish a static binding capacity value. However, expressing binding capacity in terms of dynamic loading capacity more realistically reflects practical loading limits to achieve maximal resolution under flow conditions.

Fig. 9 Bio-Scale Q Column Fig. 10

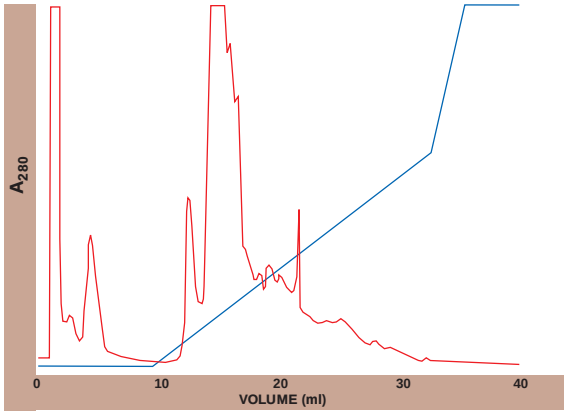


Fig. 9. Separation of proteins from human serum. Data courtesy of Dr. V. Tsang, Center for Disease Control, Atlanta, GA. Column: Bio-Scale Q2 column. Sample load: 1 ml normal serum, diluted 1:5 in buffer. Buffer A: 50 mM Tris-HCl, pH 8.0. Buffer B: A + 0.5 M NaCl. Gradient: 0-60 % B over 25 ml. Flow rate: 2 ml/min.

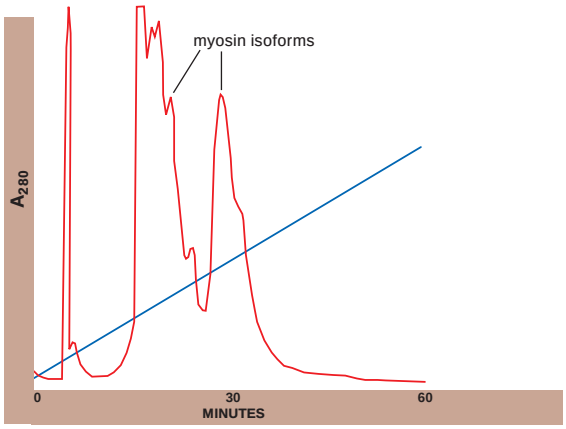


Fig.10. Purification of porcine ventricular myosin/S1. Data courtesy of Dr. H. White, Eastern Medical School, Dept. of Biochemistry, Norfolk, VA. Column: Bio-Scale Q20 column. Sample load: 60 mg. Buffer A: 50 mM Tris, 0.2 mM $MgCl_2$, 0.2 mM NaN_3, pH 7.5. Buffer B: A + 0.5 M NaCl. Gradient: 0-60 % B over 60 min. Flow rate: 4 ml/min.

Fig. 11 Bio-Scale S Column Fig. 12

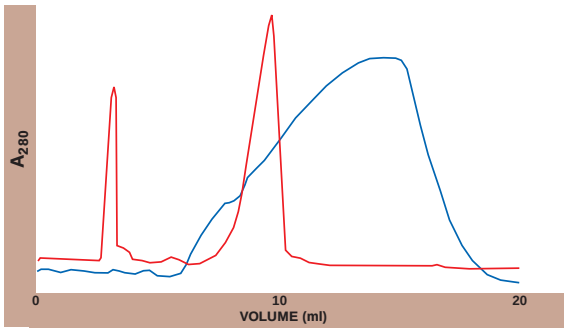


Fig. 11. Scale-up separation of lactate dehydrogenase (LDH). A: Column: Bio-Scale S2 column. Sample load: 0.25 ml, 2.3 mg of protein. Buffer A: 25 mM Tris-HCl, pH 8.1. Buffer B: A + 1.0 M NaCl. Gradient: 0-20 % over 10 ml. Flow rate: 1 ml/min.

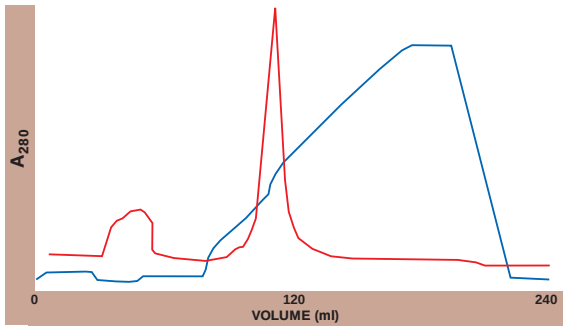


Fig. 12. Scale-up separation of lactate dehydrogenase (LDH). B: Column: Bio-Scale S20 column. Sample load: 25 ml, 230 mg of protein. Gradient: 0-20 % over 100 ml. Flow rate: 4 ml/min.

Fig. 13 Econo-Pac Q & S Columns Fig. 14

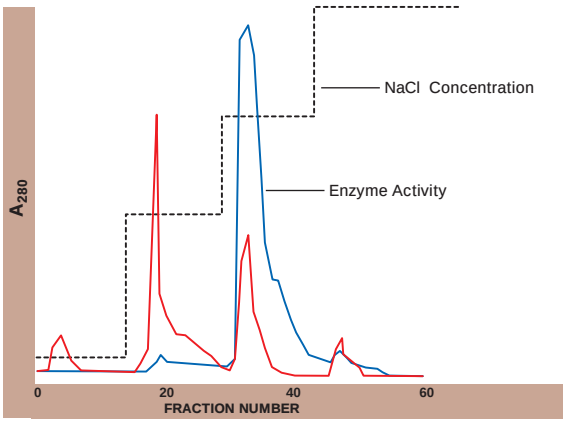


Fig. 13. Purification of Cathepsins L and L-like Proteinase from mackerel using Econo-Pac S 5 ml cartridge. Buffer. A: 20 mM sodium acetate, pH 4.0, 5 mM 2-mercaptoethanol. Protein eluted with three step gradients of 0.4 M, 0.7 M, and 1.0 M NaCl in Buffer A. Reprinted with permission from *Bioscience, Biotechnology and Biochemistry.* Data generated by Jai-Jaan Lee, *et al.* Institute of Marine Food Science, National Taiwan Ocean University, Keelung, Taiwan ROC.

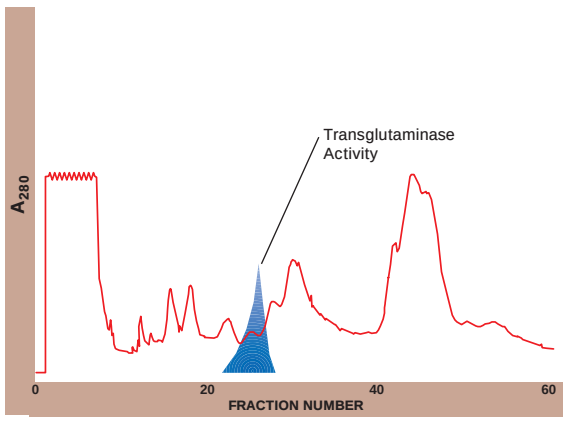


Fig. 14. Purification of sea urchin oocyte cytoplasmic extract using Econo-Pac Q 5 ml cartridge. Buffer A: 50 mM Tris, pH 7.8, 1 mM EDTA, 50 mM NaCl. Buffer B: 50 mM Tris, pH 7.8, 1 mM EDTA, 1.0 M NaCl. Gradient: 100% A for 20 minutes, 0–100% B for 150 minutes Flow rate: 0.5 ml/min. Data submitted by David Battaglia, University of Minnesota, Duluth, MN USA

Larger volumes are available upon request

Econo-Pac cartridges simplify protein purification

Ordering Information

Catalog Number	Product Description
732-0026	**Econo-Pac High Q Cartridge,** 1 x 5 ml
732-0027	**Econo-Pac High Q Cartridge,** 5 x 5 ml
732-0028	**Econo-Pac High Q Cartridge,** 5 x 1 ml
732-0066	**Econo-Pac High S Cartridge,** 1 x 5 ml
732-0067	**Econo-Pac High S Cartridge,** 5 x 5 ml
732-0068	**Econo-Pac High S Cartridge,** 5 x 1 ml
751-0001	**Bio-Scale Q2 Column**
751-0003	**Bio-Scale Q5 Column**
751-0005	**Bio-Scale Q10 Column**
751-0007	**Bio-Scale Q20 Column**
751-0009	**Top-Off Resin Kit Q,** 1 ml
751-0011	**Bio-Scale S2 Column**
751-0013	**Bio-Scale S5 Column**
751-0015	**Bio-Scale S10 Column**
751-0017	**Bio-Scale S20 Column**
751-0019	**Top-Off Resin Kit S,** 1 ml
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
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

See the current Bio-Rad catalog for additional information. Specifications are subject to change without notice. FPLC is a trademark of Pharmacia Biotech, AB.

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Life Science Group

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