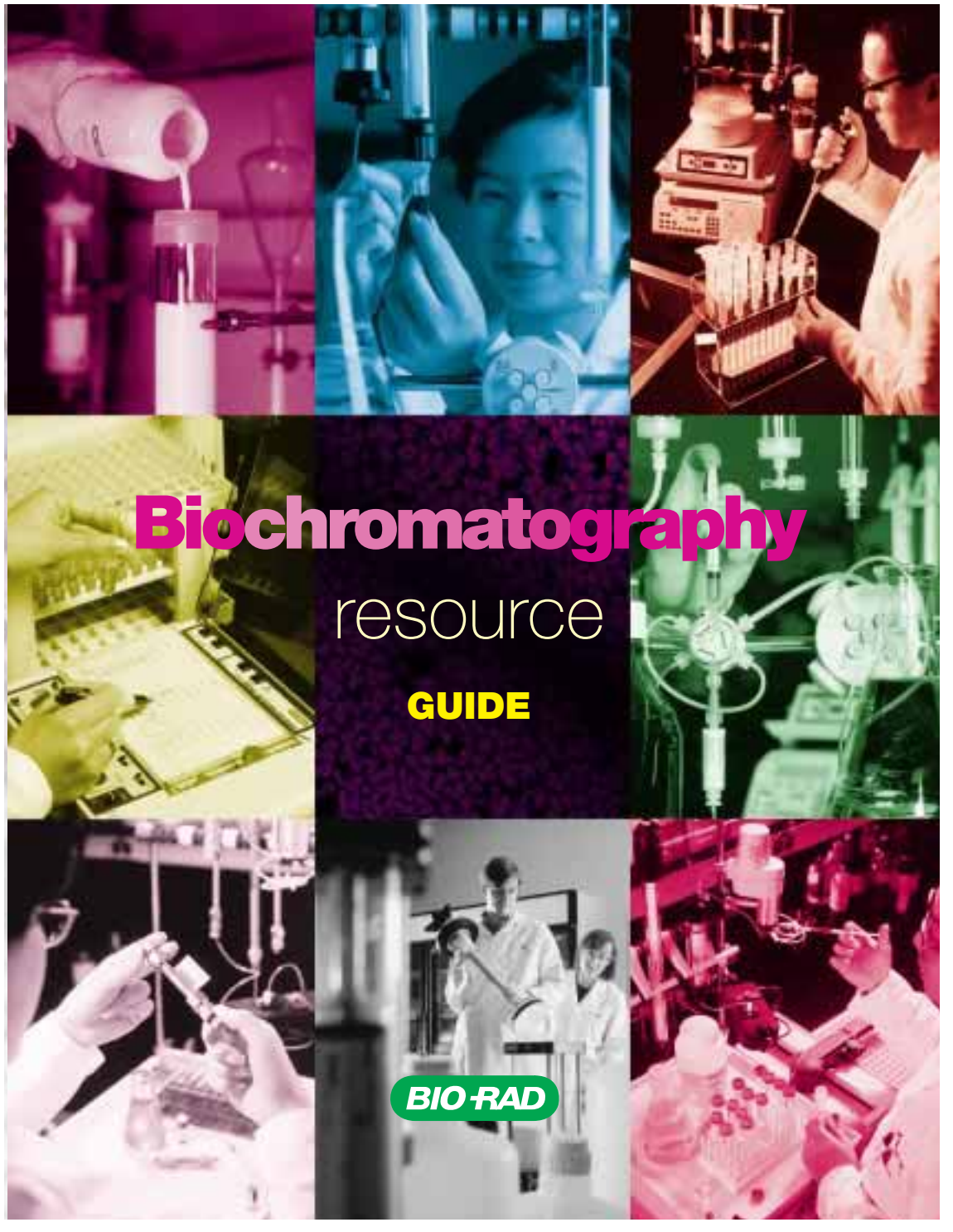

Biochromatography
resource
GUIDE
BIO-RAD

Bio-Rad Biochromatography Resource Guide

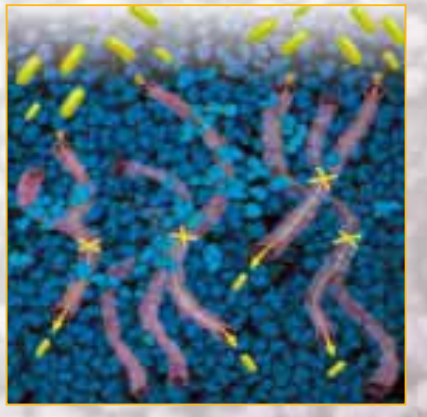
Life science. Paired together, these two simple words convey a profound meaning – the study, the discovery, the understanding of the fundamentals of our very existence.

For more than 40 years, Bio-Rad Laboratories has been at the forefront of life science research, developing the tools needed to pursue every aspect of this field. Our state-of-the-art instruments and reagents, manufactured under the most stringent quality control standards, have found homes in laboratories spanning the globe. Researchers in more than 70 countries are intimately familiar with Bio-Rad products and services.

One of the most common methods for scientific discovery is the purification of biomolecules via chromatographic separations. Bio-Rad excels in this area, offering many outstanding tools for the life scientist involved in analytical, preparative or process scale chromatography.

This guide provides a comprehensive look at Bio-Rad's biochromatography columns and supports, detailing the benefits of each, and how each fits into your lab work, from low pressure to high resolution. It should also serve as a valuable reference for general chromatography techniques and strategies.

So keep the Biochromatography Resource Guide at your side; it truly offers the support you need!



One of Bio-Rad's most recent innovations is the UNO™ column. This illustration shows proteins flowing through channels of UNO's revolutionary Continuous Bed. Capsules are biomolecules and X's are functional groups.

over 40 OF

Table OF Contents

Years

INNOVATION

GUIDE TO Biochromatographic Techniques

Bio-Rad offers **numerous chromatographic products for the purification and characterization of biomolecules, such as proteins, nucleotides and peptides. Our products include prepacked columns and cartridges for fast, convenient and economical preparation of biomolecules, and, when you need to scale up, we have all the bottled supports and empty columns you might require.**

IEX

- General biomolecule purification
- Applicable to all antibody purifications
- High capacity
- Good capture step
- Separation by charge

SEC

- Molecular weight determination
- Applicable to all antibody purifications
- Separation by molecular size
- Desalting or buffer exchange
- High resolution

HYDROXY-APATITE

- Unique selectivity
- Very high purity can often be obtained in one step
- Alternative to protein A for antibody purification

AFFINITY

- High selectivity
- High purity in a single step
- Excellent capture technique
- Separation by biological interaction

HIC

- Higher resolution alternative to ammonium sulfate precipitation
- Gentle elution conditions
- Separation by hydrophobicity of biomolecules

Prepacked

- UNO™ Q (page 19)
- UNO S (page 19)
- Bio-Scale S (page 21)
- Bio-Scale Q (page 21)
- Bio-Scale DEAE (page 21)
- Econo-Pac® Q (page 22)
- Econo-Pac S (page 22)
- Econo-Pac CM (page 22)

Bottle

- Macro-Prep® 25 Q (page 23)
- Macro-Prep 25 S (page 23)
- Macro-Prep High Q (page 24)
- Macro-Prep High S (page 24)
- Macro-Prep CM (page 24)
- Macro-Prep DEAE (page 24)

Prepacked

- Bio-Prep SE (page 31)
- Econo-Pac P-6 (page 32)
- Bio-Spin® (page 33)
- Micro Bio-Spin (page 33)

Bottle

- Macro-Prep SE (pages 34, 35)
- Bio-Gel® P (page 36)
- Bio-Gel A (page 37)

Prepacked

- Bio-Scale CHT-I (pages 41, 42)
- Econo-Pac CHT-II (page 43)

Bottle

- Macro-Prep CHT Type I, 20, 40, 80 µm (pages 44, 45)
- Macro-Prep CHT Type II, 20, 40, 80 µm (pages 44, 45)
- Bio-Gel HT gel (page 46)
- Bio-Gel HTP gel (page 46)

Ready-to-use supports

Prepacked

- Econo-Pac protein A (page 49)
- Econo-Pac blue (page 50)
- Econo-Pac DEAE blue (page 50)

Bottle

- Affi-Prep® protein A (page 51)
- Affi-Gel® protein A (page 51)
- Affi-Gel blue (page 52)
- DEAE Affi-Gel® blue (page 52)
- Affi-Gel 601 boronate (page 53)
- Affi-Prep polymyxin (page 53)

Activated coupling supports

Bottle

- Affi-Prep Hz (page 55)
- Affi-Gel Hz (page 55)
- Affi-Gel 10 (page 55)
- Affi-Prep 10 (page 55)
- Affi-Gel 15 (page 55)
- Affi-Gel 601 boronate (page 53)
- Affi-Gel 102 (page 55)

Prepacked

- Econo-Pac t-butyl HIC (page 58)
- Econo-Pac methyl HIC (page 58)
- Quantum Prep® (page 59)

Bottle

- Macro-Prep t-butyl (page 60)
- Macro-Prep methyl (page 60)

General Column and Media Selection GUIDE

Protein Purification Strategies

Many techniques are now available for protein purification and, while it is possible to design a purification process empirically, the choice of technique and determination of the sequence of operational steps can be of prime importance to the ease, quality and cost of the purification. These general guidelines can help you decide on the best purification strategy to adopt.

Purpose of Separation

A process to produce a few milligrams of enzyme for kinetic studies does not need to be very efficient or cost effective. In contrast, the process to produce kilograms of pharmaceutical product does. Therapeutic products must also be free of pyrogens, viruses and nucleic acids and be stable, efficacious and potent. Thus, the former process might use a relatively exotic affinity chromatography resin, while the latter would use more conventional materials available in large quantities.

Source of Starting Materials

In some cases, proteins are isolated directly from animal tissues and it is usually necessary to work quickly, keep materials chilled and use protease inhibitors. Often, it is more practical to work with recombinant proteins, especially when the native species exists at low levels. Some overexpressed proteins form insoluble inclusion bodies in the bacterial host.

Use different types of media, i.e., *IEX, HIC, CHT, in process development rather than several steps with the same type of media.*

Tip #1

While these bodies can consist of 70% pure protein, they must be solubilized by chaotropes like urea or guanidinium chloride. Urea is compatible with ion exchange separations and some types of affinity techniques, but guanidinium chloride must usually be removed by diafiltration or size exclusion prior to most chromatographic steps. Once pure, the protein often must be refolded to regain biological activity. Use of mammalian cell culture systems or bacterial or yeast systems with secretory signals can provide properly folded proteins.

Separations developed on smaller particles can be scaled directly to columns packed with larger particle sizes.

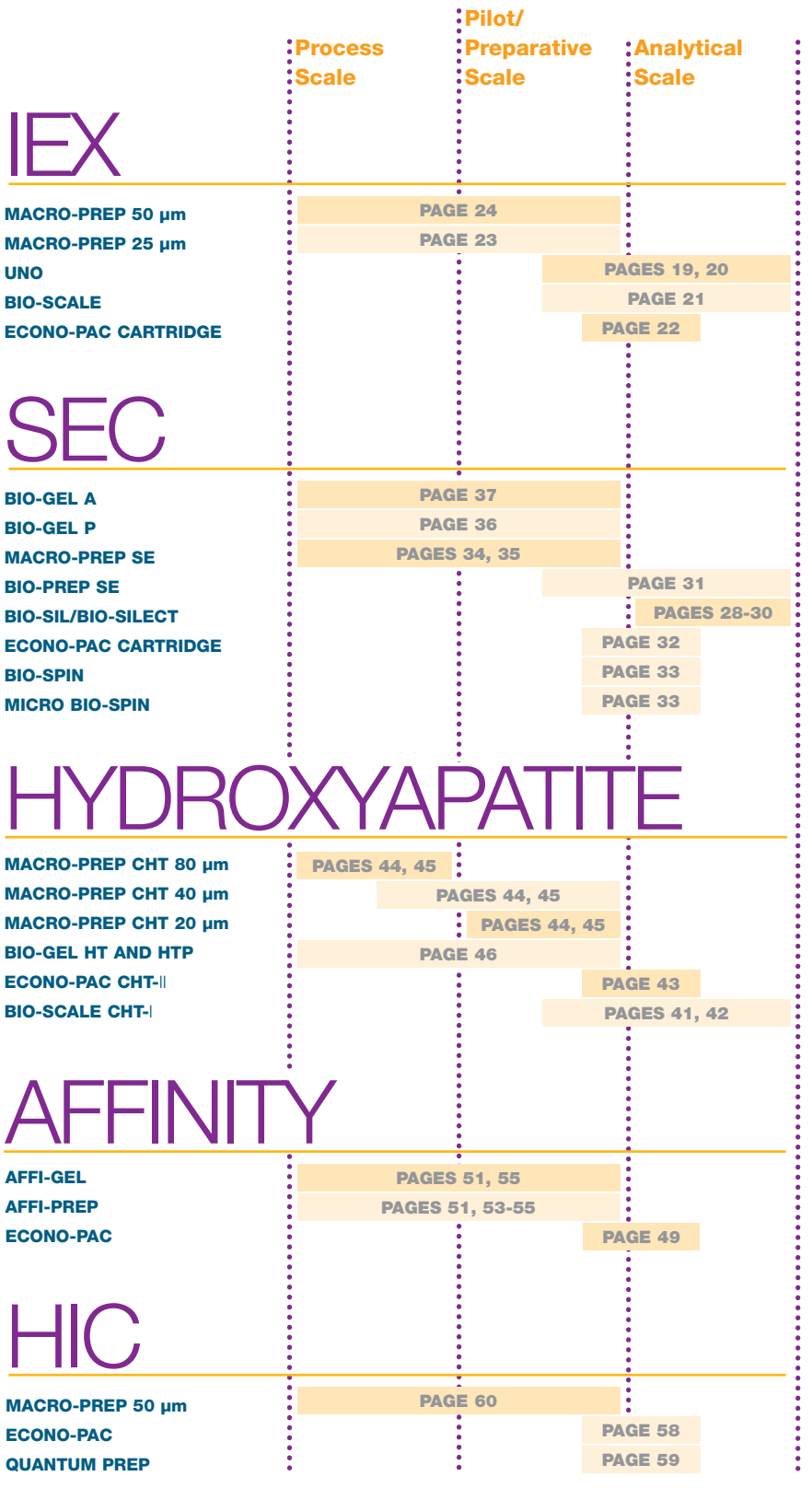
Tip #2

When equilibrating your column, do not exceed more than 50% of the recommended maximum flow rates.

Tip #3

Obtain Data on Molecule of Interest

Data on the sequence or class of molecule can be used with a variety of databases to estimate the size, charge, isoelectric point, hydrophobicity, assay techniques for, and stability of, the molecule to be purified. This information will enable choices of specific affinity media, or ion exchange conditions, selection of size exclusion range. Knowledge of the main contaminants expected is also helpful: for example, if there is little difference between the isoelectric points of the contaminants and the desired species, Ceramic Hydroxyapatite or Macro-Prep HIC resins, which separate by mechanisms other than charge difference, will be a better choice than an ion exchanger.



Use Affinity or Other Selective Techniques as Early as Possible in the Process

This will reduce feed volume, remove the bulk of the contaminants and concentrate the solution early, which will enable the use of smaller columns for subsequent steps. If this is not possible, use a capture step which will give the greatest possible reduction in feed volume (typically ion exchange or HIC).

Always begin a purification process with the most specific step and follow with more general methods.

Tip #4

Varying the Separation Mode May Give Higher Purity in Less Steps

For example, a sequential preparation scheme using ion exchange-> HIC->CHT will usually be better than three ion exchange steps.

Use a step that concentrates the product early in the process.

Tip #5

Always follow a step that dilutes the product with one that concentrates it.

Tip #6

Link Steps So that Desalting, Diafiltration or Concentration is Not Required

The high salt eluate from ion exchange can be applied directly to an HIC column, but an ammonium sulfate fraction could not be applied to an ion exchange column without desalting or dilution. If an SEC column is used, follow with an ion exchange, CHT or HIC step which will concentrate the sample and provide additional purity in one step.

For more information, request bulletins:

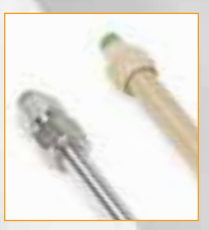
1825 Sample Prep Guide
2026 Evaluation of Different Approaches for Chromatographic Purification of Monoclonal Antibodies
2079 Non-Protein A Based Purification of Mab 414

PRODUCT Highlights

High Performance Prepacked Columns

UNO Columns
The revolutionary UNO ion exchange columns are the very first biochromatography columns to contain the unique Continuous Bed matrix. This radical departure from traditional beaded columns leads to a column designed to perform biomolecule separation at high flow rates without sacrificing resolution or capacity. For more information, see pages 19, 20.

Bio-Scale Columns
Bio-Scale columns offer reproducible high resolution separations of biomolecules. By using Macro-Prep media, methods developed on Bio-Scale columns can easily be transferred to production scale. For more information, see pages 15, 21.



Bio-Sil and Bio-Silect Columns
These analytical SE columns, prepacked with proprietary Bio-Sil silica support, provide reproducible high resolution separations of peptides, proteins and nucleic acid in the pH range of 2-8. Both the stainless steel Bio-Sil and PEEK™ Bio-Silect columns are available in a choice of fractionation ranges. For more information, see pages 28-30.

Bio-Prep SE Columns
Bio-Prep SE columns are high resolution biocompatible SE size exclusion columns, prepacked with crosslinked agarose beads for fast, reproducible separation of peptides, proteins and nucleic acids. Applications developed on the Bio-Prep SE columns can be easily transferred to production scale using Macro-Prep SE 40. For more information, see page 31.

Low Pressure Columns and Media

Convenient prepacked low pressure columns for methods scouting or first-step purification of crude samples are available. For more information, see pages 22, 43, 58.

Quantum Prep Plasmid Purification Kits
Quantum Prep plasmid purification kits easily and effectively isolate sequencing and transfection-quality plasmid DNA from 1-500 ml of bacterial cell culture. This rapid spin column technique features the novel, patented Quantum Prep matrix, which provides the highest yields of plasmid DNA. For more information, see page 59.

Prepacked Bio-Spin and Micro Bio-Spin Columns
Get rapid clean up and purification of protein and nucleic acid samples with these easy and effective spin columns. Each is packed with specially-sized Bio-Gel P polyacrylamide SE gels. For more information, see page 16.

Glass Econo-Column Columns
Econo-Column chromatography columns are the standard for high quality, affordable low pressure empty columns. A wide range of column lengths and diameters is available. Accessories include flow adaptors, funnels and glass reservoirs. For more information, see pages 12, 13.

Macro-Prep CHT Media
Macro-Prep ceramic hydroxyapatite media (CHT) is a chemically pure form of hydroxyapatite which provides the throughput, stability and reproducibility required for industrial biopharmaceutical manufacturing, plus the resolution and speed researchers demand. For more information, see pages 44, 45.

Macro-Prep IEX Media
Macro-Prep ion exchange media are designed for benchtop, pilot and process scale preparative applications. Their rigid, macroporous and hydrophilic properties provide exceptional capacity, resolution and throughput. For more information, see pages 23, 24.

Macro-Prep SE Media
Macro-Prep SE is a size exclusion media based on 40 µm spherical, crosslinked agarose beads. The high performance Macro-Prep SE is ideal for the fractionation/polishing of biomolecules, and for desalting/buffer exchange at the pilot to process scale. For more information, see pages 34, 35.

Process Separations

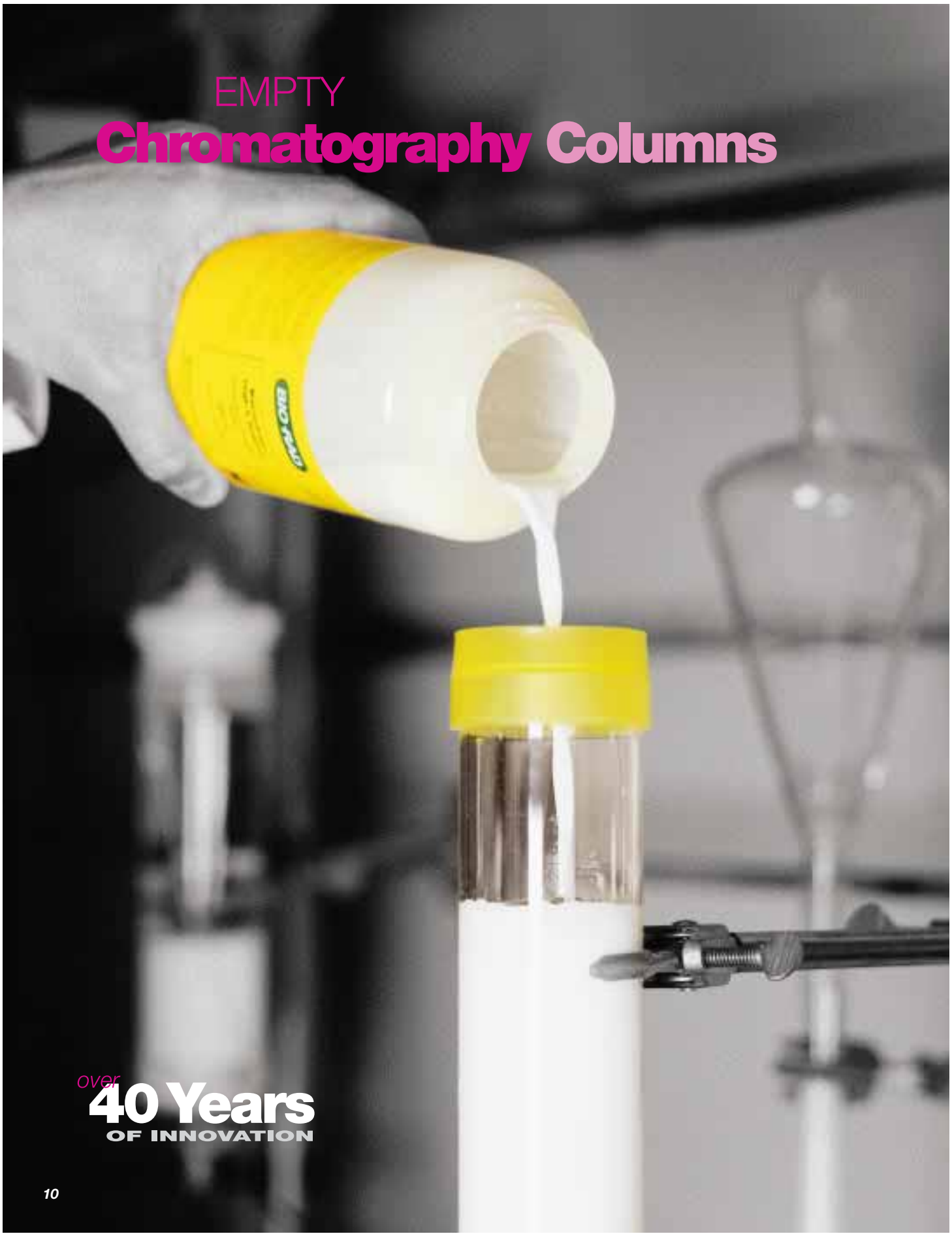
Products for Biopharmaceutical and Diagnostic Production
Bio-Rad is an established supplier of high quality process chromatography supports for the pharmaceutical and biotechnology industries. Our products are used extensively around the world in the manufacture of registered drugs and compounds.

We offer chromatography supports for production of biological molecules on every scale. Bio-Rad's chromatography supports provide the extra selectivity, throughput and capacity you need to develop your next purification process. Products such as Macro-Prep ion exchange and ceramic hydroxyapatite supports, and the Bio-Gel, Affi-Gel and AG resin lines have established Bio-Rad as a world leader in chromatography supports.

Regulatory Support
Having worked with the pharmaceutical industry for many years, Bio-Rad is intimately familiar with its particular needs. Those involved in regulatory/validation work can take advantage of Macro-Prep support's Regulatory Support Files. These files contain information essential to validation work, including general product details, biological safety information and specification test procedures.

Drug Master Files
All Macro-Prep products and many of Bio-Rad's other chromatography media are manufactured under type II Drug Master Files, which are registered with the United States Food and Drug Administration (FDA).

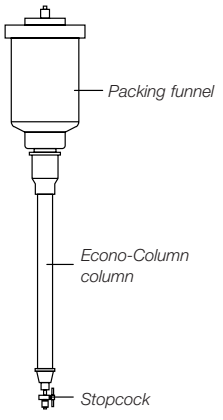
ISO 9001
The Bio-Rad Life Science Group and its design, development and manufacture of chemicals and analytical instruments, are assessed and registered by National Quality Assurance Limited against the provisions of BS EN ISO 9001:1994.


EMPTY
Chromatography Columns
over
40 Years
OF INNOVATION

Empty Column Product Selection Guide

Column	Application Requirement	Pressure Range (psi)	Volume Range (ml)	Best Used With
BIO-SCALE MT	**HIGH RESOLUTION COLUMN**	**0-1,000**	**1-22**	**HIGH PERFORMANCE SYSTEM**
ECONO-COLUMN	**GENERAL PURPOSE GLASS COLUMN**	**0-15**	**1-1,374**	**LOW PRESSURE SYSTEMS OR GRAVITY**
ECONO-PAC	**GENERAL PURPOSE PLASTIC COLUMN**	**0-15**	**1-20**	**LOW PRESSURE SYSTEMS OR GRAVITY**
POLY-PREP	**DISPOSABLE PLASTIC COLUMN**	**0-15**	**2**	**GRAVITY**
BIO-SPIN	**DISPOSABLE PLASTIC COLUMN**	**-**	**1.2**	**SWINGING BUCKET CENTRIFUGE**
MICRO BIO-SPIN	**DISPOSABLE PLASTIC COLUMN**	**-**	**0.8**	**STANDARD MICRO-CENTRIFUGE**

How to Pack a Chromatography Column



1. Hydrate or equilibrate the desired packing material in a suitable buffer according to product instructions.
2. Estimate the volume of settled material and add half that volume of degassed buffer to the gel (example: for 100 ml of settled bed, add 50 ml of buffer).
3. Pour buffer into the column. Carefully wet the bed support at the bottom of the column by opening the column outlet, making sure no air bubbles are trapped in it. Close the column outlet, leaving 5-10 cm of buffer in the column. Adjust the column on the rack to ensure that it is level.
4. Insert a packing funnel into the top of the column. Gently mix all packing material to form a suspension and pour it into the funnel. Pouring the packing material down the side of the column or down a glass rod will minimize trapped bubbles.
5. Fill the packing funnel with buffer and open the column outlet to begin packing the column under flow.
6. After the packing has settled into the column, close the column outlet and replace the packing funnel with a flow adaptor. Insert the flow adaptor, being careful not to trap air bubbles under it. Connect the flow adaptor to a pump or reservoir.
7. Open the column outlet and continue packing the column with buffer at a flow rate 10-20% higher than the anticipated experimental flow rate. The hydrodynamic pressure must not exceed the maximum for the gel type used.
8. When the bed height remains constant, close the column outlet, and adjust the flow adaptor down to the top of the bed.
9. Open the column outlet and restart the buffer flow. It may be necessary to make one or more adaptor adjustments to ensure minimal dead volumes.
10. The column is now ready to use.

Some useful equations:

Cross Sectional Area: $A = \pi r^2$

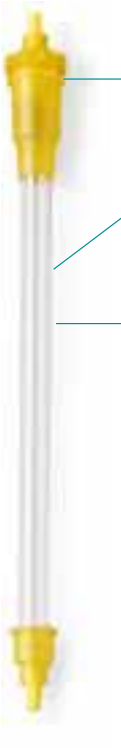
Column Volume: $CV = AL$

Linear Velocity: $u = Q / A$

Where A = column cross sectional area, CV = column bed volume, u = linear velocity (cm/hr), L = column length, D = column diameter, Q = volumetric flow rate (ml/hr)

Tip #7

Empty Chromatography Columns



Glass Econo-Column Columns

Affordable, High Quality, Low Pressure Columns

Bio-Rad offers columns ranging from 5 cm to 170 cm in length and 0.5 cm to 5.0 cm in diameter.

- Translucent polypropylene end fittings allow visualization of the entire gel bed
- Autoclavable
- Maximum operating pressure of 1 bar (14.7 psi)
- Accepts both Econo-Column funnels and flow adaptors (page 14)

Econo-Column Configurations

Econo-Column chromatography columns and accessories provide a versatile yet inexpensive low pressure chromatography system. Illustrated at right are just a few of many possible low pressure chromatography system configurations.

Some useful equations:

Retention $k' = (V_r - V_o) / V_o$

Selectivity $a = k'_2 / k'_1$

Efficiency (number of theoretical plates)

$N = 5.54 (V_r / W)^2$

Height equivalent to a theoretical plate

$HETP = L / N$

Resolution

$Rs = (V_{r2} - V_{r1}) / 0.85 (W_2 + W_1)$

Where V_o= void volume, V_r= retention volume of species r, W= peak width at half height, L= length

Tip #8

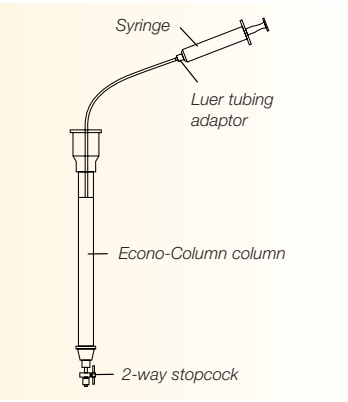


Fig. 1. Manual sample application directly to the top of the column bed.

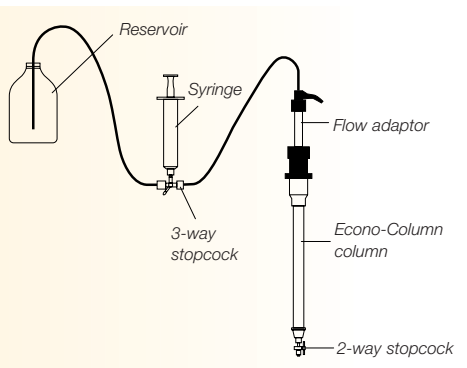


Fig. 2. Manual sample application via 3-way stopcock.

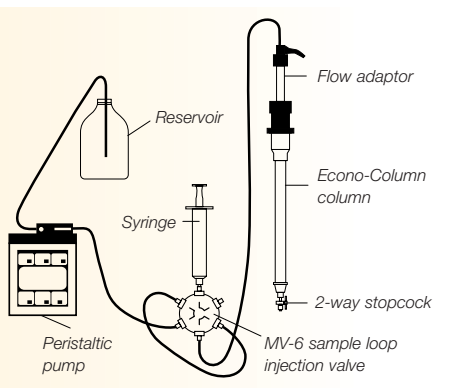


Fig. 3. Manual sample application via MV-6 sample loop injection valve.

Empty Chromatography Columns

Ordering Information

Catalog #	ID (cm)	Length (cm)	Cross-Sectional Area (cm²)	Maximum Volume (ml)	Package Size
GLASS ECONO-COLUMN COLUMNS					
737-0506	0.5	5	0.20	1	5
737-0507	0.5	5	0.20	1	2
737-0511	0.5	10	0.20	2	5
737-0512	0.5	10	0.20	2	2
737-0516	0.5	15	0.20	3	5
737-0517	0.5	15	0.20	3	2
737-0521	0.5	20	0.20	4	5
737-0522	0.5	20	0.20	4	2
737-0706	0.7	5	0.39	2	5
737-0707	0.7	5	0.39	2	2
737-0711	0.7	10	0.39	4	5
737-0712	0.7	10	0.39	4	2
737-0716	0.7	15	0.39	6	5
737-0717	0.7	15	0.39	6	2
737-0721	0.7	20	0.39	8	5
737-0722	0.7	20	0.39	8	2
737-0731	0.7	30	0.39	12	5
737-0732	0.7	30	0.39	12	2
737-0751	0.7	50	0.39	20	5
737-0752	0.7	50	0.39	20	2
737-1006	1.0	5	0.79	4	5
737-1007	1.0	5	0.79	4	2
737-1011	1.0	10	0.79	8	5
737-1012	1.0	10	0.79	8	2
737-1021	1.0	20	0.79	16	5
737-1022	1.0	20	0.79	16	2
737-1031	1.0	30	0.79	24	5
737-1032	1.0	30	0.79	24	2
737-1051	1.0	50	0.79	40	5
737-1052	1.0	50	0.79	40	2
737-1091	1.0	100	0.79	79	2
737-1093	1.0	120	0.79	103	2
737-1506	1.5	5	1.77	9	5
737-1507	1.5	5	1.77	9	2
737-1511	1.5	10	1.77	18	5
737-1512	1.5	10	1.77	18	2
737-1516	1.5	15	1.77	27	5
737-1517	1.5	15	1.77	27	2
737-1521	1.5	20	1.77	35	5
737-1522	1.5	20	1.77	35	2
737-1531	1.5	30	1.77	53	5
737-1532	1.5	30	1.77	53	2
737-1551	1.5	50	1.77	89	5
737-1552	1.5	50	1.77	89	2
737-1576	1.5	75	1.77	124	2
737-1591	1.5	100	1.77	177	2
737-1593	1.5	120	1.77	230	2
737-1598	1.5	170	1.77	301	2
737-2506	2.5	5	4.91	25	5
737-2507	2.5	5	4.91	25	2
737-2511	2.5	10	4.91	49	5
737-2512	2.5	10	4.91	49	2
737-2521	2.5	20	4.91	98	5
737-2522	2.5	20	4.91	98	2
737-2531	2.5	30	4.91	147	5
737-2532	2.5	30	4.91	147	2
737-2551	2.5	50	4.91	246	2
737-2576	2.5	75	4.91	344	2
737-2591	2.5	100	4.91	491	2
737-2593	2.5	120	4.91	589	2

Ordering Information (continued)

Catalog #	ID (cm)	Length (cm)	Cross-Sectional Area (cm²)	Maximum Volume (ml)	Package Size
GLASS ECONO-COLUMN COLUMNS (CONTINUED)					
737-5011	5.0	10	19.63	196	1
737-5021	5.0	20	19.63	393	1
737-5031	5.0	30	19.63	589	1
737-5051	5.0	50	19.63	982	1
737-5071	5.0	70	19.63	1374	1

Catalog #	Description
ECONO-COLUMN SELECTION PACKS	
737-6601	Econo Column Selection Pack A, 1 each standard column, 0.7 x 10, 20, 30 cm; 1.5 x 30, 50 cm; 2.5 x 20, 50 cm
737-6607	Econo Column Selection Pack 3, 1 each standard column, 1.0 x 20, 30, 50 cm; 1.5 x 20, 30, 50 cm

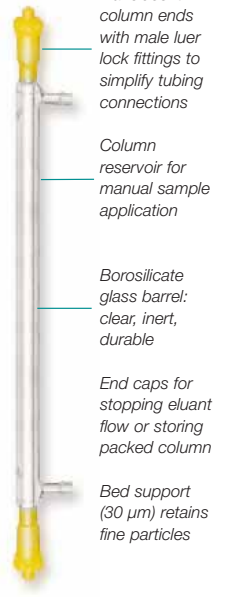
Catalog #	ID (cm)	Length (cm)	Cross-Sectional Area (cm²)	Maximum Volume (ml)	Package Size
STANDARD JACKETED COLUMNS					
737-6108	0.7	15	0.37	6	1
737-6116	1.0	15	0.79	12	1
737-6131	1.0	30	0.79	25	1
737-6151	1.5	50	1.77	89	1
OPEN-ENDED JACKETED COLUMN, INCLUDES 2 FLOW ADAPTORS					
737-6201	1.0	30	0.79	25	1

Jacketed Econo-Column Columns

For Chromatographic Applications Requiring Temperature Control

These columns feature an integral water jacket for low pressure chromatography applications requiring temperature control, such as thermal chromatography of DNA using hydroxyapatite.

- Translucent polypropylene end fittings allow visualization of the entire gel bed
- Autoclavable
- Maximum operating pressure of 1 bar (14.7 psi)
- Accepts Econo-Column flow adaptors, funnels and reservoirs



Empty Chromatography Columns

Flow Adaptors

The Easy Way to Improve Column Performance

Flow adaptors improve resolution by delivering buffer and sample directly to the top of the gel bed.

- Eliminates dead volume above the gel bed
- Protects the gel bed from disruption during sample loading
- Use with any low pressure column connected to pumps, like the BioLogic System or other low pressure systems
- Available for 1.0, 1.5, 2.5 and 5.0 cm Econo-Column chromatography columns, Jacketed Econo-Column chromatography columns and Econo-Pac columns

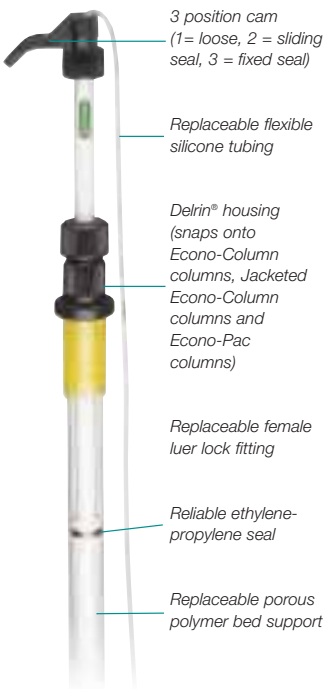
Econo-Column Funnels

For Column Packing, Loading Diluted Samples and Delivering Large Buffer Volumes

The Econo-Column funnel, constructed of durable polypropylene, forms a tight seal with Econo-Column chromatography columns up to 2.5 cm ID, jacketed Econo-Column chromatography columns, Poly-Prep columns and Econo-Pac columns.

Glass Reservoirs

Reservoirs are available in 500 ml and 1,000 ml capacities, and fit 0.5, 0.7, 1.0 and 1.5 cm ID Econo-Column chromatography columns. To make a mariotte constant pressure reservoir, close the reservoir top with a stopper containing a piece of glass tubing that extends into the reservoir. The removable upper cap has a male luer lock fitting.



Flow Adaptor

Econo-Column Reservior

Econo-Column Funnel

Ordering Information

Catalog #	*Description*	*Column ID*	*Functional Length*
FLOW ADAPTOR REPLACEMENT PARTS			
738-0014	Flow Adaptor	1.0 cm	1 to 7 cm
738-0015	Flow Adaptor	1.0 cm	1 to 14 cm
738-0016	Flow Adaptor	1.5 cm	1 to 14 cm
738-0017	Flow Adaptor	2.5 cm	1 to 14 cm
738-0018	Flow Adaptor*	5.0 cm	1 to 14 cm
738-0019	Econo-Pac Flow Adaptor	1.5 cm	
FLOW ADAPTOR REPLACEMENT PARTS			
738-0022	Flow Adaptor Maintenance Kit, for 5.0 cm flow adaptor, includes 2 bed supports, 2 O-rings		
738-0024	Flow Adaptor Maintenance Kit, for 1.0 cm adaptor with cam mechanism, includes 10 bed supports, 5 O-rings		
738-0025	Flow Adaptor Maintenance Kit, for 1.5 cm adaptor with cam mechanism, includes 10 bed supports, 5 O-rings		
738-0027	Flow Adaptor Maintenance Kit, for 2.5 cm adaptor with cam mechanism, includes 10 bed supports, 5 O-rings		
FUNNEL			
731-0003	Econo-Column Funnel, 250 ml, 5		
RESERVOIR			
737-9112	Econo-Column Reservoir, 500 ml		
737-9113	Econo-Column Reservoir, 1,000 ml		

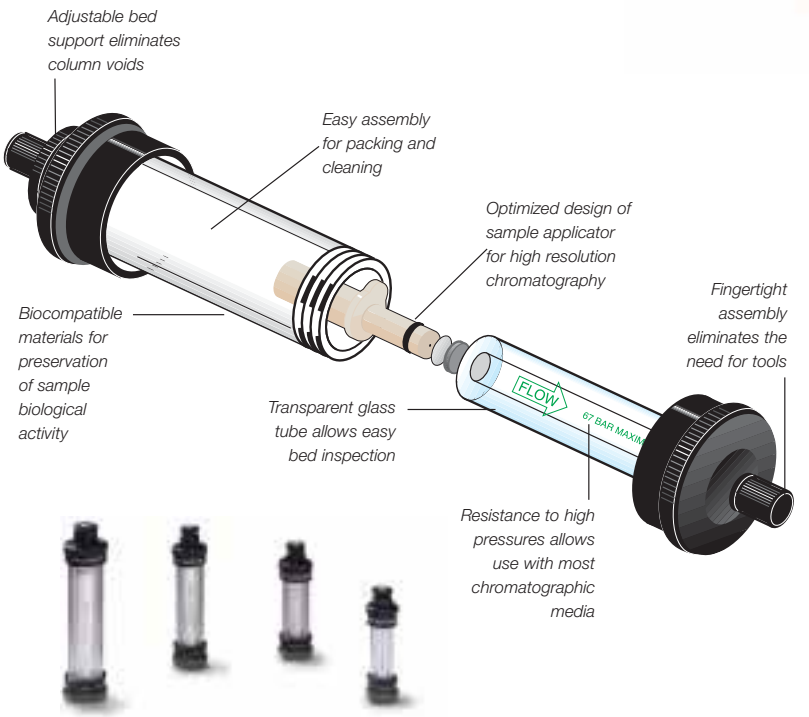
*5.0 cm flow adaptor does not include cam mechanism.

Bio-Scale MT High Resolution Columns

For Applications Requiring High Resolution and Precise Sample Loading

Bio-Scale MT empty columns provide extremely high resolution in most chromatography applications.

- Use with high performance systems such as the BioLogic HR
- Precise sample application
- Provides the low dead volume required for high resolution separations
- The four column sizes (2, 5, 10 and 20 ml) allow easy scale-up of separation and purification protocols
- Optimized design permits easy packing, bed height adjustment, sample application and equilibration



Ordering Information

Catalog #	Description	Volume Range (ml)	Column Pressure Limit (psi/bar)
BIO-SCALE COLUMNS			
751-0081	Bio-Scale MT2 Column, 7 x 52 mm	1.9–2.3	1,000/70
751-0083	Bio-Scale MT5 Column, 10 x 64 mm	4.6–5.7	750/50
751-0085	Bio-Scale MT10 Column, 12 x 88 mm	9.5–11.3	600/40
751-0087	Bio-Scale MT20 Column, 15 x 113 mm	19.4–21.9	500/34
KITS			
751-0091	Bio-Scale 2 Replacement Parts Kit, includes 5 frits, 5 distribution screens, 2 0-rings, 1 frit remover		
751-0093	Bio-Scale 5 Replacement Parts Kit		
751-0095	Bio-Scale 10 Replacement Parts Kit		
751-0097	Bio-Scale 20 Replacement Parts Kit		
750-0555	1/4 x 28 Fittings, set of 10 Nut/Ferrule-Lock Ring to connect a Bio-Scale MT to a BioLogic System		
750-0567	M6 Fittings, set of 2 nuts and 4 ferrules to connect a Bio-Scale MT to an FPLC® system		
750-0568	10-32 Fittings Kit, set of 2 nuts and 4 ferrules to connect a Bio-Scale MT column to an HPLC system		

Note: Each Bio-Scale MT column comes with a Bio-Scale Replacement Parts Kit.

Empty Chromatography Columns

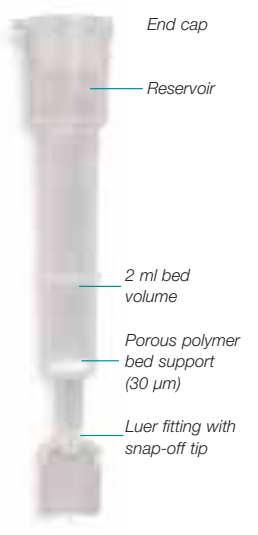
Bio-Spin and Micro Bio-Spin Columns

Remove Dye Terminators, Radiolabels and More

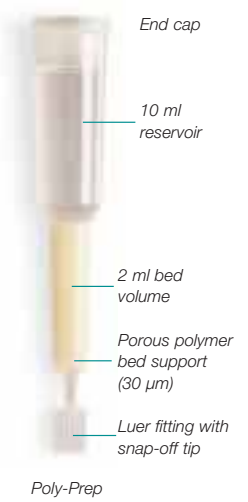
Pack these disposable polypropylene spin columns with gel filtration media to remove dye terminators, clean up DNA primers, remove radiolabels, exchange buffers and desalt proteins or DNA.

- Bio-Spin columns hold up to 1.2 ml of media and fit in standard swinging bucket centrifuges
- Micro Bio-Spin columns hold up to 0.8 ml of media and fit in standard micro-centrifuges
- Both fit standard microtubes, have snap-off tips and polyethylene bed supports
- Both are autoclavable

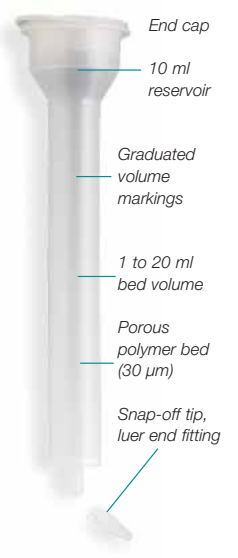
Micro Bio-Spin Column



Bio-Spin Column



Poly-Prep Column



Econo-Pac Column

Poly-Prep Columns

For Sample Preparation and Small Scale Applications

These conical, 0.8 x 4 cm polypropylene columns (9 cm total column height) hold up to 2 ml of chromatography media and 10 ml of eluant or sample in an integral reservoir.

- Ideal for work with radioisotopes and other applications where disposable products are required
- Autoclavable
- Retains fine particles (>40 µm)

Econo-Pac Columns

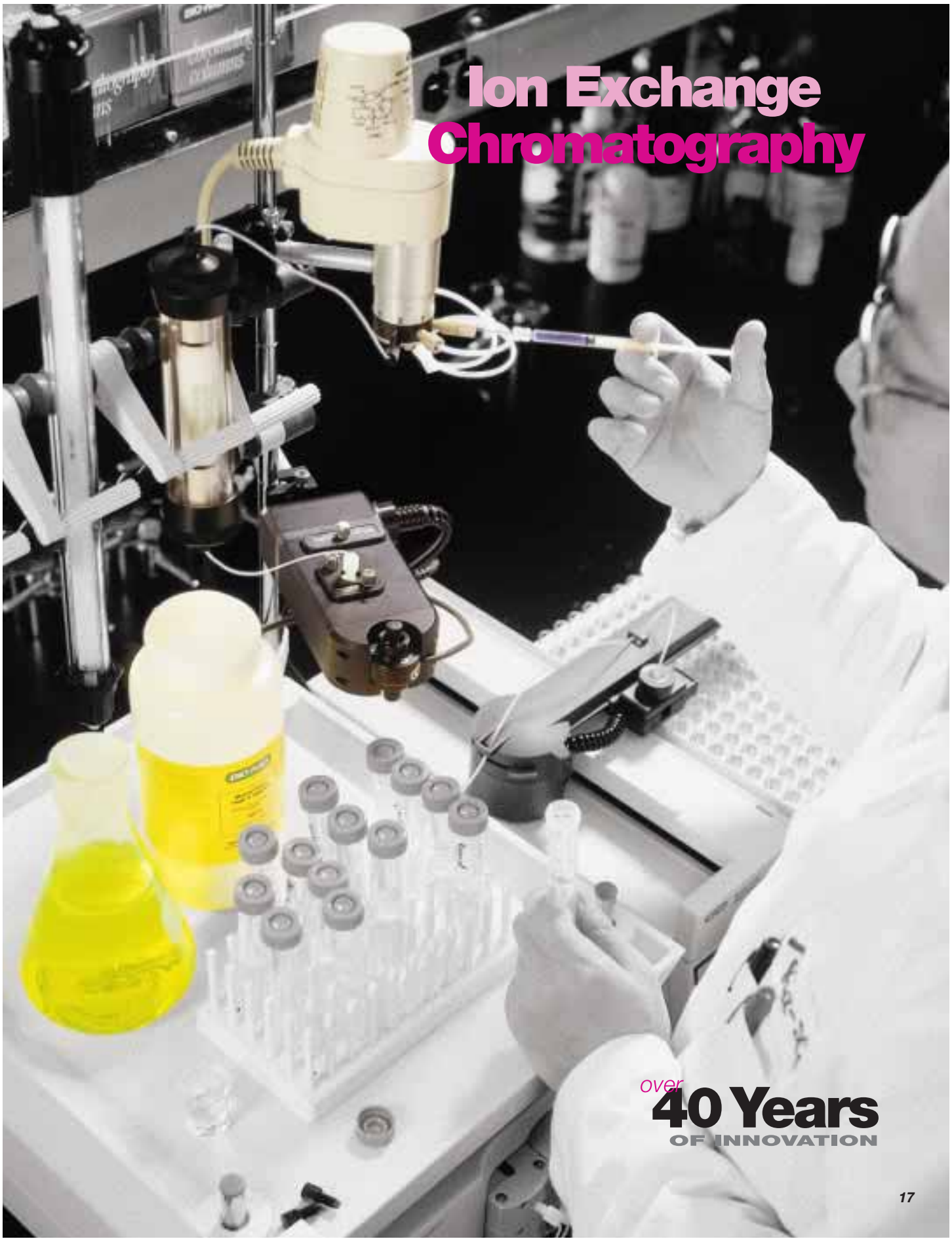
For Sample Preparation and Small Scale Applications

Econo-Pac disposable 20 ml polypropylene columns are versatile chromatography tools.

- 1.5 x 12 cm (14 cm total column height)
- Fit with a flow adaptor or use for gravity flow chromatography
- When used for open column work, a special upper bed support prevents the bed from running dry
- Bed volumes from 1 to 20 ml are possible
- Autoclavable
- Retains fine particles (>40 µm)

Ordering Information

Catalog #	*Description*	*Quantity*
BIO-SPIN AND MICRO BIO-SPIN COLUMNS		
732-6008	Bio-Spin Chromatography Columns, empty	100
732-6025	Bio-Spin Chromatography Columns, empty	1,000
732-6204	Micro Bio-Spin Chromatography Columns, empty	100
731-1660	End Caps, Micro Bio-Spin chromatography columns	100
POLY-PREP COLUMNS		
731-1550	Poly-Prep Chromatography Columns, empty, include end caps and tip closures	50
731-1553	Poly-Prep Chromatography Columns, empty, include end caps and tip closures	1,000
POLY-PREP COLUMN ACCESSORIES		
731-1555	Poly-Prep Column Stack Cap	50
731-7005	Poly Column Rack, 20 place, with removable tube rack	
732-8102	2-way Stopcock	10
731-8232	Female Luer Plug	25
ECONO-PAC COLUMNS		
732-1010	Econo-Pac Chromatography Columns, empty, include upper bed supports, end caps, tip closures	50
732-1011	Econo-Pac Chromatography Columns, empty, include upper bed supports, end caps, tip closures	500
ECONO-PAC COLUMN ACCESSORIES		
738-0019	Econo-Pac Flow Adaptor	
731-7005	Poly Column Rack, 10 place with removable tube rack	
732-8102	2-way Stopcock	10
731-8232	Female Luer Plug	25


Ion Exchange
Chromatography
over
40 Years
OF INNOVATION

Ion Exchange Chromatography

Mechanism of Action

Ion exchange chromatography (IEX) separates molecules based on their net charge. Negatively or positively charged functional groups are covalently bound to a solid support matrix producing a cation or anion exchanger. When a charged molecule is applied to an exchanger of opposite charge, it is adsorbed, while uncharged molecules or molecules of the same charge do not bind. The binding of the charged molecules is reversible and adsorbed molecules are commonly eluted with a salt gradient.

Resin Type	Cation Exchanger	Anion Exchanger
Net Charge of Molecule of Interest	+	−
Charge of Resin	−	+
Normal Running Conditions	Run at 0.5–1.5 pH units below the pI of the molecule of interest	Run at 0.5–1.5 pH units above the pI of the molecule of interest

Separation strategy for amphoteric molecules. If the amphoteric molecule is stable at a pH above its pI, an anion exchange resin is used. A cation exchange resin is used if the molecule is stable at a pH below its pI.

Both the support and the macromolecule are associated with low molecular weight ions (counterions). For binding to occur, the counterions must dissociate from both support and macromolecule. Counterions remain in equilibrium between the solution and the support, and it is the concentration and type of counterion that determines the relative affinity of the adsorbed macromolecule.

Bio-Rad's wide selection of resins allows you to choose a resin which will provide optimal resolution and efficiency for your application.

Typical IEX Application:

Purification of all biomolecules including proteins, peptides and polynucleotides.

Choosing Between Anion Exchange and Cation Exchange

Selection of an ion exchange resin depends on the properties of the macromolecules to be separated. All amphoteric macromolecules have an isoelectric point (pI), which is the pH where the molecule has an equal number of positive and negative charges. The pI of the molecule and its stability at various pH values determines the separation strategy. At a pH above its pI, the molecule of interest will be negatively charged and at a pH below its pI, the molecule will be positively charged. Thus, if the molecule is stable at a pH above its pI, an anion exchange resin is used. Conversely, if the molecule is stable at a pH below its pI, a cation exchange resin is used.

Choosing Between Strong and Weak Ion Exchangers

Anion and cation exchangers are available with either strong or weak functional groups. Here, the terms "strong" and "weak" refer to the effect of pH on the charge on the exchanger. The number of charges on a strong ion exchanger is constant regardless of the pH of the buffer. The number of charges on a weak ion exchange resin varies with pH range. The terms "strong" and "weak" do not refer to the strength with which biomolecules bind.

Bio-Rad offers both strong and weak ion exchangers since they have different selectivities.

Ion Exchange Product Selection Guide

Product	Purification Stage	Resolution	Flow Rate	Package Ionic Form	Size Range	Required Equipment
UNO COLUMN	**SEMI-PREP TO POLISHING**	**EXCELLENT**	**0.5-10 ml/min**	**Q, S**	**PREPACKED 0.2 TO 12 ml**	**MEDIUM TO HIGH PRESSURE SYSTEM**
BIO-SCALE COLUMNS	**METHOD DEVELOPMENT & POLISHING**	**VERY HIGH**	**0.5-10 ml/min**	**Q, S, DEAE**	**PREPACKED 2.0 TO 20 ml**	**MEDIUM TO HIGH PRESSURE SYSTEM**
ECONO-PAC CARTRIDGE	**SAMPLE CLEAN-UP, INTERMEDIATE**	**LOW-MEDIUM**	**0.5-5.0 ml/min**	**Q, S, CM**	**1.0 & 5.0 ml**	**SYRINGE, PERISTALTIC PUMP**
MACRO-PREP 25 MEDIA	**POLISHING**	**MEDIUM-HIGH**	**100-500 cm/hr**	**Q, S**	**10 ml TO PROCESS**	**ECONO-COLUMN**
MACRO-PREP 50 MEDIA	**CAPTURE TO POLISHING**	**MEDIUM**	**100-1,000 cm/hr**	**Q, S, CM, DEAE**	**25 ml TO PROCESS**	**ECONO-COLUMN**

UNO Q and S Columns

A Revolutionary Continuous Bed Matrix Provides Separation at High Flow Rates Without Sacrificing Resolution or Capacity

UNO columns combine the best features of traditional supports (*e.g.,* high recovery of biological activity, low backpressures, minimal nonspecific binding effects and stability over the pH range 2–12) with the exceptional speed, resolution and capacity provided by the Continuous Bed technology. UNO columns are ideal for the BioLogic HR system, ÄKTA®, FPLC® and all other medium to high pressure chromatography systems.

Fig. 1. The structure of the Continuous Bed matrix can be seen in this scanning electron micrograph (1.95k magnification). Note the fimbriated structure of the polymer nodules and the large channels between them.

In the Continuous Bed technology, advanced monomers and ionomers are polymerized directly in the column. The polymer chains aggregate into a dense, homogeneous network of nodules. The resulting rigid matrix contains interconnecting channels large enough to permit high hydrodynamic flow at low backpressures.

UNO's Homogeneous, Nonporous Continuous Bed Matrix Allows:

- Extremely fast mass transfer of proteins and eluant to the ionic functional groups
- Minimal band broadening
- Unsurpassed resolution of protein
- Minimal loss of resolution at high flow rates
- High resolution separations in about 3 minutes on a 1 ml UNO column

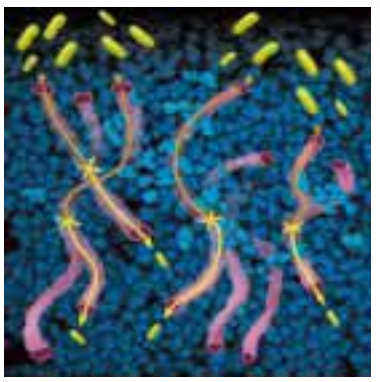


Fig. 2. Illustration of proteins flowing through channels of Continuous Bed. Capsules are biomolecule and X's are functional groups.

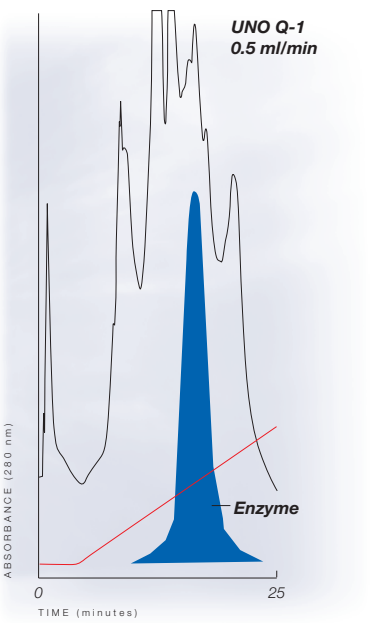


Fig. 3. (left) Resolution study: purification of phosphoinositide-3-kinase at 0.5 ml/min.

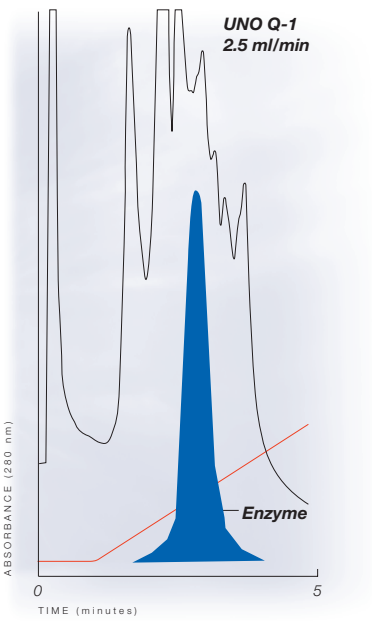


Fig. 4. (right) Speed and resolution study: same purification of phosphoinositide-3-kinase as in Figure 3, but 5 times faster.

Buffer A: 40 mM TEA, pH 7.6
Buffer B: A + 1 M KCl, pH 7.6
Gradient: 0-35% B in 20 ml

Data courtesy of A. Couvillon, Beth Israel Deaconess Medical Center, Boston, MA.

Ion Exchange Chromatography

When to Use UNO

- Best for separations requiring high throughput, such as bioassays, and high resolution preparation of macromolecules at the laboratory scale
- Enzymes, serum proteins, nucleotides and a variety of other bioactive macromolecules have been successfully purified using UNO columns

For more information, request bulletins:

2116 *UNO Ion Exchange Columns*
2118 *UNO Columns*
2205 *Purification of Phosphoinositide 3-Kinase from Rat Liver with UNO Columns*
2252 *Purification of Elongation Factors, EF-1βγδ and EF-1βγ, from Xenopus Laevis Oocytes using UNO Q1 Columns*
2258 *High Speed Separation of Isozymes of Recombinant cAMP-Dependent Protein Kinase using UNO S1 and S6 Columns*
2283 *Purification of the Components of Human Macular Carotenoid-Binding Complex using UNO Columns*
2284 *Purification of Two Isoforms of Pyruvate from Nordic Krill using UNO Q1 Column*
2303 *Purification of Insulin on UNO S1 Column*
2304 *Purification of rIL using UNO Q1 Column*

UNO Replacement Columns

UNO replacement columns provide major cost savings over the purchase of a whole new column.

UNO Polishing PEEK Columns

UNO polishing columns can serve as late-stage purification tools or be used for micropreparative separations to obtain the highest resolution and recovery from small sample loads.

- PEEK hardware maintains biomolecule integrity
- Compatible with BioLogic HR System or any medium to high pressure chromatography system
- Minimal dead volume reduces sample dilution

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

Tip #9

UNO Technical Information

Product	*Column Volume*	*Recommended Max Protein Loading (mg)*	*Recommended Flow Rate Range (ml/min)*	*Column Dimension (mm)*	*Max Operating Pressure (psi/MPa/bar)*
UNO 1 COLUMN	1.3	20	0.5–5.0	7 x 35	700/4.5/48
UNO 6 COLUMN	6	90	0.5–8.0	12 x 53	700/4.5/48
UNO 12 COLUMN	12	180	0.5–8.0	15 x 68	700/4.5/48

UNO Polishing PEEK Technical Information

Characteristics	*UNO Q Polishing*	*UNO S Polishing*
COLUMN VOLUME (ml)	0.16	0.16
RECOMMENDED MAX PROTEIN LOADING (mg)	2.0	2.0
RECOMMENDED FLOW RATE RANGE (ml/min)	0.1-1.0	0.1-1.0
COLUMN DIMENSION (mm)	4.6 x 10	4.6 x 10
MAX OPERATING PRESSURE (PSI/MPA/BAR)	200/1.3/14	200/1.3/14

Ordering Information

Catalog #	*Description*
UNO COLUMNS	
720-0001	UNO Q-1 Column
720-0003	UNO Q-6 Column
720-0005	UNO Q-12 Column
720-0021	UNO S-1 Column
720-0023	UNO S-6 Column
720-0025	UNO S-12 Column
750-0555	1/4 x 28 Fittings, 10 Nut/Ferrule-Lock Rings to connect UNO column to BioLogic HR System
750-0568	10-32 Fittings Kit, 2 nuts and 4 ferrules to connect UNO column to HPLC system
750-0567	M6 Fittings, 2 nuts and 4 ferrules to connect UNO Column to FPLC system
UNO REPLACEMENT COLUMNS	
720-0011	UNO Q-1R Column
720-0013	UNO Q-6R Column
720-0015	UNO Q-12R Column
720-0031	UNO S-1R Column
720-0033	UNO S-6R Column
720-0035	UNO S-12R Column
UNO POLISHING PEEK COLUMNS	
720-0009	UNO Q Polishing Column
720-0029	UNO S Polishing Column
750-0555	1/4 x 28 Fittings, 10 Nut/Ferrule-Lock Rings to connect UNO column to BioLogic HR System
750-0568	10-32 Fittings Kit, 2 nuts and 4 ferrules to connect UNO column to HPLC system
750-0567	M6 Fittings, 2 nuts and 4 ferrules to connect UNO Column to FPLC system

Bio-Scale Q, S and DEAE Columns

High Resolution Ion Exchange Columns for Method Development and Reproducible Separations of Proteins, Peptides and Polynucleotides

Bio-Scale columns are packed with 10 µm Macro-Prep beads allowing rapid and reproducible high resolution separations at the analytical to semi-preparative scale using the BioLogic HR System or any medium to high pressure chromatography system.

- Four column sizes (2, 5, 10 and 20 ml) for flexibility in sample loading
- Excellent base stability allows reliable, economical process development and scaled-down modeling experiments
- Methods developed on Bio-Scale columns can be easily transferred to production scale using Macro-Prep ion exchange supports
- Transparent, biocompatible hardware materials for easy column trouble-shooting
- Adjustable bed supports minimize column voids for maximum resolution
- Resin top-off kits are available to extend column life

For more information, request bulletins:

1880 *Bio-Scale Q Column Data Sheet*
1881 *Bio-Scale S Column Data Sheet*
1907 *Prepacked Ion Exchange Columns (Bio-Scale Q & S) for Protein Purification*
1913 *Developing and Scaling Up a Purification Procedure with Macro-Prep Ion Exchange Supports*
1930 *Bio-Scale DEAE Columns*

Bio-Scale Column Technical Information

Product	*Column Volume (ml)*	*Recommended Max Protein Loading (mg)*	*Recommended Flow Rate Range (ml/min)*	*Column Dimension (mm)*	*Max Operating Pressure (psi/bar)*
BIO-SCALE 2	2.0	20	0.5–3.0	7 x 52	1,000/70
BIO-SCALE 5	5.0	50	0.5–5.0	10 x 64	750/50
BIO-SCALE 10	10.0	100	0.5–7.0	12 x 88	600/40
BIO-SCALE 20	20.0	200	0.5-10.0	15 x 113	500/34

Characteristics	*Macro-Prep 10 Q*	*Macro-Prep 10 S*	*Macro-Prep 10 DEAE*
TYPE OF EXCHANGER	Strong anion	Strong cation	Weak anion
FUNCTIONAL GROUP	$-N^+(CH_3)_3$	$-SO_3^-$	$-N^+(C_2H_5)_2$
IONIC CAPACITY (µMOL/ml)	115±25	127±25	200±50.0
AVERAGE PARTICLE SIZE	10±3.0	10±3.0	10±3.0
DYNAMIC PROTEIN CAPACITY	20 mg BSA/ml	50 mg Human IgG/ml	25 mg BSA/ml
COUNTERION	Cl^-	Na^+	Cl^-

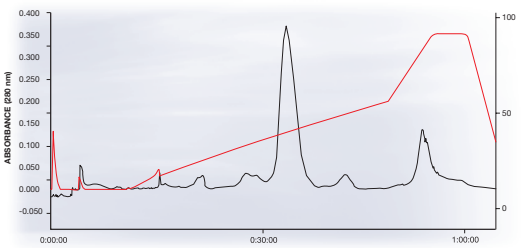


Fig. 5. Recombinant Insulin Polishing on Bio-Scale S2 in presence of acetonitrile. Buffer A = 0.1 N HAc, 40% acetonitrile; Buffer B = buffer A + 0.5 M NaCl; Gradient = 0 - 100% B in 10 column volumes; Flow rate = 2.0 ml/min.

Top-Off Resin Kits

If the top of the resin bed in your Bio-Scale column becomes fouled, and the usual hygiene steps do not restore performance, a few millimeters of the bed can be removed and replaced with fresh resin. Top-Off Resin Kits contain 1 ml of resin, frits and distribution screens for each column diameter.

Careful preparation of the sample and the buffers will maintain your column performance and lifetime. Normally washing with 1.0 M NaCl or KCl will remove most bound components.

Tip #10

Ordering Information

Catalog #	*Description*
BIO-SCALE COLUMNS	
751-0001	Bio-Scale Q2 Column
751-0003	Bio-Scale Q5 Column
751-0005	Bio-Scale Q10 Column
751-0007	Bio-Scale Q20 Column
751-0011	Bio-Scale S2 Column
751-0013	Bio-Scale S5 Column
751-0015	Bio-Scale S10 Column
751-0017	Bio-Scale S20 Column
751-0031	Bio-Scale DEAE2 Column
751-0033	Bio-Scale DEAE5 Column
751-0035	Bio-Scale DEAE10 Column
751-0037	Bio-Scale DEAE20 Column
750-0555	1/4 x 28 Fittings, 10 Nut/Ferrule-Lock Rings to connect Bio-Scale column to BioLogic HR System
750-0568	10-32 Fittings Kit, 2 nuts and 4 ferrules to connect Bio-Scale column to HPLC system
750-0567	M6 Fittings, 2 nuts and 4 ferrules to connect Bio-Scale Column to FPLC system
TOP-OFF RESIN KITS	
751-0009	Top-Off Resin Kit Q, 1 ml
751-0019	Top-Off Resin Kit S, 1 ml
751-0039	Top-Off Resin Kit DEAE, 1 ml

Econo-Pac IEX Cartridges

Prepacked Low Pressure Columns for Methods Scouting or First-Step Purification of Crude Sample

Convenient and affordable, Econo-Pac ion exchange (IEX) cartridges are prepacked low pressure chromatography columns for use with the BioLogic HR and BioLogic LP systems, any other chromatography systems and peristaltic pumps. They are ideal for:

- Methods scouting
- First step purification of crude samples
- Applications using toxic or hazardous samples where frequent column disposal is required
- Available in a 1 ml or 5 ml format
- Up to three cartridges can be connected to triple sample capacity
- Luer-lock fittings for snap-on connection to any low pressure chromatography system or directly to a syringe

For more information, request bulletins:

1826 & 1837 *Enzyme Purification with the Econo-Pac Q Cartridge*
1827 *Plasmid Purification with the Econo-Pac Q Cartridge*

Weak ion exchangers, DEAE and CM, require larger volumes of buffer to equilibrate to a new pH compared to strong ion exchangers, high S and high Q. Therefore, when changing the pH of the buffer for a weak ion exchanger, check pH of column effluent prior to loading the sample. To speed up pH equilibration, run a 10x concentration of the new running buffer/pH until the column effluent is at the proper pH, followed by 2-3 CVs of the running buffer.

Tip #11

Econo-Pac Ion Exchange Cartridge Technical Information

	Econo-Pac High Q	*Econo-Pac High S*	*Econo-Pac CM*
SUPPORT	Macro-Prep High Q	Macro-Prep High S	Macro-Prep CM
FUNCTIONAL GROUP	$-N^+(CH_3)_3$	$-SO_3^-$	$-COO^-$
PROTEIN CAPACITY	1 ml cartridge: ≥ 40 mg BSA 5 ml cartridge: ≥ 170 mg BSA	1 ml cartridge: 55 mg HSA 5 ml cartridge: 230 mg HSA	1 ml cartridge: 25 mg hemoglobin 5 ml cartridge: 125 mg hemoglobin
AVERAGE PARTICLE SIZE	50 µm (all)		
RECOMMENDED FLOW RATE (ml/min)	1 ml cartridge: 0.5-1.0 (all) 5 ml cartridge: 0.5-3.0 (all)		
MAX OPERATING PRESSURE	3.4 bar (50 psi) at 20 °C (all)		
OPERATING pH RANGE	2-12 (all)		
RECOMMENDED STORAGE CONDITIONS	50 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 0.05% NaN_3 (all)		

Ordering Information

Catalog #	*Description*	*Quantity*
ECONO-PAC ION EXCHANGE CARTRIDGES		
732-0026	Econo-Pac High Q	1 x 5 ml
732-0027	Econo-Pac High Q	5 x 5 ml
732-0028	Econo-Pac High Q	5 x 1 ml
732-0066	Econo-Pac High S	1 x 5 ml
732-0067	Econo-Pac High S	5 x 5 ml
732-0068	Econo-Pac High S	5 x 1 ml
732-0001	Econo-Pac CM	1 x 5 ml
732-0005	Econo-Pac CM	5 x 5 ml
732-0003	Econo-Pac CM	5 x 1 ml

Macro-Prep 25 S and 25 Q Media

For Benchtop to Pilot Scale Separations Where Maximum Sample Recovery and Purity are Important

Macro-Prep 25 S strong cation exchange supports and Macro-Prep 25 Q strong anion exchange supports offer high resolution separations at high flow rates with medium pressures.

- Both possess the same rigid, macroporous and hydrophilic properties of the 50 µm Macro-Prep high Q and high S supports, but in a higher resolving 25 µm bead

At the pilot and process scale, the high resolution of Macro-Prep 25 ion exchange beads:

- Reduce the number of chromatographic steps required
- Remove protein variants and other difficult contaminants which would require additional validation if present in the final formulation

For more information, request bulletins:

1913 *Developing and Scaling Up a Purification Procedure with Macro-Prep Ion Exchange Supports*
1931 *Macro-Prep Chromatography Supports*

Endotoxin levels can be reduced by running the protein-containing solution through a Macro-Prep high Q column under pH and salt conditions which do not bind the protein of interest.

Tip #12

Macro-Prep 25 S and 25 Q Media Technical Information

Support	*Macro-Prep 25 Q*	*Macro-Prep 25 S*
TYPE OF ION EXCHANGER	Strong anion	Strong cation
FUNCTIONAL GROUP	$-N^+(CH_3)_3$	$-COO^-$
IONIC CAPACITY (µeq/ml)	220±40	110±30
TYPICAL BINDING CAPACITY	> 30 mg/ml BSA	> 40 mg/ml IgG
SHIPPING COUNTERION	Cl^-	Na^+
NOMINAL PARTICLE SIZE	25 µm	25 µm
NOMINAL PORE SIZE	725 Å	725 Å
MAX LINEAR FLOW RATE	3,000 cm/hour	3,000 cm/hour
CHEMICAL STABILITY		
1.0 M HCl	> 48 hr	> 72 hr
1.0 M NaOH (20 °C)	< 24 hr	Excellent
VOLUME CHANGES		
pH 4–10	< 1%	< 1%
0.1–1.0 M NaCl	< 5%	< 4%
AUTOCLAVABLE (121 °C, 30 min)	Yes	Yes
pH STABILITY	1–14	1–14
ANTIMICROBIAL AGENT	20% ethanol	20% ethanol

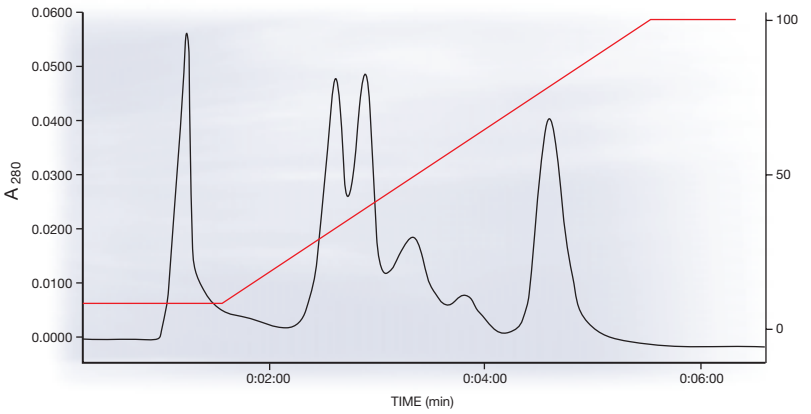


Fig. 6. Ammonium sulfate fraction of Hevea *(Rubber Plant) Cytosol on MP-25 Q (11 x 21 cm). Buffer A = 50 mM Tris, pH 8.3; Buffer B = 50 mM Tris + 1 M NaCl, pH 8.3; Flow rate = 5 ml/min.*

Ordering Information

Catalog #	*Description*
MACRO-PREP 25 Q	
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 liter
153-0024	Macro-Prep 25 Q, 5 liters
MACRO-PREP 25 S	
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 liter
153-0034	Macro-Prep 25 S, 5 liters

Macro-Prep High Q, High S, DEAE and CM Media

For Benchtop, Pilot and Process Scale Preparative Applications

The rigid, macroporous, hydrophilic properties of these supports provide exceptional capacity, resolution and throughput. The ability of Macro-Prep supports to withstand rigorous cleaning and sanitization techniques makes these supports ideal for biopharmaceutical production.

- High capacity for macromolecules
- High resolution of complex biological mixtures
- High flow rate at moderate pressures
- Low nonspecific binding
- High access to the ionic sites
- The beads have high chemical, mechanical and thermal stability, and can be cleaned with chaotropes, sanitized with NaOH and sterilized by autoclaving

Macro-Prep supports are available in a variety of package sizes and in convenient prepacked Econo-Pac cartridges (see page 22). For more information on Macro-Prep supports, request data sheet 1840 for the different derivatives and bulletin 1931.

Macro-Prep 50 Ion Exchange Media Technical Information

Support	*DEAE*	*High Q*	*CM*	*High S*
TYPE OF ION EXCHANGER	Weak anion	Strong anion	Weak cation	Strong cation
FUNCTIONAL GROUP	$-N^+(C_2H_5)_2$	$-N^+(CH_3)_3$	$-SO_3^-$	$-COO^-$
IONIC CAPACITY (µeq/ml)	175±75	400±75	160±40	210±40
TYPICAL BINDING CAPACITY	35 mg/ml of BSA	40 mg/ml of BSA	35 mg/ml of hemoglobin	70 mg/ml of human IgG
SHIPPING COUNTERION	Cl^-	Cl^-	Na^+	Na^+
NOMINAL PARTICLE SIZE	50 µm	50 µm	50 µm	50 µm
NOMINAL PORE SIZE	1,000 Å	1,000 Å	1,000 Å	1,000 Å
MAX LINEAR FLOW RATE	3,000 cm/hr	3,000 cm/hr	3,000 cm/hr	3,000 cm/hr
CHEMICAL STABILITY				
1.0 M HCl	> 72 hr	> 48 hr	> 72 hr	> 72 hr
1.0 M NaOH (20 °C)	Excellent	< 24 hr	Excellent	Excellent
VOLUME CHANGES				
pH 4–10	< 1%	< 1%	< 3%	< 1%
0.1–1.0 M NaCl	< 5%	< 5%	< 9%	< 4%
AUTOCLAVABLE (121 °C, 30 min)	Yes	Yes	Yes	Yes
pH STABILITY	1–14	1–14	1–14	1–14
ANTIMICROBIAL AGENT	20% ethanol	20% ethanol	20% ethanol	20% ethanol

For more information, request bulletins:

1913 *Developing and Scaling Up a Purification Procedure with Macro-Prep Ion Exchange Supports*
1917 *Macro-Prep Ion Exchange Purification of a Human Monoclonal IgM Antibody*
1931 *Macro-Prep Macro-Prep Chromatography Supports*
1942 *Macro-Prep Ceramic Hydroxyapatite*
2204 *Effective Removal of Negatively Charged Interfering Molecules from Proteins*
1840 A-100 *Macro-Prep High Q Support*
1840 A-200 *Macro-Prep High S Support*
1840 A-300 *Macro-Prep CM Support*
1840 A-400 *Macro-Prep DEAE Support*

Ordering Information

Catalog #	*Description*
MACRO-PREP DEAE SUPPORTS	
158-0020	Macro-Prep DEAE, 25 ml
156-0020	Macro-Prep DEAE, 100 ml
156-0021	Macro-Prep DEAE, 500 ml
156-0022	Macro-Prep DEAE, 5 liters
156-0023	Macro-Prep DEAE, 10 liters
MACRO-PREP HIGH Q SUPPORTS	
158-0040	Macro-Prep High Q, 25 ml
156-0040	Macro-Prep High Q, 100 ml
156-0041	Macro-Prep High Q, 500 ml
156-0042	Macro-Prep High Q, 5 liters
156-0043	Macro-Prep High Q, 10 liters
MACRO-PREP HIGH S SUPPORTS	
158-0030	Macro-Prep High S, 25 ml
156-0030	Macro-Prep High S, 100 ml
156-0031	Macro-Prep High S, 500 ml
156-0032	Macro-Prep High S, 5 liters
156-0033	Macro-Prep High S, 10 liters
MACRO-PREP CM SUPPORTS	
158-0070	Macro-Prep CM, 25 ml
156-0070	Macro-Prep CM, 100 ml
156-0071	Macro-Prep CM, 500 ml
156-0072	Macro-Prep CM, 5 liters
156-0073	Macro-Prep CM, 10 liters

Ion Exchange Chromatography Standards

Protein Mixtures for Easy Ion Exchange Chromatography Column Evaluation

The protein standard for anion exchange chromatography is a mixture of four proteins with isoelectric points ranging from 4.5 to 6.9. The protein standard for cation exchange chromatography is a mixture of three proteins with isoelectric points ranging from 6.9 to 10.7. Each standard is supplied as a set of six vials of a lyophilized mixture of proteins.

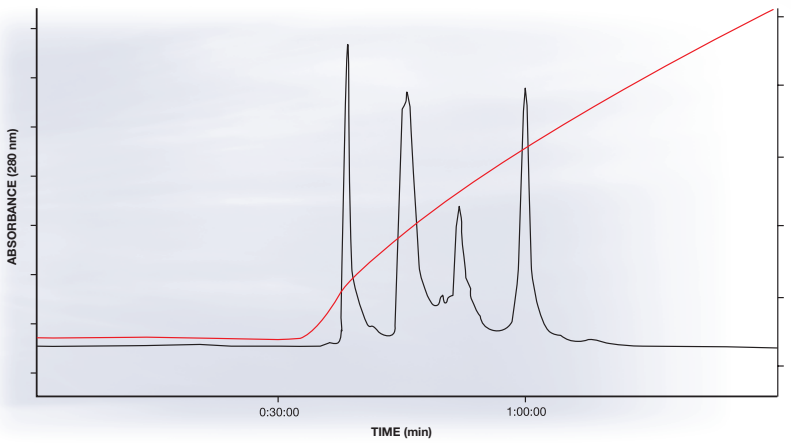


Fig. 7. Anion Exchange Standard on UNO Q1 column. Buffer A = 20 mM Bis-Tris propane, pH 8.3; Buffer B = A Tris + 1 nM NaCl; Gradient: 0-50% B in 20 ml; Flow rate = 2 ml/min.

Ordering Information

Catalog #	*Description*	*Contents*	*Molecular Weight*	*Isoelectric Point*	*For Use With*
ION EXCHANGE STANDARDS					
125-0561	Protein Standard for Anion Exchange Chromatography, 6 vials	Equine myoglobulin, 1.0 mg Conalbumin, 2.5 mg Chicken Ovalbumin, 3.0 mg Soybean trypsin inhibitor, 2.5 mg	17,000 77,000 45,000 17,500	6.9 4.9 4.6 4.5	UNO Q, Bio-Scale Q, DEAE and Econo-Pac High Q columns; Macro-Prep 25 Q, Macro-Prep High Q and DEAE supports
125-0562	Protein Standard for Cation Exchange Chromatography, 6 vials	Equine myoglobin, 1.0 mg Ribonuclease A, 4.0 mg Cytochrome C, 1.0 mg	17,000 13,500 12,000	6.9 8.7 10.7	UNO S, Bio-Scale S and Econo-Pac High S, CM columns; Macro-Prep 25 S, Macro-Prep High S, CM supports

SIZE Exclusion Chromatography

over **40 Years**
OF INNOVATION

Size Exclusion Chromatography

Mechanism of Action

Size exclusion chromatography (SEC) separates molecules based on their size. The gel media consists of spherical beads containing pores of a specific size distribution. Separation occurs when molecules of different sizes are included or excluded from the pores within the matrix. Small molecules diffuse into the pores, retarding their flow through the column, while large molecules do not enter the pores and are eluted in the column's void volume. Consequently, molecules passing through the column separate based on their size and elute in order of decreasing molecular weight.

Operating conditions and gel selection depend on the application and the desired resolution. Two methods used in SEC are group separation, including desalting and buffer exchange, and fractionation. In desalting, the molecule of interest is eluted in the void volume, while smaller molecules are retained in the gel pores. To obtain the desired separation, the gel should have an exclusion limit smaller than the molecule of interest. In fractionation, molecules of varying molecular weights are separated within the gel matrix. With this method, the molecules of interest should fall within the fractionation range of the gel.

Typical SEC Applications

- Fractionation and molecular weight determination of proteins
- Nucleic acid separations
- Removal of unincorporated nucleotides
- Polysaccharide fractionation

Resolution depends on the particle size, pore size, flow rate, column length and sample volume. Generally, the highest resolution is obtained with low flow rates (2–10 cm/hr), long narrow columns, small particle size gels, small sample volumes (1–5% of the total bed volume), a 2-fold difference in molecular weight of larger molecules and a sample viscosity that is the same as the eluant. For desalting, the sample volume can be as much as 30–40% of the total bed volume, and shorter, wider columns may be used.

Size Exclusion Product Selection Guide

Product	Purification Stage	Resolution	Linear Separation Range (kDa)	Package Size Range	Required Equipment
BIO-SIL/BIO-SILECT	**ANALYTICAL**	**EXCELLENT**	**5-1,000**	**PREPACKED 16 ml**	**MEDIUM TO HIGH PRESSURE SYSTEM**
BIO-PREP SE	**POLISHING**	**VERY HIGH**	**1-1,000**	**PREPACKED 14 ml**	**MEDIUM TO HIGH PRESSURE SYSTEM**
ECONO-PAC P6 CARTRIDGE	**DESALTING**	**GOOD**	**1-6**	**PREPACKED 5 ml**	**SYRINGE, PERISTALTIC PUMP**
ECONO-PAC 10DG COLUMN	**DESALTING**	**LOW**	**1-6**	**PREPACKED 10 ml**	**GRAVITY FLOW, PERISTALTIC PUMP**
BIO-SPIN 6	**DESALTING**	**LOW**	**EXCLUSION LIMIT 6 kDa; 98% RETENTION OF dNTPs**	**PREPACKED 1.1 ml**	**CENTRIFUGE**
MICRO BIO-SPIN 6	**DESALTING**	**LOW**	**SEE ABOVE**	**PREPACKED 0.70 ml**	**MICRO-CENTRIFUGE**
BIO-SPIN 30	**DESALTING**	**LOW**	**EXCLUSION LIMIT 40 kDa; 98% RETENTION OF dNTPs**	**PREPACKED 1.1 ml**	**CENTRIFUGE**
MICRO BIO-SPIN 30	**DESALTING**	**LOW**	**SEE ABOVE**	**PREPACKED 0.70 ml**	**MICRO-CENTRIFUGE**
MACRO-PREP SE 40	**INTERMEDIATE TO POLISHING**	**HIGH**	**1-1,000**	**25 ml TO PROCESS**	**ECONO-COLUMN**
BIO-GEL A	**INTERMEDIATE**	**GOOD**	**0.1-100**	**500 ml TO PROCESS**	**ECONO-COLUMN**
BIO-GEL P	**INTERMEDIATE**	**GOOD**	**10-50,000**	**100 g TO PROCESS**	**ECONO-COLUMN**

Bio-Sil and Bio-Silect SEC Columns

Achieve Reproducible High Resolution Separation of Peptides, Proteins and Nucleic Acid in the pH Range of 2–8

Bio-Sil and Bio-Silect analytical SEC columns are prepacked with a proprietary Bio-Sil SEC 5 µm silica support, making them ideal for sample component separation, desalting or buffer exchange, and molecular weight or molecular constant estimation.

- Unequaled reproducibility
- High degree of column-to-column and batch-to-batch consistency and reliability
- Choice of stainless steel or biocompatible PEEK plastic hardware
- Three molecular weight range-specific guard columns for maximum column protection

Reproducibility

Bio-Sil and Bio-Silect SEC column reliability is shown in a 7-year study on batch-to-batch variability where 110 SEC column production batches were compared (Figure 1). Shown below are the levels of reproducibility between individual columns from the same batch (Figure 2) and different batches (Figure 3).

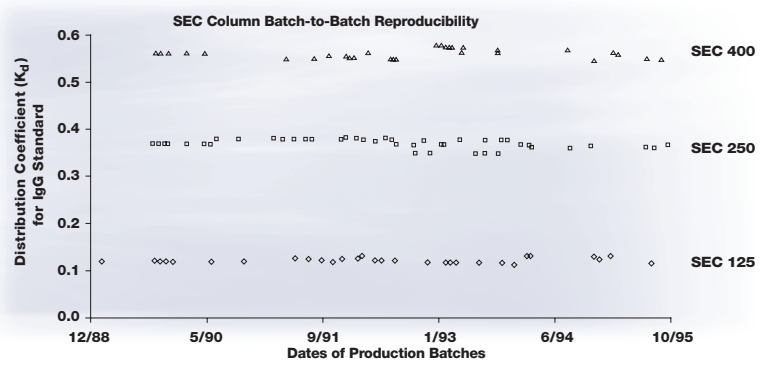


Fig. 1. SEC column batch-to-batch reproducibility. One hundred and ten SEC column production batches were used to compare batch-to-batch variability using Kd values for IgG, a common protein standard for SEC columns. Standard deviation for the SEC 125-5 was ±0.005; SEC 250-5 was ±0.010; SEC 400-5 was ±0.01.

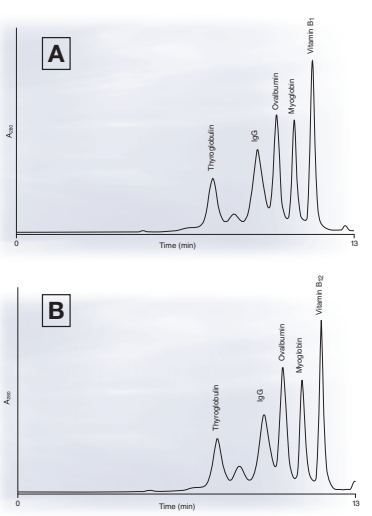


Fig. 2. Column-to-column reproducibility. Size exclusion separation of standard proteins on two different Bio-Silect SEC 400-5 columns from the same production batch.[1]

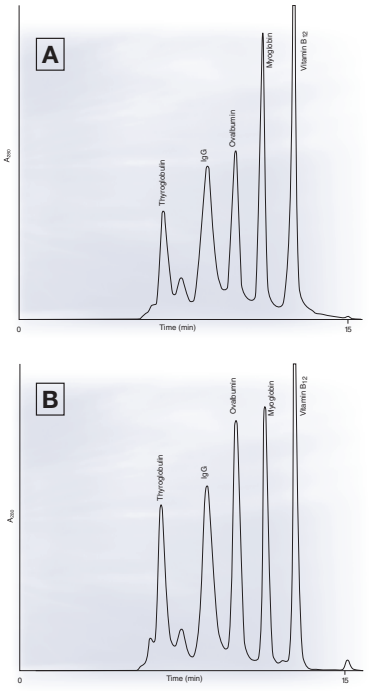


Fig. 3. Batch-to-batch reproducibility. Size exclusion separation of standard proteins on the Bio-Sil SEC 250-5 column representing different production batches from 1989 (A) and 1991 (B).[1]

Size Exclusion Chromatography

Exceptional Endurance

Both Bio-Sil and Bio-Silect columns maintain their performance over an extended period. Figure 4 demonstrates column endurance where protein standards were injected periodically onto a Bio-Silect 125-5 for over 280 hours of continuous running with negligible shift in retention (<5% variation).

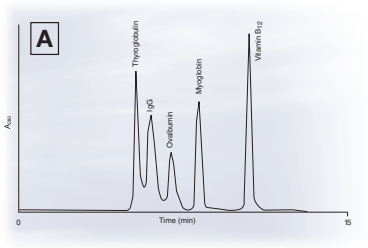


Fig. 4. Size exclusion separation of standard proteins on the Bio-Silect SEC 125-5 column at time 0 (A) and time 283 hours (B).[1]

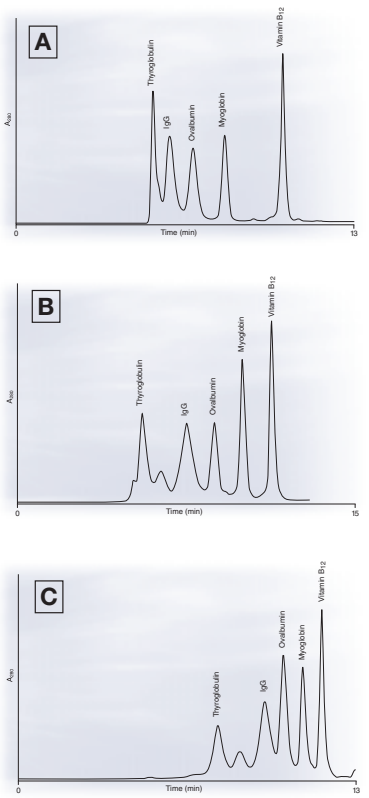


Fig. 5. Chromatograms showing the separation of standard proteins using the Bio-Silect SEC 125-5 (A), Bio-Silect SEC 250-5 (B) and Bio-Silect 400-5 (C) columns.[1]

Integrity

Bio-Sil and Bio-Silect columns are guaranteed for thermal stability to 70 °C and are stable in organic solvents typically used in HPLC, including methanol, ethanol, acetonitrile, DMSO and THF. Specific concentration limits for additional buffers and additives are as follow:

Buffer or Additive	Concentration
PHOSPHATE BUFFERS	10-500 mM
HEPES	10-500 mM
TRIS	10-500 mM
ACETATE BUFFERS	10-500 mM
UREA	6 M
GUANIDINE/HCl	6 M
SDS	1 %
AMMONIUM SULFATE	1 M

For more information, request bulletin:

1737 Bio-Compatible SEC Columns

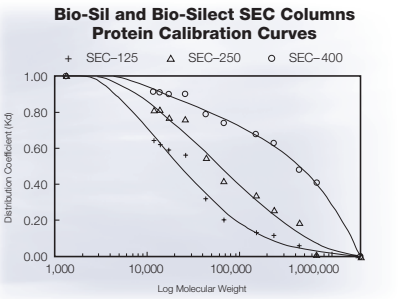


Fig. 6. Protein calibration curves for the SEC 125-5, 250, and 400-5 columns. Data points were obtained from Table 2.[1,2]

1. All chromatograms were run using the Bio-Rad Gel Filtration Standard and the following conditions: 100 mM sodium phosphate, pH 6.8, containing 150 mM sodium chloride at a flow rate of 1.0 ml/min.

2. Distribution coefficients were calculated from the following formula:

$$K_d = \frac{(t_r - t_o)}{(t_i - t_o)}$$

Where K_d is the distribution coefficient; t_r is the retention time; t_o is the void volume time (thyroglobulin); t_i is the inclusion volume time (vitamin B_{12}).

Size Exclusion Chromatography

Table 1. Protein Selectivity Data

Sample	MW	SEC 125-5		SEC 250-5		SEC 400-5	
		R_t (min)	K_d	R_t (min)	K_d	R_t (min)	K_d
VITAMIN B_{12}	1,350	10.29	1.00	11.27	1.00	11.37	1.00
CYTOCHROME C	11,700	8.55	0.64	10.25	0.81	10.83	0.91
RIBONUCLEASE A	13,700	8.45	0.62	10.21	0.81	10.79	0.91
MYOGLOBIN	17,000	8.27	0.59	10.02	0.77	10.75	0.90
CHYMOTRYPSINOGEN	25,700	8.14	0.56	9.94	0.76	10.71	0.90
OVALBUMIN	43,000	6.95	0.32	8.82	0.55	10.05	0.79
BSA	67,000	6.36	0.20	8.12	0.42	9.71	0.74
IgG	150,000	6.05	0.13	7.68	0.34	9.37	0.68
CATALASE	232,000	6.00	0.12	7.21	0.26	9.02	0.63
FERRITIN	440,000	5.68	0.60	6.85	0.19	8.10	0.48
THYROGLOBULIN	670,000	5.46	0.10	5.86	0.10	7.63	0.41
BLUE DEXTRAN	2,000,000	5.40	0.00	5.82	0.00	5.08	0.00

Technical Information

COLUMN DIMENSION	300 x 7.8 mm
PARTICLE SIZE	5 µm
BED VOLUME	14 ml
MAX LOADING CAPACITY	1.5 mg
RECOMMENDED FLOW RATE	1.0 ml/min
MAX FLOW RATE	1.5 ml/min
OPERATING PRESSURE	≤ 1,000 psi (10 bar)
MAX PRESSURE	1,500 psi (100 bar)
pH RANGE	2-8
EFFICIENCY	>17,000

Table 2. Separation Ranges for Native Proteins

Column	MW at Kd (0.5) (kDa)	MW Separation Range (kDa)
SEC 125-5	20	7-100
SEC 250-5	70	15-300
SEC 400-5	400	40-1,000

Ordering Information

Catalog #	Description	Dimensions (mm)	Molecular Weight Range (proteins)*
STAINLESS STEEL SEC COLUMNS			
125-0060	Bio-Sil SEC 125-5	300 x 7.8	5,000 - 100,000 Da
125-0062	Bio-Sil SEC 250-5	300 x 7.8	10,000 - 300,000 Da
125-0064	Bio-Sil SEC 400-5	300 x 7.8	20,000 - 1,000,000 Da
125-0072	Bio-Sil SEC 125 Guard	80 x 7.8	N/A
125-0073	Bio-Sil SEC 250 Guard	80 x 7.8	N/A
125-0074	Bio-Sil SEC 400 Guard	80 x 7.8	N/A
PEEK BIOCOMPATIBLE SEC COLUMNS			
125-0475	Bio-Silect SEC 125-5	300 x 7.8	5,000 - 100,000 Da
125-0476	Bio-Silect SEC 250-5	300 x 7.8	10,000 - 300,000 Da
125-0477	Bio-Silect SEC 400-5	300 x 7.8	20,000 - 1,000,000 Da
125-0478	Bio-Silect 125 Guard	50 x 7.8	N/A
125-0479	Bio-Silect 250 Guard	50 x 7.8	N/A
125-0480	Bio-Silect 400 Guard	50 x 7.8	N/A

*MW range values for proteins in normal saline.

Bio-Prep SE Columns

Get Fast, Reproducible Separation of Peptides, Proteins and Nucleic Acids

Bio-Prep SE 100/17 and SE 1000/17 glass columns are prepacked with Macro-Prep SE 17 µm spherical, crosslinked agarose beads.

- Ideal for use with the BioLogic HR system, or any other medium to high pressure chromatography system
- Superior separations at elevated flow rates
- Two fractionation ranges: 5,000 - 100,000 Da using Bio-Prep SE 100/17 column; 10,000 - 1,000,000 Da using Bio-Prep SE 1000/17 column
- Easy, efficient scale-up of applications developed on the Bio-Prep SE columns to production scale using Macro-Prep SE 40 (see pages 34 and 35)

When calibrating an SE column, always use the same mobile phase and sample solvent systems with the calibration standard as with the sample to be analyzed to ensure reliable, quantitative results.

Tip #13

Technical Information

	Bio-Prep SE 100/17	*Bio-Prep SE 1000/17*
LINEAR SEPARATION RANGE	5,000-100,000 Da	10,000-1,000,000 Da
EXCLUSION LIMIT	~ 200,000 Da	~ 1,500,000 Da
COLUMN DIMENSION	300 x 8 mm	
AVERAGE PARTICLE SIZE	17 µm	
BED VOLUME	15 ml	
MAX RECOMMENDED LOADING CAPACITY	0.3 ml	
RECOMMENDED FLOW RATES	0.1-1.0 ml/min	
MAX FLOW RATE	2.0 ml/min	
MAX OPERATING PRESSURE	580 psi (4 MPa, 40 bar)	
WORKING pH RANGE	3-12 long term, 1-14 short term	
OPERATING TEMPERATURES	4-40 °C	
OPERATING PRESSURE	<500 psi	

Ordering Information

Catalog #	*Description*
BIO-PREP SE COLUMNS	
732-1501	Bio-Prep SE 100/17
732-1502	Bio-Prep SE 1000/17

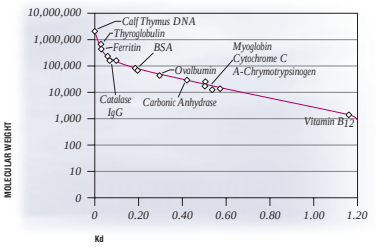


Fig. 7. Calibration curve for K_d Bio-Prep SE 100/17 gel.

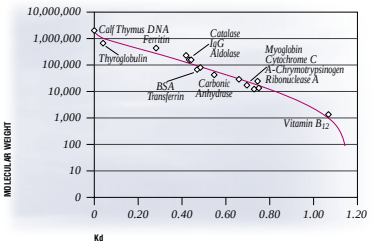


Fig. 9. Calibration curve for K_d Bio-Prep SE 1000/17 column.

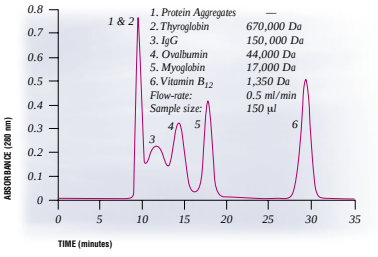


Fig. 8. Separation of a gel filtration standard using the SE 100/17 column.

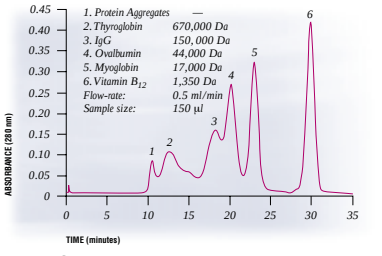


Fig. 10. Separation of a gel filtration standard using the Bio-Prep SE 1000/17 column.

Econo-Pac P6 Desalting Cartridge

Ready-to-Use Desalting Cartridge Provides Rapid and Convenient Desalting or Buffer Exchange of Biomolecules

Use these prepacked chromatography columns with a syringe, a peristaltic pump, the BioLogic System or any other chromatography system.

Technical Information

Econo-Pac P6 Desalting Cartridge	
SAMPLE VOLUME	100 µl to 3.0 ml
RECOMMENDED FLOW RATE	0.5-1.0 ml/min
MAX OPERATING PRESSURE	3.4 bar (50 psi) at 20 °C
OPERATING pH RANGE	2-10
RECOMMENDED STORAGE CONDITIONS	50 mM phosphate, pH 7.0, with 0.05% NaN_3

Econo-Pac 10DG Desalting Column

For Fast and Easy Gravity Flow, Desalting and Buffer Exchange Applications

Econo-Pac 10DG columns allow simplified desalting and buffer exchange. Easy-to-use and inexpensive, Econo-Pac prepacked columns include an upper frit, a snap-off end-tip, graduated column markings and a 30 ml total column volume.

Technical Information

Econo-Pac 10DG Column	
EXCLUSION LIMIT	6,000 Da
BED VOLUME	10 ml
TOTAL COLUMN VOLUME	30 ml
SAMPLE CAPACITY	0.5 to 3 ml

Ordering Information

Catalog #	*Description*	*Quantity*
ECONO-PAC P6 COLUMNS		
732-0011	Econo-Pac P6 Desalting cartridge	1 x 5 ml
732-0015	Econo-Pac P6 Desalting cartridge	5 x 5 ml
ECONO-PAC 10DG COLUMN		
732-2010	Econo-Pac 10DG column	30

For more information, request bulletins:

2068 *Desalting with Bio-Gel P-6DG Desalting Gel*

10DG *A Rapid and Inexpensive Procedure for Desalting Synthetic Oligonucleotides*

Always centrifuge or filter your sample with a 0.2-0.45 µm filter to remove particulates.

Tip #14

For optimal resolution, choose the smallest particle size compatible with your chromatography system.

Tip #15

Bio-Spin and Micro Bio-Spin Columns

Prepacked Columns for Rapid, Effective Clean Up of Protein and Nucleic Acid Samples

Bio-Spin and Micro Bio-Spin columns are ideal for quick and effective sample clean up and removal of salts and small molecules from purified samples—even unincorporated dye terminators from DNA sequencing reactions. The prepacked columns are packed with specially sized Bio-Gel P polyacrylamide SEC gels.

For optimal removal of unincorporated nucleotides from labeling reactions (e.g., random primer, nick translation, end labeling, fluorescent sequencing, dye terminators), the Micro Bio-Spin P-30 column is recommended.

Tip #16

When loading sample onto a Micro Bio-Spin column, it is important to apply sample directly to the center of the column, without disturbing the gel bed so as to not affect column performance.

Tip #17

The P-6 Bio-Spin and Micro Bio-Spin columns are recommended for buffer exchange, and removal of salts from protein and nucleic acid samples.

Tip #18

Technical Information

	Bio-Spin 6	*Micro Bio-Spin 6*	*Bio-Spin 30*	*Micro Bio-Spin 30*
PACKED SUPPORT	Special Grade Bio-Gel P-6 gel	Special Grade Bio-Gel P-6 gel	Special Grade Bio-Gel P-30 gel	Special Grade Bio-Gel P-30 gel
APPLICATION	Desalting and buffer exchange	Desalting and buffer exchange	Nucleotide and small molecule removal and desalting	Nucleotide and small molecule removal and desalting
BED VOLUME	1.1 ml	0.7 ml	1.1 ml	0.7 ml
EXCLUSION LIMIT	6,000 daltons; 90% recovery of 20 bases/bp, 99% retention of salts	6,000 daltons; 90% recovery of 20 bases/bp, 99% retention of salts	40,000 daltons; 95% recovery of 22 bases/bp, 99% retention of dNTPs	40,000 daltons; 95% recovery of 22 bases/bp, 99% retention of dNTPs
SAMPLE VOLUME	50-100 µl	10-75 µl	50-100 µl	10-75 µl

- Remove dye terminators from automated sequencing reactions
- Remove unincorporated nucleotides from labeling reactions
- Protein, peptide and nucleic acid desalting or buffer exchange
- Available in SSC pH 7.0 and 10 mM Tris, pH 7.4 buffers

The Micro Bio-Spin P-30 column is also available in an RNase-free form for convenient riboprobe cleanup.

Ordering Information

Catalog #	*Description*	*Quantity*
BIO-SPIN COLUMNS		
732-6002	Bio-Spin Columns with Bio-Gel P-6, in SSC buffer	25
732-6006	Bio-Spin Columns with Bio-Gel P-30, in SSC buffer	25
MICRO BIO-SPIN COLUMNS		
732-6221	Micro Bio-Spin Columns with Bio-Gel P-6 in Tris buffer	25
732-6222	Micro Bio-Spin Columns with Bio-Gel P-6 in Tris buffer	100
732-6223	Micro Bio-Spin Columns with Bio-Gel P-30 in Tris buffer	25
732-6224	Micro Bio-Spin Columns with Bio-Gel P-30 in Tris buffer	100
732-6250	Micro Bio-Spin Columns with Bio-Gel P-30 in Tris buffer, RNase-free	25
732-6251	Micro Bio-Spin Columns with Bio-Gel P-30 in Tris buffer, RNase-free	100
732-6200	Micro Bio-Spin Columns with Bio-Gel P-6 in SSC buffer	25
732-6201	Micro Bio-Spin Columns with Bio-Gel P-6 in SSC buffer	100
732-6202	Micro Bio-Spin Columns with Bio-Gel P-30 in SSC buffer	25
732-6203	Micro Bio-Spin Columns with Bio-Gel P-30 in SSC buffer	100

Size Exclusion Chromatography

Macro-Prep SE Gel

For Fractionation/Polishing of Biomolecules or for Desalting/ Buffer Exchange at the Pilot to Process Scale

Macro-Prep SE is a size exclusion gel based on biocompatible 40 µm spherical, crosslinked agarose beads. The beads are produced from highly purified agarose using a proprietary crosslinking process which produces a mechanically and chemically stable material, available in bulk quantities.

- Two fractionation ranges: 5,000-10,000 Da using the Macro-Prep SE 40/100 media; 10,000-1,000,000 Da using Macro-Prep SE 1000/40 media
- High resolution separations
- High mechanical and chemical stability
- Biocompatible crosslinked agarose
- Operational flow rates from 12-240 cm/hr
- Easy and predictable scale-up from the Bio-Prep SE columns

Macro-Prep SE is recommended for applications ranging from the separation of aggregates (dimers, trimers, *etc.*) from purified antibody solutions to the separation of complex mixtures of biomolecules directly from cell culture feedstreams.

For more information, request bulletin:

2190 *Macro-Prep SE 100/40 and 1000/40 Size Exclusion Chromatography Gels*

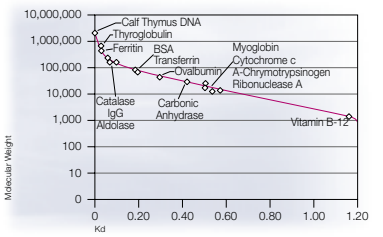


Fig. 11. Calibration curve for K_d Macro-Prep SE 100/40 gel.

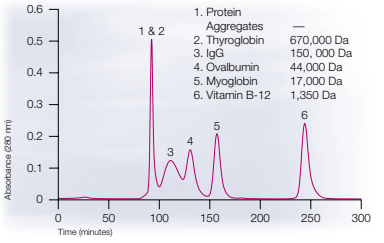


Fig. 12. Separation of a gel filtration standard using the SE 100/40 material packed in a 2.5 x 50 cm column.

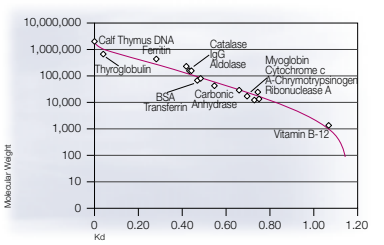


Fig. 13. Calibration curve for K_d Macro-Prep SE 1000/40 gel.

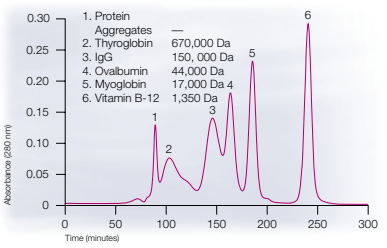


Fig. 14. Separation of a gel filtration standard using the SE 1000/40 material packed in a 2.5 x 50 cm column.

Scaling-up with the Macro-Prep SE Family

Macro-Prep SE media are available in both 17 µm (Bio-Prep SE columns, see page 31) and 40 µm particle sizes. This provides easy, efficient scale-up of applications ranging from lab scale to process scale.

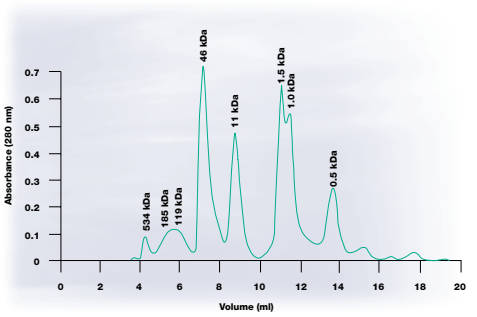


Fig. 15. Separation of milk proteins on Bio-Prep SE 100/17, 8 x 300 mm column. Sample: 0.35 mg goat milk (pH 4 supernatant), Buffer: 20 mM sodium phosphate, 0.15 M NaCl, pH 6.8. Flow rate: 0.15 ml/min.

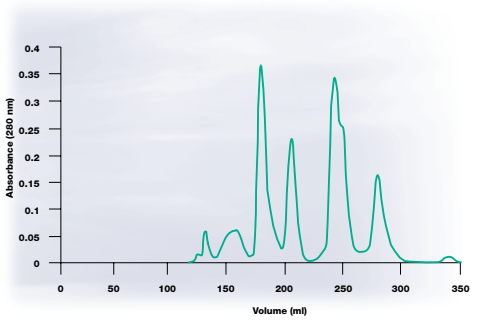


Fig. 16. Separation of milk proteins on Macro-Prep SE 100/40, 1.5 x 163 cm column. Column: Macro-Prep SE 100/40, 1.5 x 163 cm, Sample: 5.0 mg goat milk (pH 4 supernatant), Buffer: 20 mM sodium phosphate, 0.15 M NaCl, pH 6.8. Flow rate: 0.5 ml/min.

Technical Information

	Macro-Prep SE 100/40	Macro-Prep SE 1000/40
BASE MATRIX	Crosslinked agarose	Crosslinked agarose
PARTICLE SIZE (MEAN)	40±4 µm	40±4 µm
LINEAR SEPARATION RANGE	5,000–100,000 Da	10,000–1,000,000 Da
EXCLUSION LIMIT	~ 200,000 Da	~ 1,500,000 Da
OPERATING pH RANGE	1–14	1–14
OPERATING TEMPERATURES	4–40 °C, not autoclavable	4–40 °C, not autoclavable
MAXIMUM OPERATING PRESSURE	40 bar (580 psi)	30 bar (435 psi)
MAXIMUM RECOMMENDED SAMPLE VOLUME	Not to exceed 5% of the bed volume	Not to exceed 5% of the bed volume

Ordering Information

Catalog #	*Description*	*Quantity*
MACRO-PREP SE MEDIA		
160-0001	Macro-Prep SE 100/40	50 ml
160-0002	Macro-Prep SE 100/40	300 ml
160-0003	Macro-Prep SE 100/40	1 liter
160-0010	Macro-Prep SE 1000/40	50 ml
160-0011	Macro-Prep SE 1000/40	300 ml
160-0012	Macro-Prep SE 1000/40	1 liter

Bio-Gel P Gels

Ideal for High Resolution Gel Filtration

- Bio-Gel P-2 and P-6DG gels are ideal for rapid carbohydrate, peptide and protein desalting
- Bio-Gel P-4 gels are ideal for carbohydrate and peptide separation, and protein desalting
- Bio-Gel P-6, P-10, P-30, P-60 and P-100 gels are recommended for protein and polypeptide purification

Bio-Gel P polyacrylamide gels are prepared by polymerization of acrylamide and N,N'-methylene-bis-acrylamide to form beads for high resolution gel filtration. The gels are supplied dry and are available in several particle size ranges with exclusion limits from 1,800 to 100,000 Da.

Bio-Gel P Gels:

- Do not support microbial growth or leach carbohydrates
- Are extremely hydrophilic and essentially free of charge

- Are autoclavable at pH 5.5-6.5 and operate at a pH range of 2-10 at room temperature
- Are compatible with dilute organic acids, 8 M urea, chaotropes, detergents and miscible organic solvents. Alcohol, up to 20% v/v, can be used to enhance the solubility of nucleotides, peptides and tannins without altering the exclusion properties of the gels. Formamide may be used at full strength since the gels remain fully swollen in its presence.
- Bio-Gel P-6 is available in prepacked Econo-Pac cartridges for fast and easy desalting applications (see page 32).

Bio-Gel P-6DG Gels:

- Exclusion limit of 6,000 daltons
- Specifically developed for desalting and buffer exchange applications
- Provide rapid results with high sample recovery and minimal sample dilution
- Exhibit little or no interaction between the sample and support
- Bio-Gel P-6DG is also available in prepacked Econo-Pac 10DG desalting columns (see page 32)

For more information, request bulletin:

2068 Desalting with Bio-Gel P-6DG Desalting Gel

Ordering Information

Catalog #	*Description*	*Quantity*
150-4114	Bio-Gel P-2 Gel, fine	100 g
150-4115	Bio-Gel P-2 Gel, fine	500 g
150-4118	Bio-Gel P-2 Gel, extra fine	100 g
150-4120	Bio-Gel P-4 Gel, medium	100 g
150-4124	Bio-Gel P-4 Gel, fine	100 g
150-4128	Bio-Gel P-4 Gel, extra fine	100 g
150-4130	Bio-Gel P-6 Gel, medium	100 g
150-4134	Bio-Gel P-6 Gel, fine	100 g
150-4138	Bio-Gel P-6 Gel, extra fine	100 g
150-0738	Bio-Gel P-6DG Gel	100 g
150-0739	Bio-Gel P-6DG Gel	1 kg
150-4140	Bio-Gel P-10 Gel, medium	100 g
150-4144	Bio-Gel P-10 Gel, fine	100 g
150-4150	Bio-Gel P-30 Gel, medium	100 g
150-4154	Bio-Gel P-30 Gel, fine	100 g
150-4160	Bio-Gel P-60 Gel, medium	100 g
150-4164	Bio-Gel P-60 Gel, fine	100 g
150-4170	Bio-Gel P-100 Gel, medium	100 g
150-4174	Bio-Gel P-100 Gel, fine	100 g

Technical Information

Bio-Gel Product	*Hydrated Bead Size (µm)*	*Hydrated Bed/Volume Dry Gel*	*Typical Flow Rates[1] (cm/hr)*	*Fractionation Range/ Exclusion Limit*	*Exclusion Limit ds DNA*
P-2 GEL, FINE	45-90		5-10	100–1,800	
P-2 GEL, EXTRA FINE	< 45	3 ml/g	< 10	100–1,800	
P-4 GEL, MEDIUM	90-180		15-20	800–4,000	
P-4 GEL, FINE	45-90		10-15	800–4,000	
P-4 GEL, EXTRA FINE	< 45	4 ml/g	< 10	800–4,000	
P-6 GEL, MEDIUM	90-180		15-20	1,000–6,000	
P-6 GEL, FINE	45-90		10-15	1,000–6,000	
P-6 GEL, EXTRA FINE	< 45		< 10	1,000–6,000	
P-6DG GEL	90-180	6.5 ml/g	15-20	1,000–6,000	5–6 bp
P-10 GEL, MEDIUM	90-180		15-20	1,500–20,000	
P-10 GEL, FINE	45-90	7.5 ml/g	10-15	1,500–20,000	
P-30 GEL, MEDIUM	90-180		7-13	2,500–40,000	
P-30 GEL, FINE	45-90	9 ml/g	6-11	2,500–40,000	20–22 bp
P-60 GEL, MEDIUM	90-180		4-6	3,000–60,000	
P-30 GEL, FINE	45-90	11 ml/g	3-5	3,000–60,000	55 bp
P-100 GEL, MEDIUM	90-180		4-6	5,000–100,000	
P-100 GEL, FINE	45-90	12 ml/g	3-5	5,000–100,000	

1. Flow rates determined in a 1.5 x 70 cm column, using a hydrostatic pressure head to bed of 1:1.

Bio-Gel A

Agarose Gels Designed for Purification of Proteins, Peptides and Nucleic Acids, from Lab to Process Scale

The pore size of these gels is controlled by the percentage of agarose, allowing exclusion limits to range from 500 to 50,000 kDa for proteins and to 350 base pairs for nucleic acids.

- Compatible with all commonly used buffers
- Bed volume does not change significantly with high salt buffers
- Gels may be used at pH 4-13 and at temperatures from 2-30 ˚C

Ordering Information

Catalog #	*Description*	*Quantity*
151-0130	Bio-Gel A-0.5m Gel, coarse	500 ml
151-0140	Bio-Gel A-0.5m Gel, medium	500 ml
151-0150	Bio-Gel A-0.5m Gel, fine	500 ml
151-0430	Bio-Gel A-1.5m Gel, coarse	500 ml
151-0440	Bio-Gel A-1.5m Gel, medium	500 ml
151-0450	Bio-Gel A-1.5m Gel, fine	500 ml
151-0730	Bio-Gel A-5m Gel, coarse	500 ml
151-0740	Bio-Gel A-5m Gel, medium	500 ml
151-0750	Bio-Gel A-5m Gel, fine	500 ml
151-1030	Bio-Gel A-15m Gel, coarse	500 ml
151-1040	Bio-Gel A-15m Gel, medium	500 ml
151-1050	Bio-Gel A-15m Gel, fine	500 ml
151-1330	Bio-Gel A-50m Gel, coarse	500 ml
151-1340	Bio-Gel A-50m Gel, medium	500 ml
151-1901	Gel Filtration Standard	

Larger volumes and special packaging for industrial applications are available on request.

Technical Information

Product	*Grade*	*Hydrated Bead Size (µm)*	*Wet Mesh Size*	*Typical Flow Rates[2] (cm/hr)*	*Fractionation Range/Exclusion Limit (daltons)*	*Exclusion Limit Nucleic Acids[3] (base pairs)*
BIO-GEL A 0.5m GEL	Coarse	150–300	50–100	20–25	< 10,000-500,000	
	Medium	75–150	100–200	15–20	< 10,000-500,000	
	Fine	38–75	200–400	7–13	< 10,000-500,000	
BIO-GEL A 1.5m GEL	Coarse	150–300	50–100	20–25	< 10,000-1,500,000	
	Medium	75–150	100–200	15–20	< 10,000-1,500,000	
	Fine	38–75	200–400	7–13	< 10,000-1,500,000	
BIO-GEL A 5m GEL	Coarse	150–300	50–100	20–25	< 10,000-5,000,000	
	Medium	75–150	100–200	15–20	< 10,000-5,000,000	
	Fine	38–75	200–400	7–13	< 10,000-5,000,000	
BIO-GEL A 15m GEL	Coarse	150–300	50–100	20–25	< 40,000-15,000,000	
	Medium	75–150	100–200	15–20	< 40,000-15,000,000	
	Fine	38–75	200–400	7–13	< 40,000-15,000,000	
BIO-GEL A 50m GEL	Coarse	150–300	50–100	20–25	< 100,000-50,000,000	350
	Medium	75–150	100–200	5-15	< 100,000-50,000,000	350

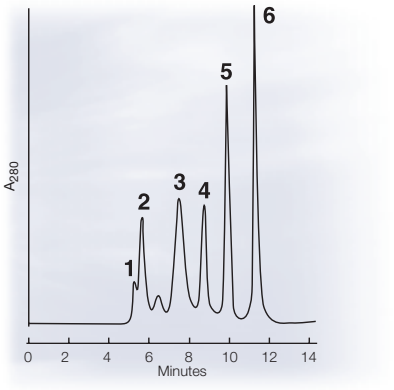
2. Flow rate determined using a 1.5 x 20 cm column and a head to bed ratio of 1:1.
3. *Hansen, J. C. and Rickett, H.,* Anal. Biochem., ***179,*** *167-170 (1989).*

Size Exclusion Chromatography Standard

A Mix of Five Molecular Weight Markers Provides Easy, Reliable Calibration of Size Exclusion Chromatography Columns

This lyophilized mixture of five molecular weight markers, ranging from 1,350 to 670,000 daltons, is a calibration standard for SEC columns used in protein purification.

- Mixture includes vitamin B_{12} and myoglobin, which are visible when applied to glass or clear plastic columns, providing a means of assuring that the column is properly packed and that the sample is eluting evenly
- Can be used with most size exclusion columns and media
- Supplied as a set of six vials, each containing 18 mg of lyophilized protein mixture



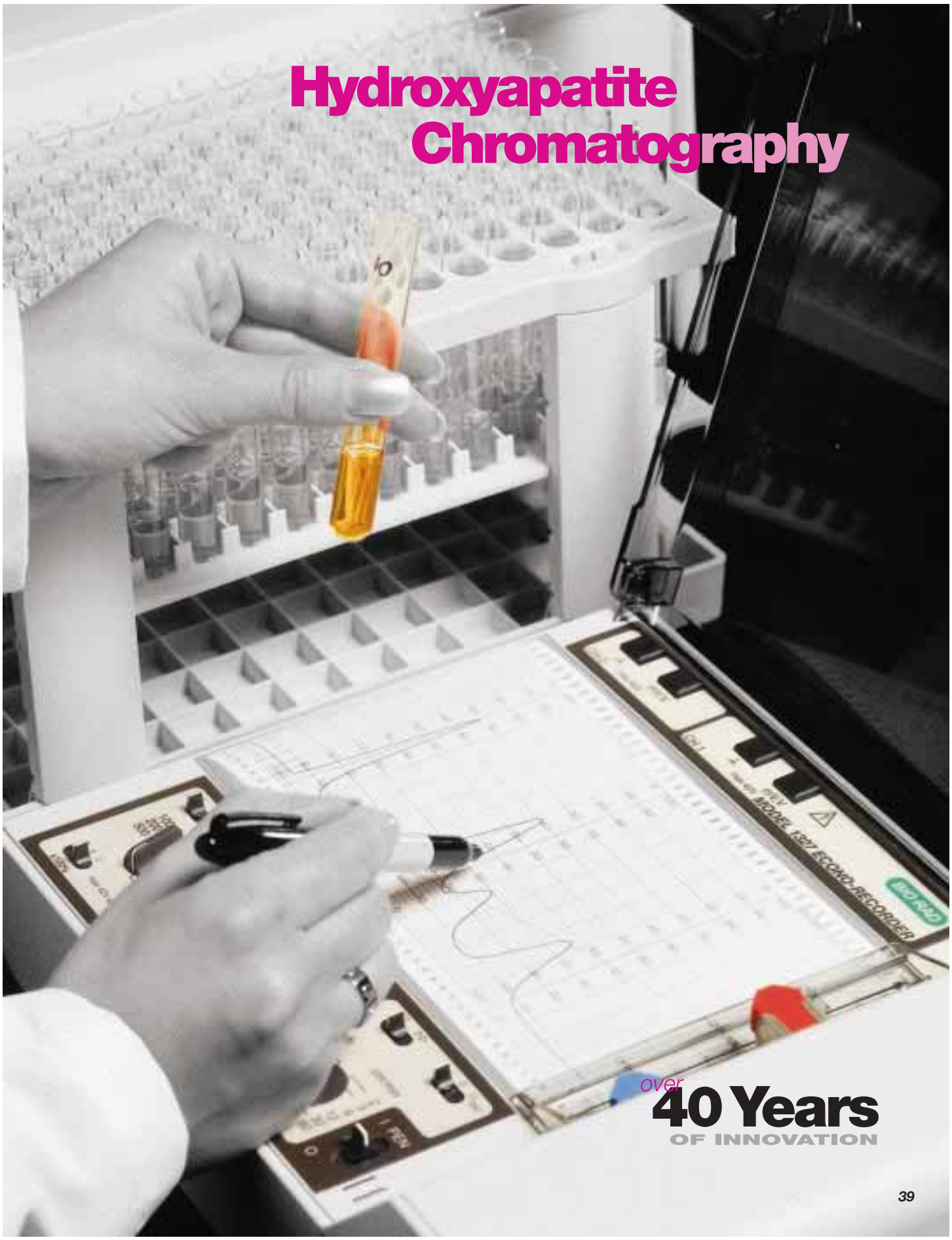
Conditions
Column: *Bio-Sil SEC 250 column, 300 x 7.8 mm*
Sample: *Gel Filtration Standard*
Mobile phase: *50 mM NaH_2PO_4, 50 mM $NaHPO_4$, 150 mM NaCl, 10 mM NaN_3, pH 6.8*
Flow rate: *1.0 ml/min*
Injection: *20 µl*

Peaks
1. *Void volume*
2. *Thyroglobulin*
3. *IgG*
4. *Ovalbumin*
5. *Myoglobin*
6. *Cyanocobalamin (Vitamin B_{12})*

Ordering Information

Catalog #	*Description*	*Contents*	*Molecular Weight, kDa*	*Isoelectric Point*	*For Use With*
151-1901	Gel Filtration Standard, 6 vials	Thyroglobulin, 5.0 mg	670,000	4.5	Bio-Sil SEC, Bio-Silect SEC, Bio-Prep SE columns; Macro-Prep SE, Bio-Gel P and A media
		Bovine gamma globulin, 5.0 mg	158,000	5.1	
		Chicken ovalbumin, 5.0 mg	44,000	4.6	
		Equine myoglobulin, 2.5 mg	17,000	6.9	
		Vitamin B_{12}, 0.5 mg	1,350	4.5	

Hydroxyapatite Chromatography



over
40 Years
OF INNOVATION

Hydroxyapatite Chromatography

Mechanism of Action

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is a form of calcium phosphate which can be used to separate and purify proteins, enzymes, nucleic acids, viruses and other macromolecules. Its unique mechanism of action involves both electrostatic interactions and the formation of calcium coordination complexes (see Figure 1).

Principal electrostatic interactions are between amine groups on the surface of a protein and phosphate sites on hydroxyapatite. At the same time, calcium coordination complexes are formed between the carboxyl groups on a protein and calcium sites on hydroxyapatite. The actual contribution by each mechanism is dependent upon the nature of the protein itself. This complex mechanism of action gives hydroxyapatite unparalleled selectivity and resolution which is evident by its ability to separate proteins determined to be homogeneous by other chromatographic and electrophoretic techniques.

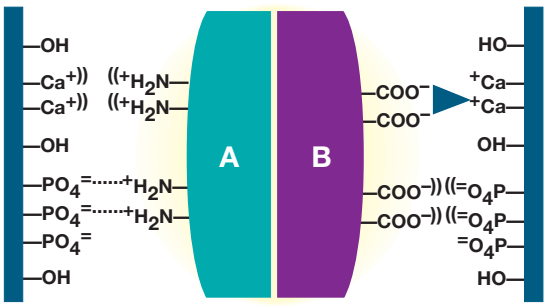


Fig. 1. Protein binding to hydroxyapatite. A is a basic protein. B is an acidic protein. Double parentheses indicate repulsion. Dotted lines indicate ionic bonds. Triangular linkages indicate coordination bonds. Data courtesy of Pete Gagnon, Validated BioSystems, Inc., Tucson, AZ.

Bio-Rad offers three different forms of hydroxyapatite, Bio-Gel HT, Bio-Gel HTP and Macro-Prep ceramic hydroxyapatite, and two types of prepacked columns. The Bio-Gel HT and Bio-Gel HTP are hydrated and dried forms of traditional crystalline hydroxyapatite while the Macro-Prep ceramic hydroxyapatite supports are spherical macroporous forms of the material.

Hydroxyapatite chromatography can be used at any stage in a purification process, from initial capture to final polishing.

Typical CHT Applications

- Separation of monoclonal and polyclonal antibodies of different classes
- Antibodies which differ in light chain composition
- Antibody fragments, isozymes and supercoiled DNA from linear duplexes
- Single-stranded from double-stranded DNA

Ceramic Hydroxyapatite (CHT) is a mechanically stable form of hydroxyapatite which can handle pressures up to 900 psi.

Tip #19

Hydroxyapatite Product Selection Guide

Product	Purification Stage	Resolution	Flow Rate	Package Size Range	Required Equipment
BIO-SCALE CHT-I	**POLISHING**	**VERY HIGH**	**0.5-10 ml/min**	**PREPACKED 2 -20 ml**	**MEDIUM TO HIGH PRESSURE SYSTEM**
ECONO-PAC CHT-II CARTRIDGE	**INTERMEDIATE**	**GOOD**	**0.5-5 ml/min**	**PREPACKED 1 AND 5 ml**	**SYRINGE, PERISTALTIC PUMP**
MACRO-PREP CHT-I & II	**CAPTURE TO POLISHING**	**HIGH**	**100-1,000 cm/hr**	**25 ml TO PROCESS**	**ECONO-COLUMN + PERISTALTIC PUMP**
DNA GRADE HTP	**CAPTURE TO INTERMEDIATE**	**GOOD**	**100 cm/hr**	**25 ml TO PROCESS**	**ECONO-COLUMN + GRAVITY**
HT/HTP	**CAPTURE TO INTERMEDIATE**	**GOOD**	**100 cm/hr**	**25 ml TO PROCESS**	**ECONO-COLUMN + GRAVITY**

Bio-Scale CHT-I Columns

Spherical 10 µm Ceramic Hydroxyapatite Resin Provides High Resolution Separations and Unique Selectivity

Bio-Scale CHT-I columns are packed with the Macro-Prep ceramic hydroxyapatite type I 10 µm support which demonstrates high affinity for basic proteins of relatively high pI and lower affinity for proteins of relatively low pI.

- Rapid, reproducible, high resolution of proteins, peptides and polynucleotides
- The spherical, ceramic nature of the CHT-I support overcomes the physical and chemical instability of traditional crystalline hydroxyapatite
- 10 µm particle size and narrow particle size distribution produce excellent resolution of biomolecules
- Four columns sizes (2, 5, 10 and 20 ml) for flexibility in sample loading
- Easy, efficient scale-up to Macro-Prep CHT, type I media (see pages 44, 45)
- Biocompatible material preserves protein integrity
- Top-Off Resin kit (optional) extends column life

An Ideal Column for Antibody Purification

The ceramic hydroxyapatite type I supports (CHT-I) are particularly well suited for the purification of antibodies, enzymes and other proteins.

- Unique selectivity lets you purify antibodies from different sources under gentle elution conditions using neutral pH sodium phosphate buffer
- Purify monoclonal antibodies that will not bind to Protein A

For more information, request bulletin:

1929 *Bio-Scale Ceramic Hydroxyapatite Type I Columns*

Separations using hydroxyapatite are typically accomplished by increasing the phosphate concentration of the eluant either as steps or as continuous gradient.

Tip #20

Hydroxyapatite Chromatography

Top-Off Resin Kit

Top-Off Resin kits significantly and inexpensively extend a column's life. If the top of the resin becomes fouled and the usual steps do not restore performances, a few millimeters of the bed can be removed and replaced with fresh resin. Top-Off Resin kits contain 1 ml of resin, frits and distribution screens for each column diameter.

Run an NaCl gradient first on CHT to see if electrostatically bound contaminants or sample can be resolved, then run phosphate gradient.

Tip #21

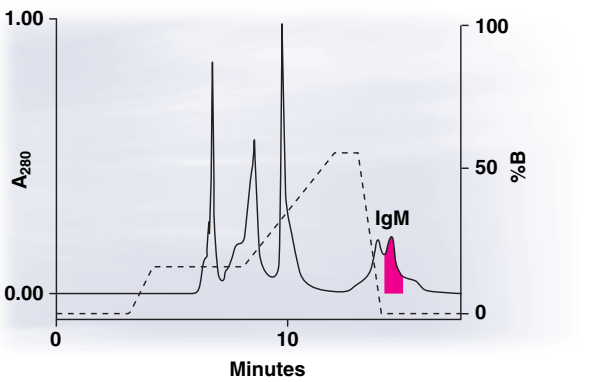


Fig. 2. IgM purification from mouse ascites using a Bio-Scale CHT20-I column

Conditions
Sample: 2.3 ml (2:1 dilution)
Flow rate: 4.6 ml/min
Buffer: A. 10 mM sodium phosphate buffer, pH 6.8
B. 500 mM sodium phosphate buffer, pH 6.8

Technical Information

Characteristics	*Bio-Scale CHT-I columns* CHT2-I	CHT5-I	CHT10-I	CHT20-I
COLUMN VOLUME (ml)	2	5	10	20
RECOMMENDED MAX PROTEIN LOADING (mg)	20	50	100	200
DYNAMIC BINDING CAPACITY (mg/LYSOZYME/COLUMN)	30	75	150	300
RECOMMENDED FLOW RATES (ml/min)	0.5-3.0	0.5-5.0	0.5-7.0	0.5-10.0
AVERAGE PARTICLE SIZES (µm)	10±3.0	10±3.0	10±3.0	10±3.0
COLUMN DIMENSION (mm)	7 x 52	10 x 64	12 x 88	15 x 113

Ordering Information

Catalog #	*Description*
751-0021	Bio-Scale CHT2-I
751-0023	Bio-Scale CHT5-I
751-0025	Bio-Scale CHT10-I
751-0027	Bio-Scale CHT20-I

TOP-OFF RESIN KIT

751-0029	Top-off Resin Kit CHT-I, 1 ml

Econo-Pac CHT-II Cartridges

Convenient Prepacked Low Pressure Columns for Methods Scouting or First Step Purification of Crude Sample

Econo-Pac CHT-II cartridges are prepacked low pressure chromatography columns for use with the BioLogic System, a peristaltic pump or any chromatography system.

- Packed with Macro-Prep CHT media
- Available in 1 ml or 5 ml formats
- Up to three cartridges can be connected in series to triple sample capacity
- Luer-lock fittings for snap-on connection to any low pressure chromatography system or directly to a syringe

The Macro-Prep CHT media packed in Econo-Pac cartridges is also available in bulk for easy scale-up.

See page 44 for more information on Macro-Prep CHT supports.

Don't use the following with the Macro-Prep CHT supports: buffers below pH 5.5; chelating agents; ionic detergents or unbuffered solutions such as DI or WFI.

Tip #22

Macro-Prep CHT Type I (CHT-I) materials work best with basic proteins and Macro-Prep CHT Type II (CHT-II) materials are best for separation of nucleic acids and large proteins.

Tip #23

Technical Information

SUPPORT	Macro-Prep Ceramic Hydroxyapatite, Type II
AVERAGE PARTICLE SIZE	20 µm
FUNCTIONAL GROUP	Ca^{+2}, PO_4^{-3}
PROTEIN CAPACITY	1 ml cartridge: ≥ 3 mg BSA or 6 mg lysozyme 5 ml cartridge: ≥ 15 mg BSA or 30 mg lysozyme
RECOMMENDED FLOW RATE (ml/min)	1 ml cartridge: 0.6-0.8 5 ml cartridge: 0.5-1.0
MAX OPERATING PRESSURE	3.4 bar (50 psi) at 20 °C
OPERATING pH RANGE	5.5-14

Ordering Information

Catalog #	*Description*	*Package Size*
732-0081	Econo-Pac Ceramic Hydroxyapatite, type II	1 x 5 ml
732-0085	Econo-Pac Ceramic Hydroxyapatite, type II	5 x 5 ml
732-0083	Econo-Pac Ceramic Hydroxyapatite, type II	5 x 1 ml

Macro-Prep Ceramic Hydroxyapatite (CHT)

This Chemically Pure Hydroxyapatite Provides Exceptional Throughput, Stability, Reproducibility and Speed

Macro-Prep ceramic hydroxyapatite (CHT) is a spherical, macroporous form of hydroxyapatite that offers the throughput, stability and reproducibility required for industrial biopharmaceutical manufacturing, plus the resolution and speed researchers demand.

Sintered at high temperatures, it yields a physically and chemically robust support with high protein binding capacity and unparalleled selectivity. Use reproducibly for hundreds of cycles in both industrial scale columns and analytical columns run at high flow rates and backpressures as high as 900-1,500 psi.

Choose Your Type

Macro-Prep CHT is available in two distinct types, Type I and Type II, and three particle sizes, 20, 40 and 80 µm. Both types retain the unique separation properties of crystalline hydroxyapatite, and possess some unique properties of their own. Type I material has a higher protein binding capacity, and, in particular, a higher capacity for acidic proteins. Type II material often provides superior selectivity and resolution in the separation of many larger proteins, plasmid DNA, single stranded DNA, double stranded DNA and viruses.

- Unique selectivity/high resolution
- High throughput
- Physical and chemical stability
- High reproducibility
- Two types and three particle sizes for any application

For more information, request bulletins:

1842 & 1927	*Macro-Prep Ceramic Hydroxyapatite*
1971	*Macro-Prep Ceramic Hydroxyapatite Supports*
1986	*Effect of pH on Gradient Elution of Proteins on Two Types of Macro-Prep Ceramic Hydroxyapatite*
2156	*Ceramic Hydroxyapatite–A New Dimension in Chromatography of Biological Molecules*

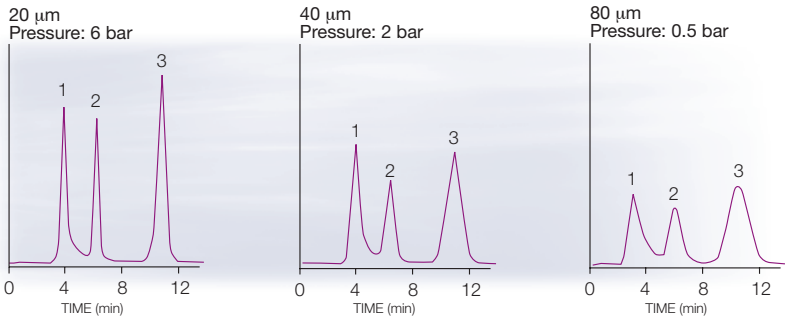


Fig. 3. Effect of particle size on separation of proteins.

Column: *4 x 100 mm*
Sample: *10 µl [10 mg/ml BSA (1), 1.3 mg/ml lysozyme (2), 5 mg/ml cytochrome c (3)]*
Buffer: *A: 1 mM sodium phosphate (pH 6.8)*
B: 400 mM sodium phosphate (pH 6.8)
Gradient: *0–75% buffer B in 15 min*
Linear
flow rate: *478 cm/h*

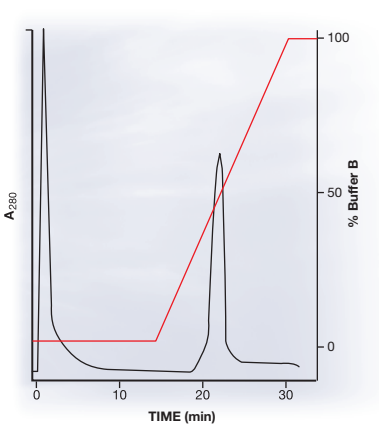


Fig. 4. Separation of antihuman ß-2 microglobulin monoclonal antibody (IgG_1) from mouse ascites. Sample: 100 µl of undiluted ascites fluid applied to a 2 ml column of Macro-Prep ceramic hydroxyapatite, type I, 40 µm. Buffer A: 10mM sodium phosphate, pH 6.8, Buffer B: 500 mM sodium phosphate, pH 6.8.

Hydroxyapatite Chromatography

Technical Information

Characteristics	*Type I*	*Type II*
FUNCTIONAL GROUPS	Ca^{+2}, PO_4^{-3}	Ca^{+2}, PO_4^{-3}
DYNAMIC BINDING CAPACITY	> 25 mg/g lysozyme	> 12.5 mg/g lysozyme
TYPICAL IgG BINDING CAPACITIES @ 500 cm/hr	10–50 mg/ml	5–25 mg/ml
PARTICLE SIZES AVAILABLE	20, 40 and 80 µm (nominal)	20, 40 and 80 µm (nominal)
NOMINAL PORE DIAMETER	600–800 Å	800–1,000 Å
MAX OPERATING PRESSURE	60 bar (900 psi)	100 bar (1,500 psi)
RECOMMENDED OPERATING FLOW RATES	10–1,500 cm/hr	10–1,500 cm/hr
pH STABILITY	5.5–14	5.5–14
REGENERATION	0.4–1.0 M phosphate buffer	0.4–1.0 M phosphate buffer
SANITIZATION	1–2 M NaOH	1–2 M NaOH
AUTOCLAVABLE	Yes; 20 min @ 121 °C	Yes; 20 min @ 121 °C
CHEMICAL COMPATIBILITY		
1 M NaOH	24+ hr	24+ hr
8 M UREA	24+ hr	24+ hr
8 M GUANIDINE-HCl	24+ hr	24+ hr
ETHANOL OR METHANOL	24+ hr	24+ hr
100% ACETONITRILE	24+ hr	24+ hr

Note: A small amount of an appropriate counter-ion such as 0.1–10 mM $NaPO_4$ - should be added to all unbuffered solutions.

Macro-Prep CHT Type I (CHT-I) materials typically provide greater capacity for most proteins compared to the Type II materials.

Tip #24

Ordering Information

Catalog #	*Description*	*Package Size*
158-2000	Macro-Prep Ceramic Hydroxyapatite, Type I, 20 µm	10 g
157-0020	Macro-Prep Ceramic Hydroxyapatite, Type I, 20 µm	100 g
157-0021	Macro-Prep Ceramic Hydroxyapatite, Type I, 20 µm	1 kg
157-0025	Macro-Prep Ceramic Hydroxyapatite, Type I, 20 µm	5 kg
158-4000	Macro-Prep Ceramic Hydroxyapatite, Type I, 40 µm	10 g
157-0040	Macro-Prep Ceramic Hydroxyapatite, Type I, 40 µm	100 g
157-0041	Macro-Prep Ceramic Hydroxyapatite, Type I, 40 µm	1 kg
157-0045	Macro-Prep Ceramic Hydroxyapatite, Type I, 40 µm	5 kg
158-8000	Macro-Prep Ceramic Hydroxyapatite, Type I, 80 µm	10 g
157-0080	Macro-Prep Ceramic Hydroxyapatite, Type I, 80 µm	100 g
157-0081	Macro-Prep Ceramic Hydroxyapatite, Type I, 80 µm	1 kg
157-0085	Macro-Prep Ceramic Hydroxyapatite, Type I, 80 µm	5 kg
158-2200	Macro-Prep Ceramic Hydroxyapatite, Type II, 20 µm	10 g
157-2000	Macro-Prep Ceramic Hydroxyapatite, Type II, 20 µm	100 g
157-2100	Macro-Prep Ceramic Hydroxyapatite, Type II, 20 µm	1 kg
157-2500	Macro-Prep Ceramic Hydroxyapatite, Type II, 20 µm	5 kg
158-4200	Macro-Prep Ceramic Hydroxyapatite, Type II, 40 µm	10 g
157-4000	Macro-Prep Ceramic Hydroxyapatite, Type II, 40 µm	100 g
157-4100	Macro-Prep Ceramic Hydroxyapatite, Type II, 40 µm	1 kg
157-4500	Macro-Prep Ceramic Hydroxyapatite, Type II, 40 µm	5 kg
158-8200	Macro-Prep Ceramic Hydroxyapatite, Type II, 80 µm	10 g
157-8000	Macro-Prep Ceramic Hydroxyapatite, Type II, 80 µm	100 g
157-8100	Macro-Prep Ceramic Hydroxyapatite, Type II, 80 µm	1 kg
157-8500	Macro-Prep Ceramic Hydroxyapatite, Type II, 80 µm	5 kg

Larger quantities available upon request.

Hydroxyapatite Chromatography

Bio-Gel HT and HTP Supports

Prepared by the Method of Tiselius[1]; Can Be Used to Purify Virtually Any Macromolecule

The Bio-Gel HT is traditional crystalline hydroxyapatite suspended in 10 mM sodium phosphate buffer containing 0.02% NaN_3 and should be stored at 4 °C. The Bio-Gel HTP is essentially Bio-Gel HT which has been dried using our unique process. It may be stored without refrigeration, and when suspended in buffer, has characteristics similar to Bio-Gel HT. Both gels can be used with a wide range of aqueous and organic solvents, and can be sanitized with sodium hydroxide and/or autoclaved.

DNA Grade Bio-Gel HTP gel is a dry powder with a smaller particle size than HTP gel, which significantly increases its capacity and enhances its selectivity for double-stranded DNA. Due to its small particle size, it is recommended for batch mode chromatography or for use with very short columns.

- Unique selectivity
- Chemical and thermal stability
- Ideal for both batch and column operations

Technical Information

Characteristics	*Bio-Gel HTP Gel*	*Bio-Gel HTP, DNA Grade*	*Bio-Gel HT Gel*
FLOW RATE* cm/hr	> 25	> 5	> 35
BSA ADSORBED (mg per dry gram)**	10	10	10
CALF THYMUS DNA ADSORBED (µg per dry gram)	> 500	> 800	> 500
HYDRATED VOLUME	2-3 ml/g	2-3 ml/g	-

** Flow rate determined on a 1.5 x 10 cm column with 40 cm hydrostatic pressure. ** Batchwise uptake.*

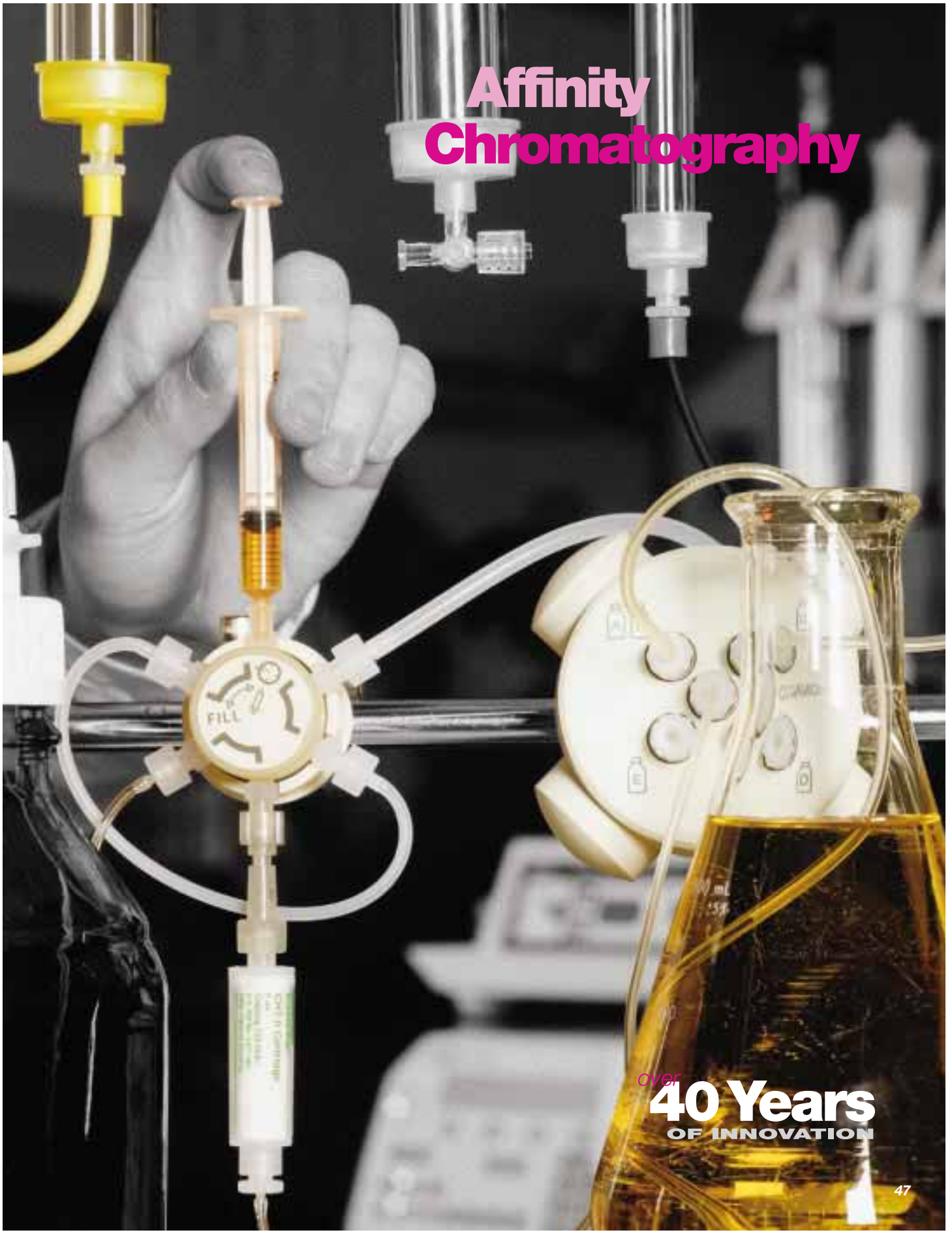
1. Tiselius, A., Hjerten, S. and Levin, O., Arch. Biochem. Biophys., ***65****, 132 (1956).*

Ordering Information

Catalog #	*Description*
130-0150	Bio-Gel HT Hydroxyapatite, hydrated, 250 ml
130-0151	Bio-Gel HT Hydroxyapatite, hydrated, 500 ml
130-0420	Bio-Gel HTP Hydroxyapatite, powder, 100 g
130-0421	Bio-Gel HTP Hydroxyapatite, powder, 1 kg
130-0425	Bio-Gel HTP Hydroxyapatite, powder, 5 kg
130-0520	Bio-Gel HTP Hydroxyapatite, DNA grade, 100 g

Larger volumes and special packaging for industrial applications are available upon request.

Affinity Chromatography



Affinity Chromatography

Mechanism of Action

In affinity chromatography, a ligand is covalently bound to a solid matrix which is packed in a chromatography column. A mixture of components is then applied to the column. The unbound contaminants, which have no affinity for the ligand, are washed through the column, leaving the desired component (protein, peptide, DNA fragment, *etc.*) bound to the matrix.

Elution is accomplished by changing the pH and/or salt concentration or by applying organic solvents or a molecule which competes for the bound ligand.

Bio-Rad offers affinity gels for a wide range of biomolecular applications.

Ready-to-use supports have a specific ligand pre-coupled to an affinity support. These supports are available in two types:

- Affi-Gel supports are based on a crosslinked agarose matrix
- Affi-Prep supports are based on a pressure stable methacrylate polymer matrix

Activated supports allow you to make the affinity matrix of your choice by coupling a ligand such as an antibody, antigen, enzyme or receptor with a support.

For more information, request bulletin:

1099 *Immunoaffinity Chromatography with Affinity Supports*

Ready-to-Use Affinity Supports Product Selection Guide

Ready-to-use affinity supports, available with specific ligands already coupled to a base matrix, offer convenience and dependability. Each ligand exhibits its own specificity and can be used to isolate specific molecules.

Media	Specificity	Functional Group	Capacity	Matrix	Working pH	Pressure Limit
AFFI-GEL PROTEIN A GEL	IgG	Protein A (2 mg/ml)	See page 51	Crosslinked agarose	2-10	15 psi (1 bar)
AFFI-PREP PROTEIN A	IgG	Protein A (2 mg/ml)	See page 51	Pressure stable polymer	2-10	1,000 psi (70 bar)
AFFI-GEL BLUE GEL	Albumin, many nucleotide requiring enzymes and other proteins	Cibacron Blue® F3GA (1.9 mg/ml)	≥ 11 mg/ml	Crosslinked agarose	2-10	15 psi (1 bar)
DEAE AFFI-GEL BLUE GEL	Albumin and serum proteins	Cibacron Blue F3GA and DEAE	0.14 ml serum/ml gel	Crosslinked agarose	2-10	15 psi (1 bar)
CM AFFI-GEL BLUE GEL	Albumin and serum proteins	Cibacron Blue F3GA and CM	0.17-0.5 ml serum/ml gel	Crosslinked agarose	2-11	15 psi (1 bar)
AFFI-GEL HEPARIN GEL	General, including growth factors, coagulation factors, DNA/RNA specific enzymes, lipase, lipoproteins and proteases	Heparin	≥ 1.2 mg/ml of human antithrombin	Crosslinked agarose	5-10	15 psi (1 bar)
AFFI-PREP POLYMYXIN SUPPORT	Endotoxins	Polymyxin (2-4 mg/ml)	≥ 5 mg/ml	Pressure stable polymer	2-10	1,000 psi (70 bar)
AFFI-GEL 601 GEL	Cis-Diols	Boronate (1.05±0.15 meq/ml)	130 µmoles sorbitol/ml	Crosslinked agarose	2-10	15 psi (1 bar)

Econo-Pac Affi-Prep Protein A Cartridges

For Rapid Purification and Isolation of Monoclonal and Polyclonal Antibodies from Small Amounts of Ascites Fluid or Serum

Use these prepacked chromatography columns with a syringe, a peristaltic pump, the BioLogic System or any other chromatography system. Up to three cartridges can be connected in a series to triple sample capacity.

Econo-Pac Protein A Cartridges Technical Information

Support	*Affi-Prep Protein A*
PROTEIN CAPACITY	1 ml cartridge: ~7 mg mouse monoclonal IgG_1 or 16 mg human IgG 5 ml cartridge: ~34 mg mouse monoclonal IgG_1 or 70 mg human IgG
RECOMMENDED FLOW RATE (ml/min)	1 ml cartridge: 0.1-0.5 5 ml cartridge: 0.5-1.5
MAX OPERATING PRESSURE	3.4 bar (50 psi) at 20 °C
OPERATING pH RANGE	2-14
RECOMMENDED STORAGE CONDITIONS	50 mM phosphate, pH 7.0, with 0.05% NaN_3

Econo-Pac Protein A Column

Achieve Fast, Easy Gravity-flow Purification of Monoclonal Antibodies from Small Amounts of Ascites Fluid or Serum

Econo-Pac protein A columns allow simplified antibody purification. Each prepacked column includes an upper frit, a snap-off end-tip, graduated column markings and a 30 ml total column volume. For best performance, use a flow adaptor (see page 14).

For more information, request bulletin:

1836 *Antibody Purification with the Econo-Pac Protein A Cartridge*

Econo-Pac Protein A Column Technical Information

Packed Support	*Affi-Gel Protein A*
BED VOLUME	2 ml
TOTAL COLUMN VOLUME	30 ml
CAPACITY	10-14 mg mouse IgG_1

Don't overload your column, as both the resolution and column lifetime will decrease. For larger loads, either change to a larger column or perform several runs with a reduced loading.

Tip #25

Econo-Pac Protein A Kit

Everything You Need to Purify Monoclonal Antibodies from Ascites Fluid or Serum in One Complete Kit

The Econo-Pac protein A kit includes an Affi-Gel protein A column, reusable Econo-Pac 10DG desalting columns (for sample preparation and buffer exchange of the purified antibody) and MAPS II buffers – enough reagents to purify 300 mg of mouse IgG_1.

Ordering Information

Catalog #	*Description*	*Quantity*
ECONO-PAC AFFI-PREP PROTEIN A CARTRIDGES		
732-0091	Econo-Pac Protein A Cartridge	1 x 5 ml
732-0093	Econo-Pac Protein A Cartridge	5 x 1 ml
ECONO-PAC PROTEIN A COLUMN		
732-2022	Econo-Pac Protein A Column	5
ECONO-PAC PROTEIN A KIT		
732-2020	Econo-Pac Protein A Kit	1

Dye Affinity Columns

Econo-Pac Cartridges

These prepacked columns can be used with a syringe, a peristaltic pump, the BioLogic System or any other chromatography system.

- Econo-Pac blue cartridges are prepacked with Affi-Gel blue for rapid albumin removal and purification of serum proteins and enzymes
- Econo-Pac DEAE blue cartridges are prepacked with DEAE Affi-Gel blue for IgG-type antibody purification from both serum and ascites samples
- Up to three cartridges can be connected in a series to triple sample capacity

Econo-Pac Serum IgG Purification Column

FAST, EASY GRAVITY-FLOW PURIFICATION OF IgG FROM OTHER SERUM PROTEINS AND PLASMINOGEN WITH ONLY RESIDUAL TRANSFERRIN CONTAMINATION

Econo-Pac serum IgG prepacked purification columns allow simplified antibody purification.

- Can be used 8-10 times
- Include an upper frit, a snap-off end-tip, graduated column markings, and a 30 ml total column volume
- For best performance, use a flow adaptor (see page 14)

Econo-Pac Serum IgG Purification Kit

EVERYTHING YOU NEED TO PURIFY IgG FROM SERUM

Includes 5 Econo-Pac serum IgG purification columns, a reusable Econo-Pac 10DG desalting column for sample preparation, and premixed buffers for human and rabbit IgG applications.

- Up to 13 ml of serum can be purified at a time

Econo-Pac Cartridges Technical Information

	Econo-Pac Blue	*Econo-Pac DEAE Blue*
SUPPORT	Affi-Gel blue	DEAE Affi-Gel blue
SERUM CAPACITY	0.3-1.0 ml	0.3-1.0 ml
RECOMMENDED FLOW RATE	2 ml/min	2 ml/min
MAX OPERATING PRESSURE	0.68 M bar (10 psi) at 20 °C	0.68 M bar (10 psi) at 20 °C
OPERATING pH RANGE	2-10	2-10
RECOMMENDED STORAGE CONDITIONS	20 mM sodium phosphate, pH 8.0, with 0.01% NaN_3 at 4 C°	20 mM sodium phosphate, pH 8.0, with 0.01% NaN_3 at 4 C°

Econo-Pac Serum IgG Purification Column Technical Information

Packed Support	*DEAE Affi-Gel Blue Gel*
BED VOLUME	2 ml
TOTAL COLUMN VOLUME	30 ml
CAPACITY	3 ml of serum per column per run

Ordering Information

Catalog #	*Description*	*Quantity*
ECONO-PAC CARTRIDGES		
732-0101	Econo-Pac Blue	1 x 5 ml
732-0105	Econo-Pac Blue	5 x 5 ml
732-0031	Econo-Pac DEAE Blue	1 x 5 ml
732-0035	Econo-Pac DEAE Blue	5 x 5 ml
ECONO-PAC SERUM IgG PURIFICATION COLUMN		
732-2026	Econo-Pac Serum IgG Purification Column	5
ECONO-PAC SERUM IgG PURIFICATION KIT		
732-2027	Econo-Pac Serum IgG Purification Kit	1

Affi-Gel and Affi-Prep Protein A Supports

Produce Highly Purified Immunoglobulins (IgG), Selectively Remove IgG Prior to Analysis of Other Immunoglobulin Classes or Adsorb Immune Complexes for Antigen Purification

Affi-Gel Protein A Support is based on crosslinked agarose and is intended for low pressure applications such as laboratory scale purification using a peristaltic pump or gravity flow elution.

Affi-Prep Protein A Support is based on a pressure-stable macroporous polymer and is suitable for pilot and process scale applications.

Protein A produced from *Staphylococcus aureus* binds to the Fc region of immunoglobulins, especially mammalian IgG.

- High purity IgGs are obtained
- Supports show high affinity for mammalian IgG
- High capacities with Affi-Gel protein A for mouse IgG_1 and other subclasses are obtained using MAPS optimized buffer solutions
- Using the MAPS II buffer system, up to 10 mg of IgG_1 per ml of gel can be purified; this is 8-10 times higher than published standard methods

Additional Benefits of Affi-Prep Protein A:

- Can be run at high flow rates
- Pressure stability up to 1,000 psi (70 bar)
- Available in Econo-Pac Protein A cartridges and an Econo-Pac Protein A column (see page 49)

To reduce effects of the low pH buffers used to elute from Protein A supports, collect in fraction tubes containing a neutralizing buffer.

Tip #26

Capacities of Protein A Supports

IMMUNOGLOBLIN	Affi-Gel Protein A (mg/ml)	Affi-Prep Protein A (mg/ml)
MOUSE IgG_1	6-8	8-10
MOUSE IgG_{2a}	8-10	13-15
MOUSE IgG_{2b}	8-10	13-15
MOUSE IgG_3	8-10	13-15
MOUSE IgM*	3-5	5-7
HUMAN IgG	20	16-23
SHEEP IgG		9-16
BOVINE IgG		9-16
EQUINE IgG		9-16
GOAT IgG		9-16
RABBIT IgG		2-16
DOG IgG		9-16
PORCINE IgG		9-16

**Approximately 50% of all mouse IgMs bind using MAPS buffer system.*

Ordering Information

Catalog #	*Description*	*Quantity*
AFFI-GEL AND AFFI-PREP PROTEIN A SUPPORTS		
153-6153	Affi-Gel Protein A Agarose	5 ml
153-6154	Affi-Gel Protein A Agarose	50 ml
156-0006	Affi-Prep Protein A Support	5 ml
156-0005	Affi-Prep Protein A Support	25 ml
153-6159	Affi-Gel Protein A MAPS II Kit, includes 5 ml Affi-Gel protein A gel, Affi-Gel Protein A MAPS II buffers, 1 x 10 cm Econo-Column column, to purify 500 mg of mouse IgG_1	
153-6160	Affi-Gel Protein A MAPS II Buffers, includes 471 g binding buffer (1,500 ml), 25 g elution buffer (1,100 ml), 400 ml regeneration buffer	
153-6164	Affi-Prep Protein A MAPS II Buffers, includes 471 g binding buffer (1,500 ml) and 25 g elution buffer (1,100 ml)	
153-6161	Protein A MAPS II Binding Buffer, makes 5 L	
153-6162	Protein A MAPS II Elution Buffer, makes 5 L	
153-6166	Affi-Gel Protein A MAPS II Regeneration Buffer, makes 5 L	

Affi-Gel Blue Affinity Gels

Ideal for Albumin Removal (50-100 Mesh) and Enzyme Purification (100-200 Mesh)

Affi-Gel blue affinity gel is a crosslinked agarose bead with Cibacron blue F3GA dye covalently attached.

- Functions as an ionic, hydrophobic, aromatic or sterically active binding site in various applications
- Proteins and peptides are bound and released with great specificity by manipulating the composition of the buffers
- Available in an Econo-Pac blue column (see page 50)

For more information, request bulletins:

1092 *DEAE Affi-Gel Blue Gel and CM Affi-Gel Blue Gel for IgG Purification*
1107 *Affi-Gel Blue Affinity Chromatography Gel for Enzyme and Blood Protein Purification*

DEAE Affi-Gel Blue Gel

A Bifunctional Affinity Gel for Single Step IgG Purification from Serum

DEAE Affi-Gel blue gel is a bifunctional gel consisting of Cibacron blue F3GA dye covalently attached to DEAE Bio-Gel A agarose gel. The dye binds albumin, proteases and complement proteins while the DEAE group binds the remaining acidic proteins.

- Economical alternative to protein A affinity chromatography
- Minimal sample preparation (dialysis or desalting)
- No detectable protease activity in the eluted IgG fraction
- Available in Econo-Pac blue cartridges and Econo-Pac serum IgG purification columns (see page 50)

CM Affi-Gel Blue Gel

For Rapid Removal of ≥ 90% of Albumin and All Plasminogen in Serum Samples

CM Affi-Gel blue gel consists of Cibacron blue F3GA dye covalently coupled to CM Bio-Gel A agarose gel.

- Binds both albumin and serum proteases
- Provides a convenient initial step in the purification of serum proteins
- No sample preparation required
- Over 80% yield of stable antiserum, free of albumin and protease activity

For more information, request bulletin:

1092 *DEAE Affi-Gel Blue Gel and CM Affi-Gel Blue Gel for IgG Purification*

Ordering Information

Catalog #	*Description*	*Quantity*
AFFI-GEL BLUE AFFINITY GELS		
153-7301	Affi-Gel Blue Gel, 50-100 mesh	100 ml
153-7302	Affi-Gel Blue Gel, 100-200 mesh	100 ml
DEAE AFFI-GEL BLUE GEL		
153-7307	DEAE Affi-Gel Blue Gel	100 ml
CM AFFI-GEL BLUE GEL		
153-7304	CM Affi-Gel Blue Gel	100 ml

Affi-Prep Polymyxin Support

Developed for Endotoxin Removal in Research and Process Scale Applications

Affi-Prep polymyxin supports contain 2-4 mg of USP grade polymyxin per ml. This matrix binds endotoxins from a number of strains of gram negative bacteria including *E. coli, Salmonella abortus, Salmonella minnesota,* and *Serratia marcesens.*

- Linear flow rates up to 2,000 cm/hr
- Pressure stability up to 1,000 psi (70 bar)
- High chemical stability (sanitization with 0.1 N sodium hydroxide)

For more information, request bulletin:

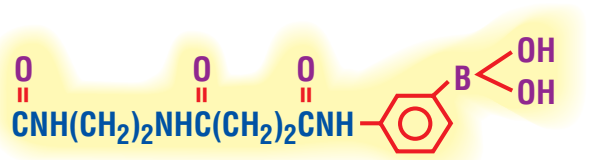
1298 Affi-Prep High Performance Affinity Media

Affi-Gel 601 Boronate Derivatized Polyacrylamide Gel

For Highly Efficient Separations of Low Molecular Weight Compounds

Affi-Gel 601 boronate derivatized polyacrylamide gel has an affinity for coplanar adjacent hydroxyl groups (cis-diols) and provides very efficient separations of low molecular weight compounds such as nucleotides, nucleosides, catecholamines and sugars.

- High binding capacity for diols
- Sorbitol capacity of 130 µm/ml



For more information, request bulletin:

1066 Affi-Gel 601 Affinity Chromatography Gel

Ordering Information

Catalog #	*Description*	*Quantity*
AFFI-PREP POLYMYXIN SUPPORT		
156-0010	Affi-Prep Polymyxin Support	25 ml
AFFI-GEL 601 BORONATE DERIVATIZED POLYACRYLAMIDE GEL		
153-6101	Affi-Gel 601 Gel	5 g

Activated Supports Product Selection Guide

Create custom affinity matrices by immobilizing the ligand of your choice. Activated supports add flexibility and convenience to affinity chromatography.

Media	Specificity	Functional Group	Capacity	Matrix	Working pH	Pressure Limit
AFFI-GEL 10 GEL	**-NH_2 FORMS STABLE COVALENT BONDS VIA PRIMARY AMINES; MOST EFFICIENT FOR COUPLING PROTEINS WITH pI FROM 6.5 TO 11**	**N-HYDROXY-SUCCINAMIDE ≥ 10 µ MOLES/ml OF GEL**	**35 mg/ml**	**CROSSLINKED AGAROSE**	**3-10**	**15 psi (1 bar)**
AFFI-GEL 15 GEL	**-NH_2 FORMS STABLE COVALENT BONDS VIA PRIMARY AMINES; MOST EFFICIENT FOR COUPLING PROTEINS WITH pI BELOW 6.5**	**N-HYDROXY-SUCCINAMIDE ≥ 9 µ MOLES/ml OF GEL**	**35 mg/ml**	**CROSSLINKED AGAROSE**	**3-10**	**15 psi (1 bar)**
AFFI-PREP 10 SUPPORT	**-NH_2 FORMS STABLE COVALENT BONDS VIA PRIMARY AMINES; MOST EFFICIENT FOR COUPLING PROTEINS WITH pI FROM 6.5 TO 11**	**N-HYDROXY-SUCCINAMIDE**	**≥ 7.5 mg/ml HUMAN IgG**	**PRESSURE STABLE POLYMER**	**2-10**	**1,000 psi (70 bar)**
AFFI-GEL HZ GEL	**OXIDIZED CARBOHYDRATES USED FOR THE IMMOBILIZATION OF IMMUNOGLOBULINS AND OTHER GLYCOPROTEINS VIA CARBOHYDRATE MOIETIES**	**HYDRAZIDE**	**1-5 mg/ml**	**CROSSLINKED AGAROSE**	**2-10**	**15 psi (1 bar)**
AFFI-PREP Hz SUPPORT	**OXIDIZED CARBOHYDRATES USED FOR THE IMMOBILIZATION OF IMMUNOGLOBULINS AND OTHER GLYCOPROTEINS VIA CARBOHYDRATE MOIETIES**	**HYDRAZIDE**	**1-5 mg/ml**	**PRESSURE STABLE POLYMER**	**2-10**	**1,000 psi (70 bar)**

Prior to immobilizing a precious protein to an activated support, perform a test coupling, using a small quantity of your ligand, with a carrier protein such as BSA.

Tip #27

Affi-Gel 10, Affi-Gel 15 and Affi-Prep 10 Affinity Supports

For Fast, Efficient Coupling of Ligands Via Primary Amines
The Affi-Gel 10 and Affi-Gel 15 gels offer:

- Spontaneous coupling
- Aqueous and anhydrous coupling conditions
- Protein coupling completed within 4 hours at 4 ˚C
- Protein coupling capacity from 1 to 35 mg/ml gel

For more information, request bulletin:

1085 Affi-Gel 10 and 15 Activated Supports

Affi-Prep 10 is a pressure stable affinity support ideal for process scale and high performance applications with features including:

- Spontaneous coupling
- Linear flow rates to 2,000 cm/hr
- Pressure stability to 1,000 psi (70 bar)
- Protein coupling capacity > 7.5 mg/ml gel

Affi-Gel 10 and Affi-Prep 10 supports are most efficient for coupling neutral or basic proteins with isoelectric points from 6.5-11. Affi-Gel 15 is recommended for coupling acidic proteins with isoelectric points below 6.5.

For more information, request bulletin:

1085 Affi-Gel 10 and 15 Activated Supports
1099 Immunoaffinity Chromatography with Affinity Supports
1298 Affi-Prep High Performance Affinity Media

Affi-Gel Hz and Affi-Prep Hz Activated Supports

For Greater Specificity and Higher Antigen Binding Capacity
Affi-Gel Hz and Affi-Prep Hz couple IgG molecules via carbohydrate moieties on the Fc region. Fc attachment results in greater specificity of antigen-antibody interaction and 100-300% higher antigen binding capacity compared to other supports.

- Stable covalent hydrazide bonds
- Mild oxidation without antibody activity alteration
- High antigen binding capacity
- pH stability
- Process scale and medium to high pressure applications with Affi-Prep Hz

For more information, request bulletin:

1424 Affi-Gel Hz Immunoaffinity Kit

Affi-Gel 102 Carbodiimide-Activated Support

Support for use with EDAC coupling reagent to immobilize ligands containing primary amines or terminal carboxyl groups. These supports offer alternative chemistries, flexibility and economy.

Affi-Gel 102 is an amino terminal crosslinked agarose gel with a 6-atom, hydrophilic arm.

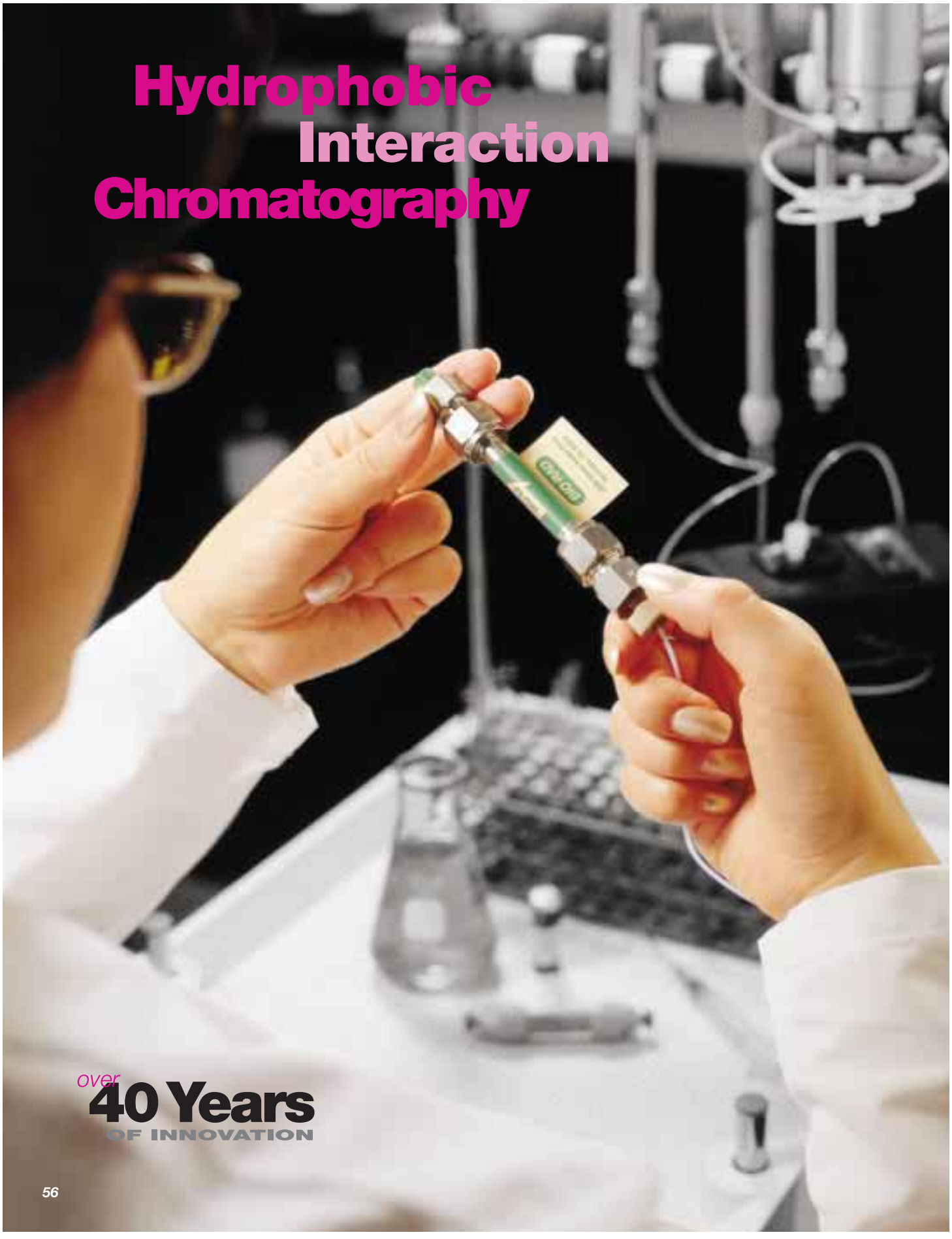
- EDAC carbodiimide coupling reagent
- Use with carboxyl containing ligands

Ordering Information

Catalog #	*Description*	*Quantity*
AFFI-GEL 10, AFFI-GEL 15 AND AFFI-PREP 10		
153-6099	Affi-Gel 10 Gel	25 ml
153-6046	Affi-Gel 10 Gel	4 x 25 ml
153-1000	Affi-Gel 10 Gel	1 liter
153-6051	Affi-Gel 15 Gel	25 ml
153-6052	Affi-Gel 15 Gel	4 x 25 ml
153-1500	Affi-Gel 15 Gel	1 liter
153-6098	Affi-Gel 10/15 Combination, includes: Affi-Gel 10 gel	2 x 25 ml
	Affi-Gel 15 gel	2 x 25 ml
156-0002	Affi-Prep 10 Support	1 x 25 ml
156-0001	Affi-Prep 10 Support	4 x 25 ml
156-0003	Affi-Prep 10 Support	500 ml
AFFI-GEL HZ AND AFFI-PREP HZ		
153-6047	Affi-Gel Hz Hydrazide Gel	25 ml
153-6060	Affi-Gel Hz Immunoaffinity Kit, includes 5 ml Affi-Gel Hz gel, 2 x 25 mg Affi-Gel Hz oxidizer, 25 ml Affi-Gel Hz coupling buffer concentrate, 2 Econo-Pac 10DG desalting columns, 1 x 10 cm Econo-Column column, instructions	
153-6054	Affi-Gel Hz 10x Coupling Buffer Concentrate	500 ml
153-6055	Affi-Gel Oxidizer, 250 mg, sodium periodate for use with Affi-Gel or Affi-Prep Hz support	
156-0015	Affi-Prep Hz Hydrazide Support	5 ml
156-0016	Affi-Prep Hz Hydrazide Support	25 ml
156-0017	Affi-Prep Hz Hydrazide Support	500 ml
AFFI-GEL 102		
153-2401	Affi-Gel 102 Gel	50 ml
153-0990	EDAC	5 g

Note: Larger volumes and special packaging for industrial applications are available upon request

Hydrophobic Interaction Chromatography



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Hydrophobic Interaction Chromatography

Mechanism of Action

Hydrophobic interaction chromatography (HIC) separates molecules based on their hydrophobicity. Sample molecules containing hydrophobic and hydrophilic regions are applied to an HIC column in a high salt buffer. The high salt concentration in the buffer promotes a favorable environment for binding by interacting with water molecules to reduce the solvation of the other molecules in the solution.

As solvation decreases, exposure of hydrophobic regions in the molecules increases and these molecules are then adsorbed by the beads. The more hydrophobic the molecule, the less salt needed to promote binding. Usually a decreasing salt gradient is used to elute samples from the column. As the ionic strength decreases, the exposure of the hydrophilic regions of the molecules increases and molecules elute from the column in order of increasing hydrophobicity. Sample elution may also be completed by adding mild organic modifiers or detergents to the elution buffer.

Hydrophobic Interaction Product Selection Guide

Product	Purification Stage	Resolution	Available Functional Groups	Flow Rate	Package Size Range	Required Equipment
ECONO-PAC HIC CARTRIDGES	**INTERMEDIATE**	**GOOD**	**METHYL AND t-BUTYL**	**0.5-5.0 ml/min**	**PREPACKED 1 AND 5 ml**	**SYRINGE, PERISTALTIC PUMP**
QUANTUM PREP PLASMID KITS	**SAMPLE PREPARATION**	**SEQUENCING QUALITY DNA**	**SiO_2**	**SPIN COLUMN**	**MINI-, MIDI- AND MAXIPREPS**	**CENTRIFUGE**
MACRO-PREP HIC SUPPORTS	**INTERMEDIATE TO POLISHING**	**GOOD**	**METHYL AND t-BUTYL**	**3,000 cm/hour**	**25 ml TO PROCESS**	**PERISTALTIC PUMP**

Hydrophobic Interaction Chromatography

Econo-Pac HIC Cartridges

Convenient Prepacked Low Pressure Columns for Methods Scouting or First Step Purification of Crude Sample

Econo-Pac HIC cartridges can be used with the BioLogic System, a peristaltic pump or any other chromatography system. Their low cost and convenience make these cartridges practical for:

- Methods scouting
- First step purification of crude samples
- Applications using toxic samples where frequent column disposal is required

In addition:

- Up to three cartridges can be connected in series to triple sample capacity
- Luer-lock fittings allow snap-on connection to any low pressure chromatography system or directly to a syringe

Technical Information

	Econo-Pac t-Butyl HIC Cartridges	*Econo-Pac Methyl HIC Cartridges*
SUPPORT	Macro-Prep t-Butyl	Macro-Prep Methyl HIC
FUNCTIONAL GROUP	$-C(CH_3)_3$	$-CH_3$
AVERAGE PARTICLE SIZE	50 µm	50 µm
PROTEIN CAPACITY	1 ml cartridge: ≥ 15 mg HSA 5 ml cartridge: 65 mg HSA	1 ml cartridge: 25 mg HSA 5 ml cartridge: 110 mg HSA
RECOMMENDED FLOW RATE (ml/min)	1 ml cartridge: 0.5-1.0 5 ml cartridge: 0.5-3.0	
MAXIMUM OPERATING PRESSURE	3.4 bar (50 psi) at 20 °C	
OPERATING pH RANGE	2-12	
RECOMMENDED STORAGE CONDITIONS	50 mM Tris-HCl, pH 8.0, 0.1 M NaCl, 0.05% NaN_3	

Ordering Information

Catalog #	*Description*	*Quantity*
ECONO-PAC HIC CARTRIDGES		
732-0056	Econo-Pac t-Butyl HIC	1 x 5 ml
732-0057	Econo-Pac t-Butyl HIC	5 x 5 ml
732-0058	Econo-Pac t-Butyl HIC	5 x 1 ml
732-0051	Econo-Pac Methyl HIC	1 x 5 ml
732-0055	Econo-Pac Methyl HIC	5 x 5 ml
732-0053	Econo-Pac Methyl HIC	5 x 1 ml

The media prepacked in Econo-Pac cartridges is also available in bulk for easy scale-up.

Quantum Prep Plasmid Purification Kits

Convenient, Ready-to-Use Kits for Isolating and Purifying High Purity Plasmid DNA from Bacterial Cell Cultures

Quickly extract and purify plasmid DNA from bacterial cell cultures. Quantum Prep plasmid kits are based on a modified alkaline lysis procedure and the Quantum Prep chromatographic matrix—a novel, patented preparation of purified diatomaceous earth. Plasmid DNA released from lysed bacterial cells selectively binds to the Quantum Prep matrix in the presence of chaotropic salts. Denatured contaminants and salts are washed away with alcohol, and purified DNA is eluted with low-salt solutions or water.

- Convenient spin column format eliminates time-consuming and laborious phenol/chloroform extraction and cesium chloride purifications
- Purified DNA is suitable for sequencing, cloning, PCR and transfection/transformation protocols
- Available in three convenient sizes for all major DNA purification applications
- Gel Slice Kit is also available for the rapid and efficient removal of DNA bands from agarose gel slices

Plasmid yield can be improved by growing high copy number plasmids in rich media such as Terrific Broth. While this may overload anion exchange-based plasmid purification kits, resulting in reduced yield and purity of DNA, the unique diatomaceous earth-based Quantum Prep matrix permits yields as high as 25-40 µg per miniprep using recombinant clones.

Tip #28

The use of bacterial strains deficient in the endonuclease 1 gene product (endA1 genotype), such as DH5∝F', is recommended for improving the quality of plasmid DNA prepared from minipreps.

Tip #30

To accurately determine the yield of purified DNA, it is recommended that the concentration be determined by both UV spectroscopy (A_{260}/A_{280}) and analysis by agarose gel electrophoresis.

Tip #31

Technical Information

	Miniprep Kit	*Midiprep Kit*	*Maxiprep Kit*
APPLICATION	Sequencing, cloning	Cloning, sequencing, transfections	Cloning, sequencing, transfections
CELL CULTURE VOLUME	1-3.0 ml	20-40 ml	100-500 ml
DNA YIELD	Up to 25 µg	Up to 300 µg	Up to 3 µg
SEPARATION TIME	15 min	45 min	90 min
PLASMID PREPS/KIT	100	20	10

To improve the yield of plasmid DNA, preheat the elution buffer to 65-70 ˚C.

Tip #29

Ordering Information

Catalog #	*Description*
QUANTUM PREP PLASMID PURIFICATION KITS	
732-6100	Quantum Prep Plasmid Miniprep Kit, 100 preps
732-6120	Quantum Prep Plasmid Midiprep Kit, 20 preps
732-6130	Quantum Prep Plasmid Maxiprep Kit, 10 preps
732-6160	Quantum Prep Gel Slice Kit, 100 preps

Hydrophobic Interaction Chromatography

Macro-Prep HIC Supports

Robust Methacrylate-Based 50 µm Beads for Protein and Peptide for Semi-prep to Process Scale Purification

Macro-Prep HIC supports are available derivatized with both methyl and t-butyl functional groups. The weakly hydrophobic methyl support is ideal for purifying strongly hydrophobic macromolecules, while the mildly hydrophobic t-butyl support is recommended for macromolecules with few or weakly hydrophobic regions.

- Chemically and thermally stable
- Can withstand acids, bases, chaotropes, detergents and autoclaving
- Can be cleaned and sanitized in place with ethanol or sodium hydroxide
- Available in convenient 1 and 5 ml prepacked Econo-Pac cartridges (see page 58)

For more information, request bulletin:

1841 B-100 Macro-Prep HIC Support

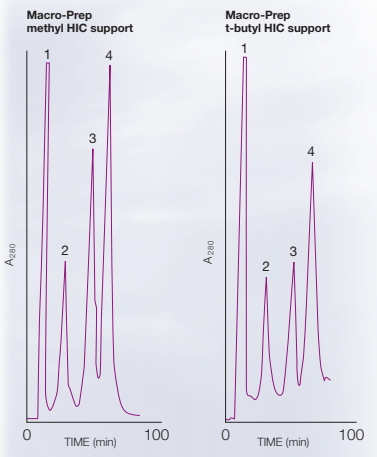
Hydrophobic Interaction Chromatography Standard

This standard contains a mixture of three proteins for easy hydrophobic interaction chromatography column testing and troubleshooting.

Technical Information

	Methyl HIC Support	*t-Butyl HIC Support*
FUNCTIONAL LIGAND	$-CH_3$	$-C(CH_3)_3$
BINDING CAPACITY	> 25 mg/ml HSA	> 15 mg/ml HSA
NOMINAL PARTICLE SIZE	50 µm	50 µm
RECOMMENDED MAXIMUM LINEAR FLOW RATE	3,000 cm/hr	3,000 cm/hr
AUTOCLAVABLE (121 °C, 30 min)	Yes	Yes
pH STABILITY	1-14	1-14
REGENERATION	70% ethanol	70% ethanol
SANITIZATION	1 M NaOH	1 M NaOH

Column: *1 x 10 cm (4.5 ml)*
Sample: *50 µl (0.6 mg total protein)*
Buffer: *A: 0.1 M Na_2PO_4, pH 7.0*
B: A + 1.85 M $(NH_4)_2SO_4$, pH 7.0
Gradient: *100 % B for 10 min*
100–0 % B in 90 min
Linear flow rate: *38 cm/h*



Retention times (min)

	Methyl	*t-Butyl*
1. Cytochrome c	*7.30*	*10.41*
2. Ovalbumin	*32.25*	*40.22*
3. Amylase	*62.81*	*71.50*
4. Ferritin	*81.49*	*90.90*

Fig. 1. The two different ligands provide alternative selectivities for easier optimization of separation.

Ordering Information

Catalog #	*Description*	*Contents*	*Molecular Weight*	*Isoelectric Point*	*For Use With*
151-1905	Protein Standard for Hydrophobic Interaction Chromatography, 6 vials	Cytochrome c, 1.4 mg Equine myoglobulin, 1.0 mg Lysozyme, 0.8 mg	12,000 17,000 14,300	10.7 6.9 10.0	Econo-Pac HIC cartridges; Macro-Prep HIC supports

Ordering Information

Catalog #	*Description*
MACRO-PREP METHYL HIC SUPPORTS	
158-0080	Macro-Prep Methyl HIC Support, 25 ml
156-0080	Macro-Prep Methyl HIC Support, 100 ml
156-0081	Macro-Prep Methyl HIC Support, 500 ml
156-0082	Macro-Prep Methyl HIC Support, 5 L
156-0083	Macro-Prep Methyl HIC Support, 10 L
MACRO-PREP T-BUTYL HIC SUPPORTS	
158-0090	Macro-Prep t-Butyl HIC Support, 25 ml
156-0090	Macro-Prep t-Butyl HIC Support, 100 ml
156-0091	Macro-Prep t-Butyl HIC Support, 500 ml
156-0092	Macro-Prep t-Butyl HIC Support, 5 L
156-0093	Macro-Prep t-Butyl HIC Support, 10 L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