PROTEIN PURIFICATION

Profinity™ IMAC Resins

- Unique open pore structure facilitates purification of large MW proteins and protein-protein interaction studies
- Excellent purity of target proteins
- Stability from pH 1 to 14
- Compatibility with denaturing agents, detergents, and reducing agents
- Available in uncharged and nickel-precharged forms
- Based on patented UNOsphere™ technology

Selective Profinity IMAC Resins Provide Ultrahigh-Purity Recombinant His-Tagged Proteins

Introduction

Revolutionary advances in genomics and proteomics have made possible the expression of large amounts of proteins of interest, usually in a tagged format, that allow standard chromatography schemes to be used for their purification. The most prevalent affinity tag by far is the polyhistidine tag (His-tag). His-tagged proteins are purified primarily by immobilized metal affinity chromatography (IMAC).

Ni-charged Profinity IMAC resin, because of its optimized ligand density and open pore structure, interacts with high stringency with recombinant His-tagged proteins of a wide molecular weight range. This unique affinity support is based on Bio-Rad's innovative UNOsphere resin* (Figure 1), which enables Profinity IMAC resins to exhibit excellent flow properties without compromising binding, capacity, recovery, or purity.

Resin Characteristics

The Profinity IMAC bead is a 60 µm particle derivatized with iminodiacetic acid (IDA), which functions as the chelating ligand (Figure 2). The chemical structure of IDA allows highly selective binding of recombinant His-tagged proteins when charged with Ni^{2+} or other transition metals. As a result, target proteins can often be purified close to homogeneity in a single step.

The selectivity of Profinity IMAC resin provides specific purification of recombinant His-tagged proteins rather than naturally occurring His-containing proteins.

Characteristics like the polymeric nature, optimized IDA ligand density, and open pore structure of the Profinity IMAC bead result in superb mechanical strength, high selectivity for target proteins, low nonspecific binding, and the ability to perform purifications at extremely fast flow rates (see Specifications). These features lend a number of benefits to the Profinity IMAC resin and help distinguish it from other commercial IMAC adsorbents.

Profinity IMAC resin is stable across the full pH range of 1 to 14 and is compatible with all reagents used in the purification of His-tagged proteins, such as denaturing agents, detergents, and reducing agents. The resin is available in two forms: uncharged and precharged with Ni^{2+}.

* US patent 6,423,666.

BIO-RAD

Specifications

Functional ligand	IDA
Base bead	UNOsphere base matrix
Form	50% suspension in 20% EtOH, precharged with Ni^{2+} or uncharged
Particle size	45–90 µm
Mean particle size	60 µm
Metal ion capacity	12–30 µmol Cu^{2+}/ml
Dynamic binding capacity*	≥15 mg/ml
Recommended linear flow rate	≤600 cm/hr at 25°C
Maximum operating pressure**	7.5 bar (109 psi)
pH stability, uncharged resin*** (up to 200 hr)	1–14
Chemical compatibilities	See Table
Storage	4°C to ambient temperature
Shelf life in 20% EtOH	>1 year at ambient temperature
Operational temperature	4–40°C
Autoclaving conditions	0.1 M sodium acetate at 120°C for 30 min

* Binding capacity was determined by $Q_{10\%}$ determination under the following conditions (dynamic binding capacity will vary from protein to protein):

Column volume	1 ml (7 mm ID x 2.6 cm) column
Sample	1.8 mg/ml pure 32 kD His-tagged protein
Flow rate	1 ml/min loading 2 ml/min wash and elution
Loading buffer	50 mM sodium phosphate, 300 mM NaCl, 5 mM imidazole (pH 8.0)
Wash buffer	Same as loading except 10 mM imidizole
Elution buffer	Same as loading except 250 mM imidazole

** Profinity IMAC resin was packed in a 1.1 x 30 cm column to a bed height of 20 cm with 20 mM sodium phosphate buffer up to 3 bar (43 psi). Flow rates were increased stepwise by 200 cm/hr and held for 2 min at each step. The pressure-flow curve for Profinity IMAC becomes nonlinear at pressures above 7.5 bar (109 psi).

*** Ligand density and protein binding capacity are essentially unchanged after resin is treated with pH 1–14 buffers or other solutions for up to 200 hr.

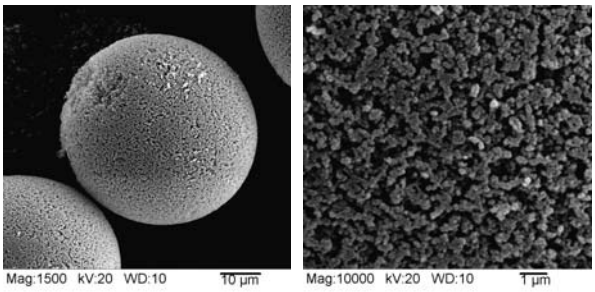

Fig. 1. Scanning electron micrograph of UNOsphere base bead. Left, 1,500x magnification; right, 10,000x magnification.

Table. Chemical compatibilities for binding of target protein to Profinity IMAC Ni-charged resin.*

Reagent	Concentration**
Buffer Reagents	
Tris	50 mM
HEPES	50 mM
MOPS	50 mM
Sodium or potassium phosphate	50 mM
Chelating Agents	
EDTA, EGTA	0.1 mM
Sulfhydryl Reagents	
β-Mercaptoethanol	30 mM
DTT	5 mM
TCEP	10 mM
Detergents	
Nonionic detergents (Triton, Tween, NP-40)	5%
Cationic detergents (CTAB)	1% (care must be taken to avoid protein precipitation)
Zwitterionic detergents (CHAPS, CHAPSO)	5%
Anionic detergents (SDS, Sarkosyl)	1%
Denaturing Agents	
Guanidine HCl	6-M
Urea	8-M
Other Additives	
NaCl	2 M (at least 300 mM NaCl should be included in buffers)
$MgCl_2$	100 mM (HEPES or Tris should be used to prevent precipitation)
$CaCl_2$	10 mM (HEPES or Tris should be used to prevent precipitation)
Glycerol	20%
Ethanol	20%
Imidazole	25 mM in wash buffer 500 mM for elution
Citrate	80 mM

* In order to determine the efficacy of protein binding and recovery, tests were performed using a purified 75 kD protein in a static mode. All tests were carried out using Micro Bio-Spin™ columns packed with Profinity IMAC resin and eluted by centrifugation.

** Profinity IMAC binding capacities are unaffected with typical reagents used for His-tagged protein purification, up to the concentrations given.

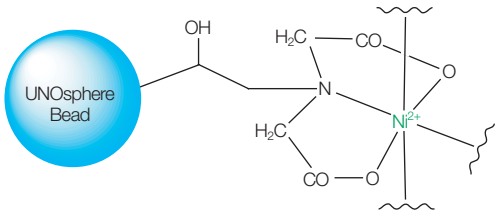

Fig. 2. Partial structure of Profinity IMAC resin. Illustration of UNOsphere bead with coupled IDA functional ligand, shown charged with Ni^{2+}. Wavy lines indicate available binding sites.

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High Chemical Compatibility

The chemical stability of Profinity IMAC permits use of a wide range of reagents. As shown in Figure 3, binding capacity, purity, and recovery are unaffected even by strong reducing agents, such as 5 mM DTT. The Table lists the compatibility of Profinity IMAC resin (up to the concentrations given) with some reagents that are commonly used for recombinant protein purification.

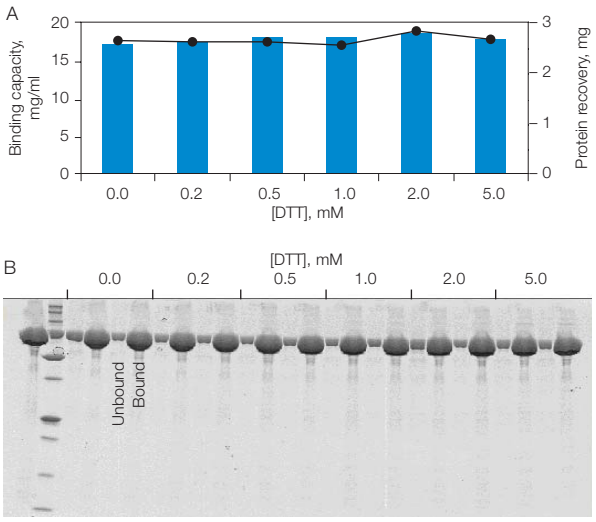

Fig. 3. Performance of Profinity IMAC in the presence of strong reducing agents. Purified protein (~2.5 mg) was loaded onto a Micro Bio-Spin column packed with 100 µl of preequilibrated Profinity IMAC resin. The binding buffer was 50 mM potassium phosphate, 300 mM NaCl (pH 8.0) and the elution buffer was the binding buffer plus 500 mM imidazole; the concentration of DTT in the buffers differed as indicated. A, protein binding capacity and recovery. ▬, binding capacity; —●—, protein recovery. B, SDS-PAGE analysis of duplicate samples. Unbound and bound fractions are shown in alternating lanes.

Performance Benefits

Inherent structural characteristics along with the chemical stability of Profinity IMAC resin provide a variety of benefits. Purity, the most important benefit, results from the stringency of interaction provided by the resin's optimal ligand density. Profinity IMAC specifically selects for recombinant His-tagged proteins over naturally occurring His-containing proteins, resulting in greater target protein purity. A clear indication of the higher purity achieved by Profinity IMAC resin over other commercial adsorbents is shown in Figure 4. Using Quantity One® software, purity levels of up to 93% were determined quantitatively for samples from each resin.

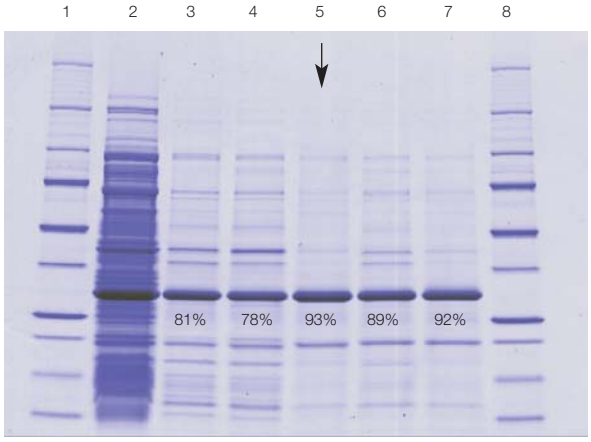

Fig. 4. Purification of a putative aminopeptidase protein using different IMAC resins. An insoluble 32 kD protein obtained from *Anabaena* sp. strain PCC 7120 (courtesy of Dr Ray Stevens, University of California, Berkeley, CA, USA) was expressed in *E. coli* and purified under denaturing conditions. *E. coli* lysate was loaded onto Micro Bio-Spin columns containing individual IMAC resins and purified. The binding buffer was 50 mM potassium phosphate, 300 mM NaCl, 8 M urea (pH 8.0), and the elution buffer was binding buffer plus 250 mM imidazole. To determine purity of the target protein, 3 µg of sample eluate from each column was loaded and separated on a Criterion™ gel, stained with Coomassie Blue, then quantitated using Quantity One software. Lanes 1 and 8, 10 µl Precision Plus Protein™ standards; lane 2, 3 µl crude lysate; lane 3, Ni-charged, high-binding-capacity agarose-based resin from supplier A (IDA ligand); lane 4, uncharged agarose-based resin from supplier A (IDA ligand), charged with Ni^{2+}; lane 5, Profinity IMAC Ni-charged resin; lane 6, Ni-charged agarose-based resin from supplier B (NTA ligand); lane 7, Co^{2+}-charged tetradentate agarose (supplier C). The purity obtained for each resin is indicated; arrow highlights result obtained with Profinity IMAC.

Profinity IMAC and UNOsphere Technology

UNOsphere beads, on which Profinity IMAC resin is based, were designed to achieve high productivity (grams drug or target per operational hour per liter of support). UNOsphere media may be run with solutions of different viscosity at high flow rates, well within the pressure limits of low-pressure column and chromatography systems (Figure 5).

The dynamic binding capacities of Profinity IMAC and other IMAC adsorbents were evaluated using a purified soluble protein — NGG1P interacting factor 3 (NIF-3; 32 kD). For evaluation, the A_{280} for the purified protein is first measured. Dynamic binding capacity is determined by continuously loading a sample of known concentration to a column and monitoring the protein in the column flow-through. When the quantity of the protein in the flow-through exceeds 10% ($Q_{10\%}$ or 10% breakthrough), sample application stops. Next, the column was washed and eluted. The amount of protein in mg that has been applied up to a certain breakthrough point is a measure of dynamic binding capacity of the IMAC resin. The broader

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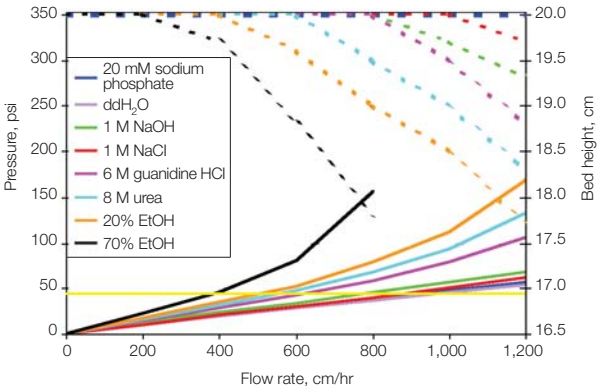
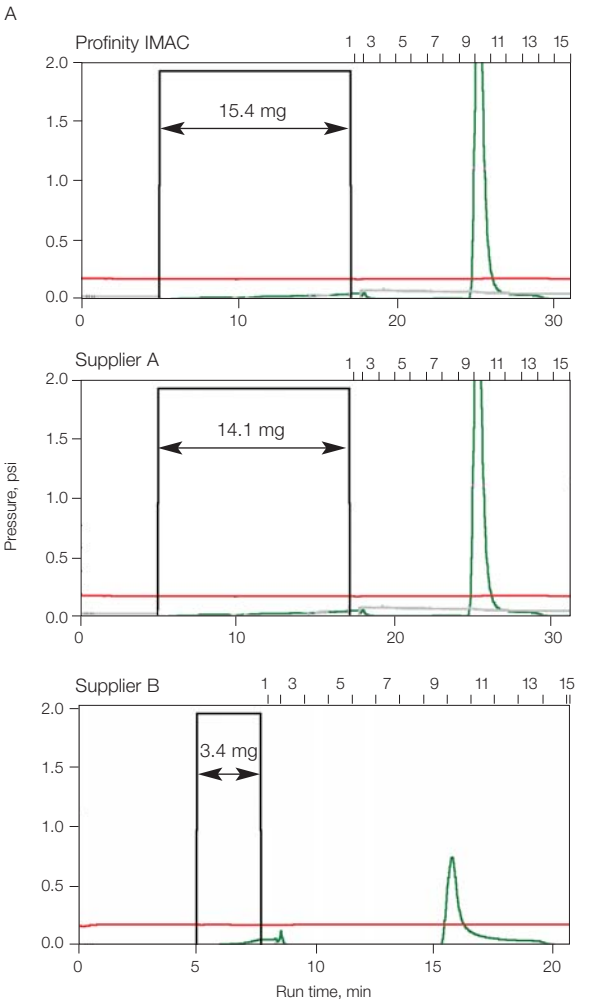


Fig. 5. Shrink-swell test on Profinity IMAC resin. Common reagents used in His-tagged protein purifications were run on a 1.1 x 30 cm Amicon column packed with Profinity IMAC resin to a bed height of 20 cm. System pressure and column bed height compression were recorded for each flow rate. Flow rates were increased stepwise to 200 cm/hr and held for 2 min at each step. All tests were performed on a BioLogic DuoFlow Maximizer™ system. Yellow horizontal line indicates 43 psi (3 bar), the maximum recommended operating pressure.

the peak obtained during loading without product breakthrough (see arrows in Figure 6A), the greater the binding capacity is for that resin. The level of saturation of the columns was determined by using a breakthrough capacity measured at 10% ($Q_{10\%}$) and shows how the capacity of Profinity IMAC resin outperforms products from other suppliers (Figure 6).

Excellent flow properties, high maximum operating pressures, and high flow rates for fast cleaning, sanitizing, and reequilibration are among the other benefits provided by the polymeric nature and open pore structure of Profinity IMAC resin. A good example of the productivity of Profinity IMAC resin is the consistency with which His-tagged dynamic binding capacity and selectivity at various flow rates are maintained. Figure 7 demonstrates the rapid binding kinetics of this resin, whether the column is run at 150 cm/hr or at 600 cm/hr. Regardless of flow



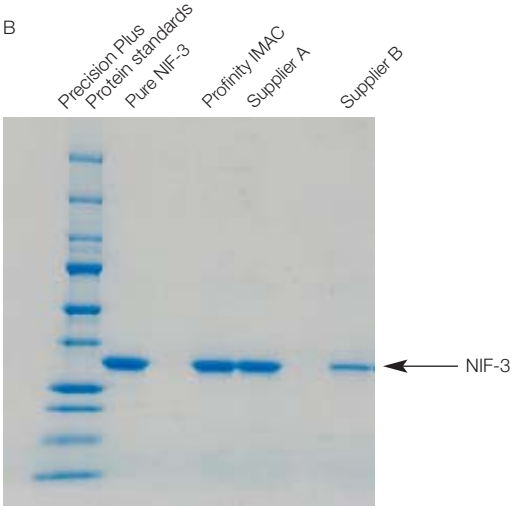


Fig. 6. Comparison of dynamic binding capacity for a His-tagged NIF-3 protein. A, chromatograms. B, SDS-PAGE analysis. A sample (1.8 mg/ml) of a purified 32 kD soluble protein, NIF-3, was continuously loaded using a BioLogic DuoFlow™ system onto 1 ml columns containing either Profinity IMAC (IDA ligand), an uncharged agarose-based resin from supplier A (IDA ligand) that was charged with Ni^{2+} before chromatography, or a Ni-charged agarose-based resin from supplier B (NTA ligand) until $Q_{10\%}$ breakthrough was achieved (black arrows indicate time to breakthrough; values are protein loaded). A BioLogic DuoFlow system was used to load the sample in 50 mM sodium phosphate, 300 mM NaCl, 5 mM imidazole (pH 8.0) at 1 ml/min. After 10% breakthrough, the columns were washed in loading buffer with 10 mM imidazole and eluted in loading buffer with 250 mM imidazole, all at 2 ml/min. For SDS-PAGE, a 10 µl aliquot of the sample load or eluate was loaded per lane.

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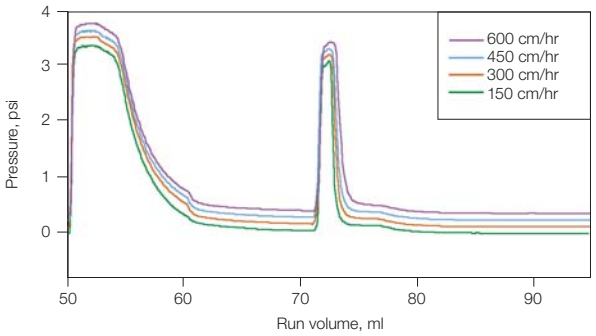

Fig. 7. Consistent binding capacity and selectivity for a His-tagged protein at different flow rates. Crude *E. coli* lysate was loaded onto a 1 ml column (Amersham HR 5/5) preequilibrated with loading buffer (50 mM potassium phosphate and 0.3 M NaCl, pH 8.0) using the BioLogic DuoFlow system. The His-tagged protein was eluted with buffer containing 50 mM potassium phosphate, 0.3 M NaCl, and 500 mM imidazole (pH 8.0). Elution was monitored at 280 nm. Proteins eluted from the column were collected in 1 ml fractions.

rate, the target protein was still eluted in the same number of fractions. Accordingly, the dynamic binding capacity at 600 cm/hr was comparable to that obtained when the column was run at 150 cm/hr at 20.3 and 21.8 mg/ml, respectively.

Flow vs. backpressure properties of Profinity IMAC resin are also advantageous when regeneration, cleaning, sanitization, and reequilibration are necessary (Figure 8).

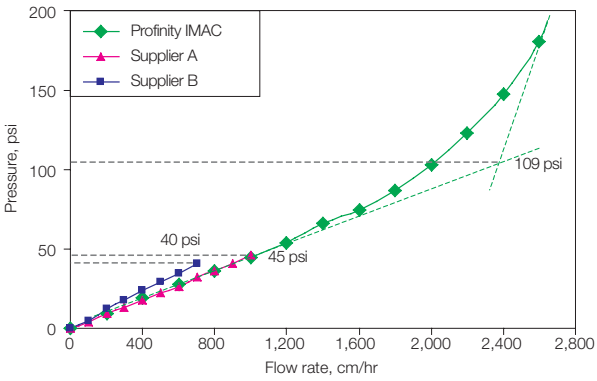

Fig. 8. Comparison of maximum operating pressure for different IMAC resins. The pressure values shown are the estimated maximum operating pressures for each resin. Beyond these pressures, the column beds are seriously compressed and damaged. Resins were converted to a 50% (v/v) slurry in 20 mM sodium phosphate buffer and packed in a 1.1 x 20 cm column to a bed height of 20 cm up to 43 psi (3 bar). Flow rates were then increased stepwise by 200 cm/hr and held for 2 min at each step. The pressure-flow curve for Profinity IMAC resin became nonlinear only at pressures above 109 psi (7.5 bar), the point defined as the intersection of the two tangents on the pressure-flow curve.

Repeated Cycling Stability Ensures Reproducible Results

Profinity IMAC resin may be used many times without affecting the quality or performance of this resin. To demonstrate the durability of the material, Profinity IMAC resin was subjected to 201 cycles of use — the sample was loaded onto the column at the beginning of cycle 1 and after every interval of 50 wash cycles. The resulting chromatogram (Figure 9) demonstrates that Profinity IMAC resin, after repeated cycling, delivers consistent and reproducible separation results.

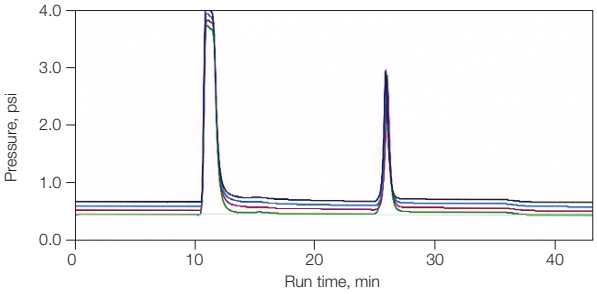

Fig. 9. BioLogic DuoFlow system overlay report of a cycling study performed using Profinity IMAC resin. A 1 ml column packed with Profinity IMAC was subjected to 201 cycles of use. Sample (250 µl lysate containing 3.51 mg of 75 kD recombinant His-tagged protein) was loaded onto the column at cycle 1 and after every interval of 50 wash cycles, which included the following steps:

Strip	50 mM EDTA, 50 mM sodium phosphate, 300 mM NaCl (pH 7.5)
Clean	50 mM sodium acetate, 300 mM NaCl (pH 4.0)
Charge	100 mM $NiSO_4$ (pH 4.0)
Clean	50 mM sodium acetate, 300 mM NaCl (pH 4.0)
Equilibrate	50 mM sodium phosphate, 300 mM NaCl (pH 8.0)
Sanitize	1.0 N NaOH (this step skipped before sample injection)

Recommended Columns for Packing

Profinity IMAC and Ni-charged IMAC resins are available in both small and large packs, ranging from 10 ml to 1 L. The resin is easy to pack and may be used in medium-pressure, gravity-flow, and spin columns. Bio-Rad offers a variety of columns, including glass Econo-Column® columns for gravity and low-pressure purifications, Bio-Scale™ MT high-resolution columns for medium-pressure purifications, and empty Bio-Spin® and Micro Bio-Spin columns for gravity and spin-column purifications.

Storage, Shelf Life, and Stability

Profinity IMAC resin is stable at room temperature and across the pH range (1–14). The resin may be stored in any of the following solutions:

- 1 N NaOH (up to 200 hr)
- 1% acetic acid and 0.12 M phosphoric acid, pH 1.5 (up to 200 hr)
- 2% benzyl alcohol
- 20% ethanol

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Ordering Information

Catalog #	Description
Profinity IMAC Resins*	
156-0121	Profinity IMAC Resin, 10 ml
156-0123	Profinity IMAC Resin, 50 ml
156-0125	Profinity IMAC Resin, 500 ml
156-0127	Profinity IMAC Resin, 1 L
156-0131	Profinity IMAC Ni-Charged Resin, 10 ml
156-0133	Profinity IMAC Ni-Charged Resin, 25 ml
156-0135	Profinity IMAC Ni-Charged Resin, 100 ml
156-0137	Profinity IMAC Ni-Charged Resin, 500 ml

Related Products

Catalog #	Description
Bio-Scale Columns	
751-0081	Bio-Scale MT2 Column, 7 x 52 mm
751-0083	Bio-Scale MT5 Column, 10 x 64 mm
751-0085	Bio-Scale MT10 Column, 12 x 88 mm
751-0087	Bio-Scale MT20 Column, 15 x 113 mm
Micro Bio-Spin Columns	
732-6204	Micro Bio-Spin Chromatography Columns, empty, 100
731-1660	End Caps, for Micro Bio-Spin chromatography columns, 1,000
Econo-Column Selection Packs**	
737-6601	Econo-Column Selection Pack A, includes 7 columns, 1 each of 0.7 x 10, 20, and 30 cm; 1.5 x 30 and 50 cm; 2.5 x 20 and 50 cm
737-6607	Econo-Column Selection Pack B, includes 6 columns, 1 each of 1.0 x 20, 30, and 50 cm; 1.5 x 20, 30, and 50 cm
BioLogic DuoFlow Systems	
760-0037	BioLogic DuoFlow Basic System, 100/120 V, includes Dell controller and monitor, USB Bitbus communicator, F10 workstation, MX-1 mixer, 3-tray rack, AVR7-3 sample inject valve, fittings kit, UV detector with 5 mm flow cell and 254/280 nm filters, conductivity monitor, starter kit, UNO® Q1 column, instructions
760-0036	BioLogic DuoFlow Basic System, 100/120 V, for Japan and Korea only
760-0038	BioLogic DuoFlow Basic System, 220/240 V
760-0047	BioLogic DuoFlow Standard System, 100/120 V, same as 760-0037 with BioFrac™ fraction collector, diverter valve, two F1 racks
760-0046	BioLogic DuoFlow Standard System, 100/120 V, for Japan and Korea only
760-0048	BioLogic DuoFlow Standard System, 220/240 V
BioLogic DuoFlow QuadTec™ Systems	
760-1137	BioLogic DuoFlow QuadTec Basic System, 100/120 V, includes Dell controller and monitor, USB Bitbus communicator, F10 workstation, MX-1 mixer, 3-tray rack, AVR7-3 sample inject valve, fittings kit, QuadTec UV/Vis detector with 3 mm PEEK flow cell, instrument control module (ICM), system cables 25 and 26 (RS-232 and ICM power), conductivity monitor, starter kit, UNO Q1 column, instructions

Catalog #	Description
BioLogic DuoFlow QuadTec Systems, cont.	
760-1136	BioLogic DuoFlow QuadTec Basic System, 100/120 V, for Japan and Korea only
760-1138	BioLogic DuoFlow QuadTec Basic System, 220/240 V
760-1147	BioLogic DuoFlow QuadTec Standard System, 100/120 V, same as 760-1137 with BioFrac fraction collector, diverter valve, two F1 racks
760-1146	BioLogic DuoFlow QuadTec Standard System, 100/120 V, for Japan and Korea only
760-1148	BioLogic DuoFlow QuadTec Standard System, 220/240 V
BioLogic DuoFlow Maximizer Systems	
760-2237	BioLogic DuoFlow Maximizer 20 System, 100/120 V, includes Maximizer valve unit, Maximizer mixer, pH monitor, Dell controller and monitor, USB Bitbus communicator, F10 workstation, MX-1 mixer, 3-tray rack, AVR7-3 sample injection valve, fittings kit, UV detector with 5 mm flow cell and 254/280 nm filters, conductivity monitor, starter kit, UNO Q1 column, BioFrac fraction collector, diverter valve, two F1 racks, instructions
760-2236	BioLogic DuoFlow Maximizer 20 System, 100/120 V, for Japan and Korea only
760-2238	BioLogic DuoFlow Maximizer 20 System, 220/240 V
760-2247	BioLogic DuoFlow Maximizer 80 System, 100/120 V, same as 760-2237 with F40 workstation replacing F10 workstation
760-2246	BioLogic DuoFlow Maximizer 80 System, 100/120 V, for Japan and Korea only
760-2248	BioLogic DuoFlow Maximizer 80 System, 220/240 V
BioLogic DuoFlow Pathfinder™ Systems	
760-2257	BioLogic DuoFlow Pathfinder 20 System, 100/120 V, includes Maximizer valve unit, Maximizer mixer, pH monitor, Dell controller and monitor, USB Bitbus communicator, F10 workstation, MX-1 mixer, 3-tray rack, AVR7-3 sample inject valve, fittings kit, QuadTec UV/Vis detector with 3 mm PEEK flow cell, system cable 25 (RS-232), conductivity monitor, starter kit, UNO Q1 column, BioFrac fraction collector, diverter valve, two F1 racks, instructions
760-2256	BioLogic DuoFlow Pathfinder 20 System, 100/120 V, for Japan and Korea only
760-2258	BioLogic DuoFlow Pathfinder 20 System, 220/240 V
760-2267	BioLogic DuoFlow Pathfinder 80 System, 100/120 V, same as 760-2257 with F40 workstation replacing F10 workstation
760-2266	BioLogic DuoFlow Pathfinder 80 System, 100/120 V, for Japan and Korea only
760-2268	BioLogic DuoFlow Pathfinder 80 System, 220/240 V

* Larger volumes for industrial applications are available on request.

** Many more Econo-Column chromatography columns are available. The complete selection can be found in the current Life Science Research Products catalog.

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