Product Data Sheet

BioFox™ Mini-Columns

Catalog Number: 10200, 10201 and 10222 to 10231

AthenaES®
a division of Athena Environmental Sciences, Inc.

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Product Description

The BioFox™ Mini-Columns are pre-packed 1 ml columns. The columns are packed with BioFox™ 40 µm agarose resins for size exclusion (SEC), ion exchange (IEX), immotilized metal affinity (IMAC) and affinity chromatography (ACT). These resin beads are made using a proprietary cross-linking process that yields a highly porous, physically stable resin. The beads can withstand pressures up to 40 bar (580 psi). The high pressure tolerance coupled with a narrow particle size range allows for high resolution separations without loss of process time.

Ion Exchange

Ion exchange chromatography is the process of separating biomolecules based on their ionic state. For the target molecule to bind to a given resin, it must have a net charge. Therefore, the pH of the solution should be at least 1 pH unit above or below the pI of the molecule. In anion exchange chromatography the negatively charged molecule binds to positively charged resin whereas in cation exchange chromatography the positively charged molecule binds to negatively charged resin. For the separation of proteins, the practical pH range is 5.5 to 9.0. Bound molecules are desorbed from the resin by neutralizing the charge with increasing concentrations of counter ions, often NaCl or KCl.

Size Exclusion

Size exclusion chromatography is the separation molecules based on their size. The SEC Mini-Columns are filled with a non-derivatized 40 µm agarose resin with exclusion limit ratings of 150, 1,200 and 10,000 kDa. These columns are ideal for rapid desalting and buffer exchange procedures of 0.05 to 2.0 mg of protein. They are also suitable for the screening of aggregates in protein samples and after refolding procedures as well as for the quick assessment of the molecular mass of a target protein.

Immobilized Metal Affinity

Immobilized metal affinity chromatography is an affinity chromatography procedure used for separating proteins that can bind cation metals. The metal ions are immobilized on the agraose resin using a chelation chemistry that permits coordination between two of the non-complexed valencies of the metal ion with histidine or cystidine residues of the protein. These resins are particularly useful for single-step purification of recombinant proteins to which a hexa-His tag has been attached at the N- or C-terminus of the peptide sequence. This tag provides high affinity binding to the immobilized metal thereby enhancing the efficiency of the affinity separation. The IMAC Mini-Columns are available with IDA or TREN metal cheleating compounds with a high or low ligand density.

Affinity

The ACT Mini-Columns are filled with 40 µm agarose resins that are pre-activated for the attachment of ligands, proteins or antibodies. The coupling is via a bromhydrin reaction that covelently attaches the desired ligand or protein to the solid support. Coupling is via an amine, sufhydryl or hydroxyl group in an aqueous buffer at neutral or alkaline pH and at room temperature. Purification of the target protein is by its affinity to the immobilized ligand. This resin is particularly suitable for immunoaffinity or protein-protein affinity chromatography.

Specifications:

Resin Properties	Specification
Particle Size (µm)	32-60
Agarose Content	7.5-7.8%
Flow Rate (ml/min)	0.1-5.0
pH Stability	1-14
Solvent Resistance	
100% ethanol, 100% methanol, 6 M Guanidine HCl, 30% acetonitrile, 70% formic acid, 1 M NaOH, 0.1 M HCl, 5% SDS, 5% mercaptoethanol, 30% acetic acid, 0.1% trifluoroacetic acid	

Catalog Numbers:

(Columns are sold in boxes of six.)

Column Type	Cat. No.
Mix & Match: Pick any six columns	10200
Immobilized Metal Affinity Screening Kit - 2 TREN High, 1 TREN Low, 2 IDA High, 1 IDA Low	10201
40Q Strong Anion Exchange	10222
40S Strong Cation Exchange	10223
40DEAE Weak Anion Exchange	10224
SEC 40/1,200 kDa Exclusion	10225
SEC 40/150 kDa Exclusion	10226
SEC 40/10,000 kDa Exclusion	10227
IMAC-TREN 3 high, 3 low	10228
IMAC-IDA 3 high, 3 low	10229
ACT Affinity	10230
Bulk Columns package of 10 with flow adapters and stop plugs	10231

BioFox™ Resins:

Available as bulk resins or prepacked columns. Visit *www.athenaes.com/Biofox-Resins.php* for more information.

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Instructions for Use:

The BioFox™ Mini-Columns are intended for small-scale separations and methods development and scouting. Before using the Mini-Column, remove the storage solution (20% ethanol) by flushing it with deionized water or low ionic strength buffer. Washing is best done at 1 ml/min for 15 min (15 CV). When not in use, the Mini-Columns should be stored in a solution of 20% ethanol. Sanitize with 0.5 N NaOH at a flow rate of 0.25 ml/min for 1 h.

Resin Preparation: Prior to the first chromatographic run or following storage, the resin should be washed and equilibrated at the desired starting conditions. Prepare Buffer A, the "Equilibration" or "Wash" Buffer, and Buffer B, the "Elution" Buffer, at the desired pH and ionic strengths. Wash the Column with 5 CV (5 ml) of Buffer A at a flow rate of 1 ml/min. Switch to Buffer B and wash with 5 CV (5 ml) at a flow rate of 1 ml/min. Equilibrate the resin in Buffer A by washing with 10 CV at 1 ml/min (10 ml). Suitable buffers and salts are listed in the table to the right.

IMAC Mini-Columns should be charged with the desired metal ion by washing the resin with 5 CV of 100 mM cation salt followed by 5 CV of water. Pre-cycle as described below. TREN and IDA resins can be charged with Ni, Co, Ca, Cu, Zn or Fe metals.

The ACT affinity resin is prepared by reacting with the desired ligand under neutral to alkaline conditions. The ACT resin employs bromhydrin chemistry to couple amine, sulfhydryl or hydroxyl groups to the bead. Prepare the ligand in 50 mM phosphate, borate or carbonate at a pH between 7 and 9 (use pH 12 for -OH coupling). Do not use an amine or organic acid buffers. React the resin with the ligand solution for 16 to 20 h at room temperature. Wash the column with buffer containing excess glycine, hydroxylamine or ethanolamine and eqilibrate in the desired starting buffer.

Generalized Chromatographic Separation: Absorption fractionation of a protein solution (IEX, IMAC or ACT) involves three basic phases: Loading, washing, eluting. Ideally, before loading the protein solution onto the resin, the solution should be exchanged into Buffer A. This can be done by dialysis, gel filtration (desalting), diafiltration or dilution. To perform the separation, wash the column with Buffer A to establish a baseline absorbance at 280 nm. Load the sample at a flow of 0.1 to 5.0 ml/min (20-1,000 cm/h) until the absorbance at 280 nm returns to baseline. The optimum flow rate should be determined empirically, however, 1 ml/min is typical and a good starting point. Once the absorbance has returned to baseline, wash the resin with 5 to 10 CV (5 to 10 ml) of Buffer A. Elute the bound protein by applying increasing amounts of Buffer B. Initial separations should be done using a 10-20 CV (10-20 ml) linear gradient of 0-100% Buffer B or a step gradient in 100 mM or 1 pH unit increments. Continue washing with 100% Buffer B for an addtional 5 CV (5 ml). Collect 0.5 to 1.0 CV fractions during the elution and Buffer B wash phase. Switch to Buffer A and wash the resin for 5 CV (5 ml) to reequilibrate the resin.

Buffer Recommendations:

Size Exclusion	
25-100 mM Buffer pH 5.0 to 9.0	100-500 mM NaCl, KCl, Na- or K- phosphate
Ion Exchange	
pH Range	**Buffer**
Anion Exchange	
8.5-9.0	Diethanolamine
7.5-8.5	Tris
6.5-7.5	Imidazole
5.5-6.5	Histidine
5.0-5.5	Pyridine
Cation Exchange	
7.5-8.5	Tricine
6.5-7.5	MOPS
5.5-6.5	MES
5.0-5.5	Acetic Acid
Adjust pH with HCl, NaOH or KOH. Use 1 M NaCl or KCl as the displacing ions.	
Immobilized Metal Affinity	
Equilibration Buffer	50 mM K_2HPO_4, 300 mM NaCl, 10 mM imidazole pH 7.0
Elution Buffer	50 to 250 mM Imidazole pH 6.5
Affinity	
Equilibration Buffer (protein-dependent)	neutral pH with moderate ionic strength (50-250 mM)
Elution Buffer (protein-dependent_	low/high pH, competitive displacement, chaotrophic salts

Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information see full Material Safety Data Sheet.