

# Affinity

CHROMATOGRAPHY

# columns AND media



Product profile

## Affinity Chromatography (AC)

Affinity Chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand attached to a chromatographic matrix. Affinity Chromatography can be used whenever a suitable ligand is available.

The target protein(s) is specifically and reversibly bound by a complementary binding substance (ligand). The sample is applied under conditions that favour specific binding to the ligand. Unbound material is washed away, and the bound target protein is recovered by changing conditions to those favouring desorption. Desorption is performed specifically, using a competitive ligand, or non specifically, by changing the pH, ionic strength or polarity. Proteins are concentrated during binding and collected in a purified, concentrated form. The key stages in a separation are shown in Figure 1.

Affinity Chromatography may also be used to remove specific contaminants, for example Benzamidine Sepharose FF (high sub) removes serine proteases such as trypsin, thrombin and factor Xa, and Blue Sepharose HP removes albumin.

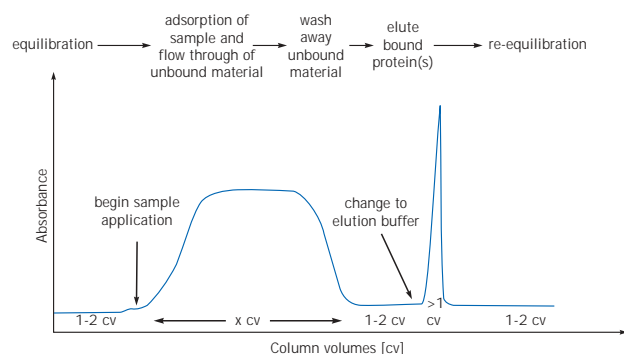


Figure 1. Typical affinity separation.

## Media selection

Parameters such as scale of purification and commercial availability of affinity matrices should be considered when selecting affinity media.

HiTrap affinity columns are ideal for method optimization or small scale purification of target proteins using well established protocols.

Affinity media can be prepared by coupling a ligand to a selected gel matrix. HiTrap NHS-activated HP is designed specifically to facilitate this process and is supplied with a recommended coupling procedure for coupling primary amines.

For separations of glycoproteins and polysaccharides, media screening may be required to select the correct specificity.

## Immunoglobulins

While protein A and protein G affinity media are similar in many respects, their specificities for IgG differ. Protein G affinity media are the better choice for general purpose capture of antibodies since they bind IgG from a broader range of eukaryotic species and bind more subclasses of IgG. Species-specific examples include stronger binding of polyclonal IgG from cow, sheep and horse to protein G. Polyclonal rat IgG, human IgG<sub>3</sub> and mouse IgG<sub>1</sub> are bound by protein G but not by protein A. Generally, protein G has greater affinity for IgG and minimal binding of albumin resulting in cleaner preparations and greater yield.

Conversely, protein A may be the better choice for isolating certain subclasses of IgG or for removing cross-species IgG contaminants from horse or foetal calf serum, for example.

Purification of human and mouse IgM is possible by the use of HiTrap IgM Purification HP 1 ml column. The thiophilic adsorption media with 2-mercaptopyridine coupled to Sepharose HP is designed for one-step purification protocol resulting in 80–95% pure IgM.

Purification of IgY from egg yolk is easily performed using HiTrap IgY Purification HP 5 ml column. The purity is over 70% in one-step using this special designed medium.

## Fusion proteins

Expression of fusion proteins is needed when larger quantities of target protein are required for further characterization. We offer products to facilitate every step in this process, from choosing the correct expression system through to selecting the most suitable purification solution for GST and His-tagged proteins.

Purification of a glutathione S-transferase fusion protein is simple, using mild elution conditions that minimize the risk of damage to the functionality of the target protein. The GST-tag is easily detected and can be removed in one-step if required.

For routine purification of larger quantities of GST-tagged proteins, GSTrap FF, prepacked HiTrap 1 ml and 5 ml columns with Glutathione Sepharose 4 FF and HisTrap or HiTrap Chelating HP, for His-tagged proteins, provide the ideal solution. The columns are compatible with ÄKTA design chromatography systems to ensure reproducible results under optimized conditions.

## Optimization parameters

1. Select correct specificity for target protein.
2. Follow manufacturer's recommendations for binding and elution conditions.
3. Select optimum flow rate for sample application to achieve efficient binding.
4. Select optimum flow rate for elution to maximize recovery.
5. Select maximum flow rate for column regeneration to minimize run times.

## Media and prepacked columns for group specific purification

Ordering information	Product	Binding capacity per ml media (approx.)	Particle diameter $\mu\text{m}$	Maximum operating flow rate <sup>1</sup>	Maximum operating pressure	pH stability <sup>2</sup>		Application areas, purification, isolation or removal of the following substances	
						Long term	Short term		
<b>Code No.</b>	<b>Prepacked columns</b>	<b>Column size</b>							
17-0412-01 17-0413-01	HiTrap™ Blue HP	5 x 1 ml 1 x 5 ml	20 mg human albumin	24–44	4 ml/min 20 ml/min	0.3 MPa, 3 bar	4–12	3–13	Albumin, broad range of nucleotide-requiring enzymes, coagulation factors.
17-0406-01 17-0407-01	HiTrap Heparin HP	5 x 1 ml 1 x 5 ml	3 mg antithrombin III	24–44	4 ml/min 20 ml/min	0.3 MPa, 3 bar	5–10	5–10	Antithrombin III and other coagulation factors, lipoproteins, lipases, protein synthesis factors, DNA binding proteins.
17-5189-01	HiPrep™ 16/10 Heparin FF	20 ml	2 mg bovine antithrombin III	45–165	10 ml/min	0.15 MPa, 1.5 bar	4–12	4–13	Antithrombin III and other coagulation factors, lipoproteins, lipases, protein synthesis factors, DNA binding proteins.
17-5130-02 17-5130-01 17-5131-01	GSTrap™ FF	2 x 1 ml 5 x 1 ml 1 x 5 ml	10 mg recombinant GST or 11 mg GST fusion protein (M, 43 000)	45–165	4 ml/min 15 ml/min	0.3 MPa, 3 bar	3–12	3–12	Glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
17-5234-01	GSTPrep™ FF 16/10	20 ml	See above	45–165	10 ml/min	0.15 MPa, 1.5 bar	3–12	3–12	Glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
17-5112-01	HiTrap Streptavidin HP	5 x 1 ml	Biotin >300 nmol, 6 mg biotinylated BSA	24–44	4 ml/min	0.3 MPa, 3 bar	4–9	2–10.5	Biotinylated substances, such as biotin-tagged proteins.
17-5143-02 17-5143-01 17-5144-01	HiTrap Benzamidin FF (high sub)	2 x 1 ml 5 x 1 ml 1 x 5 ml	$\geq$ 35 mg trypsin	45–165	4 ml/min 20 ml/min	0.3 MPa, 3 bar	2–8	1–9	Trypsin and trypsin-like serine proteases (e.g. thrombin and factor Xa).
<b>Code No.</b>	<b>Media</b>	<b>Pack size</b>							
17-0700-01	2',5' ADP Sepharose™ 4B	5 g	0.4 mg glucose-6-phosphate dehydrogenase	45–165	75 cm/h	0.02 MPa, 0.2 bar	4–10	4–10	NADP <sup>+</sup> - dependent dehydrogenases and other enzymes which have affinity for NADP <sup>+</sup> , e.g. glucose-6-phosphate dehydrogenase.
27-3608-02	Agarose Wheat Germ Lectin	5 ml	Ligand concentration 1–2 mg	45–165	75 cm/h	0.02 MPa, 0.2 bar	4–9	4–9	Molecules containing N-acetyl-glucosamine. Chitobiose core of N-linked oligosaccharides, (GlcNAc $\beta$ 1,4GlcNAc $_{1-2}$ ) $\beta$ GlcNAc and sterically related residues like glycoproteins, membrane proteins, glycolipids, lipoproteins, polysaccharides.
17-0620-01	5' AMP Sepharose 4B	5 g	10 mg lactate dehydrogenase	45–165	75 cm/h	0.02 MPa, 0.2 bar	4–10	4–10	NAD <sup>+</sup> - dependent enzymes, aldehyde and formate dehydrogenases, ATP-dependent enzymes, cAMP dependent protein kinases.
17-0524-01	Arginine Sepharose 4B	25 ml	Ligand concentration 14–20 $\mu\text{mol}$ arginine/ml	45–165	75 cm/h	0.02 MPa, 0.2 bar	2–13	2–13	Serine proteases, purification and isolation of coagulation factors, plasminogen and plasminogen activator.
17-0568-01	Benzamidin Sepharose 6B	25 ml	13 mg trypsin	45–165	75 cm/h	0.03 MPa, 0.3 bar	3–11	2–13	Trypsin and trypsin-like serine proteases (e.g. thrombin and factor Xa).
17-5123-10	Benzamidin Sepharose 4 FF (high sub)	25 ml	$\geq$ 35 mg trypsin	45–165	400 cm/h	0.1 MPa, 1 bar	2–8	1–9	Trypsin and trypsin-like serine proteases (e.g. thrombin and factor Xa).
17-0948-01	Blue Sepharose 6 FF <sup>8</sup>	50 ml <sup>7</sup>	$\geq$ 18 mg human albumin	45–165	400 cm/h	0.1 MPa, 1 bar	4–12	3–13	Albumin, broad range of nucleotide-requiring enzymes, coagulation factors. Ideal for scale up applications.
17-0529-01	Calmodulin Sepharose 4B	10 ml	Ligand concentration 1 mg/ml	45–165	75 cm/h	0.02 MPa, 0.2 bar	4–9	4–9	ATPases, protein kinases, phosphodiesterases, neurotransmitters, interferon, calmodulin-binding peptide (CBP) fusion protein.
17-0440-03 17-0440-01	Con A Sepharose 4B	5 ml 100 ml <sup>7</sup>	20–45 mg thyroglobulin	45–165	75 cm/h	0.02 MPa, 0.2 bar	4–9	4–9	Molecules containing branched mannoses, carbohydrates with terminal mannose or glucose, ( $\alpha\text{Man}$ - $\alpha\text{Glc}$ -GlcNAc) and sterically related residues like glycoproteins, membrane proteins, glycolipids, lipoproteins, polysaccharides, hormones, $\alpha_1$ -antitrypsin, interferon.
17-0956-01	Gelatin Sepharose 4B	25 ml <sup>7</sup>	1 mg plasma fibronectin	45–165	75 cm/h	0.02 MPa, 0.2 bar	3–10	3–10	Fibronectin.
17-5132-01 17-5132-02 17-5132-03	Glutathione Sepharose 4 FF	25 ml 100 ml 500 ml	10 mg recombinant GST or 11 mg GST fusion protein (M, 43 000)	45–165	450 cm/h	0.1 MPa, 1 bar	3–12	3–12	Glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
17-0756-01	Glutathione Sepharose 4B	10 ml	8 mg horse liver GST	45–165	75 cm/h	0.02 MPa, 0.2 bar	3–13	3–13	Glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
17-0467-01	Heparin Sepharose CL-6B	10 g	2 mg antithrombin III	45–165	150 cm/h	0.05 MPa, 0.5 bar	5–10	5–10	Antithrombin III and other coagulation factors, lipoproteins, lipases, protein synthesis factors, DNA binding proteins.
17-0998-01	Heparin Sepharose 6 FF <sup>8</sup>	50 ml <sup>7</sup>	2 mg bovine antithrombin III	45–165	400 cm/h	0.1 MPa, 1 bar	4–12	4–13	Antithrombin III and other coagulation factors, lipoproteins, lipases, protein synthesis factors, DNA binding proteins.
17-0969-01	IgG Sepharose 6 FF <sup>8</sup>	10 ml <sup>7</sup>	2 mg protein A	45–165	400 cm/h	0.1 MPa, 1 bar	3–10	3–10	Recombinant fusion proteins containing a protein A tag.
17-0444-01	Lentil Lectin Sepharose 4B	25 ml	16–35 mg thyroglobulin	45–165	75 cm/h	0.02 MPa, 0.2 bar	3–10	3–10	Molecules containing branched mannoses with fucose linked $\alpha(1,6)$ to the N-acetyl-glucosamine, ( $\alpha\text{Man}$ - $\alpha\text{Glc}$ -GlcNAc) and sterically related residues like glycoproteins, membrane proteins, glycolipids, lipoproteins, polysaccharides, hormones, $\alpha_1$ -antitrypsin, interferon.
17-0690-01	Lysine Sepharose 4B	15 g	0.6–0.7 mg rRNA	45–165	75 cm/h	0.02 MPa, 0.2 bar	2–11	2–11	rRNA, plasminogen and plasminogen activator.
17-0528-01	Red Sepharose CL-6B	10 g	2 mg rabbit LDH	45–165	150 cm/h	0.05 MPa, 0.5 bar	4–12	3–13	NADP <sup>+</sup> - dependent enzymes, aldehyde reductase, dihydrofolate reductase, carboxy-peptidase G, interferon, plasminogen and plasminogen activator.
17-5113-01	Streptavidin Sepharose HP	5 ml	Biotin >300 nmol, 6 mg biotinylated BSA	24–44	150 cm/h	0.3 MPa, 3 bar	4–9	2–10.5	Biotinylated substances, such as biotin-tagged proteins and biotin-tagged DNA.

## Media and prepacked columns for isolation and purification of immunoglobulins

Ordering information	Product	Binding capacity <sup>4</sup> per ml media (approx.)	Particle diameter $\mu\text{m}$	Maximum operating flow rate <sup>1</sup>	Maximum operating pressure	pH stability <sup>2</sup>		Application areas	
						Long term	Short term		
<b>Code No.</b>	<b>Prepacked columns</b>	<b>Column size</b>							
17-0402-01 17-0402-03 17-0403-01	HiTrap Protein A HP	5 x 1 ml 2 x 1 ml 1 x 5 ml	20 mg human IgG	24–44	4 ml/min 20 ml/min	0.3 MPa, 3 bar	3–9	2*–10	Isolation and purification of classes, subclasses and fragments of IgG from many different species. The applications of protein G include practically all applications of protein A.
17-0404-01 17-0404-03 17-0405-01	HiTrap Protein G HP	5 x 1 ml 2 x 1 ml 1 x 5 ml	25 mg human IgG	24–44	4 ml/min 20 ml/min	0.3 MPa, 3 bar	3–9	2*–9	Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Unlike protein A, protein G binds human IgG <sub>3</sub> and has a stronger affinity to mouse IgG <sub>1</sub> and rat IgG.
17-5079-01 17-5079-02 17-5080-01	HiTrap rProtein A FF	5 x 1 ml 2 x 1 ml 1 x 5 ml	50 mg human IgG	45–165	4 ml/min 20 ml/min	0.3 MPa, 3 bar	3–10	2*–11	Recombinant protein A exhibits similar Fc region specificity to that of native protein A but shows enhanced binding capacity.
17-5110-01	HiTrap IgM Purification HP	5 x 1 ml	5 mg human IgM	24–44	4 ml/min	0.3 MPa, 3 bar	3–11	2*–13	HiTrap IgM Purification HP is used for purification of monoclonal IgM from hybridoma cell culture and human IgM.
17-5115-01	HiTrap IgY Purification HP	1 x 5 ml	20 mg pure IgY/ml gel or 1/4 egg yolk/5 ml gel	24–44	20 ml/min	0.3 MPa, 3 bar	3–11	2*–13	HiTrap IgY Purification HP is used for purification of IgY from egg yolk.
<b>Code No.</b>	<b>Kits (including buffers)</b>	<b>Included column</b>							
17-1128-01	MABTrap™ Kit	HiTrap Protein G HP, 1 ml	25 mg human IgG	24–44	4 ml/min	0.3 MPa, 3 bar	3–9	2*–9	MABTrap Kit includes all necessary buffers for ten purifications using a syringe.
17-1362-01	RPAS Purification Module	HiTrap Anti-E Tag, 5 ml	0.7 mg ScFv	24–44	20 ml/min	0.3 MPa, 3 bar	3–9	3–10	The RPAS Purification Module is used for purification of soluble, functional mouse single chain fragments variable (ScFv) antibodies bearing a C-terminal 13 amino acid peptide tag (E Tag).
<b>Code No.</b>	<b>Media</b>	<b>Pack size</b>							
17-0780-01 17-0963-03	Protein A Sepharose CL-4B	– 1.5 g 25 ml	16–25 mg human IgG 2 mg mouse IgG	45–165	150 cm/h	0.02 MPa, 0.2 bar	3–9	2*–10	Sepharose Fast Flow (FF) media are ideal for scale up applications.
17-0974-01 17-0974-04	Protein A Sepharose 4 FF <sup>8</sup>	– 5 ml 25 ml <sup>7</sup>	35 mg human IgG, 3–10 mg mouse IgG	45–165	400 cm/h	0.1 MPa, 1 bar	3–9	2*–10	Sepharose High Performance (HP) media are ideal for high resolution purifications.
17-1279-01 17-1279-02	rProtein A Sepharose FF <sup>5, 8</sup>	– 5 ml 25 ml <sup>7</sup>	50 mg human IgG, 8–20 mg mouse IgG	45–165	400 cm/h	0.1 MPa, 1 bar	3–10	2*–11	Immunoprecipitation Starter Pack includes 2 ml Protein A Sepharose FF and 2 ml of Protein G Sepharose FF.
17-0618-01 17-0618-02	Protein G Sepharose 4 FF <sup>8</sup>	– 5 ml 25 ml <sup>7</sup>	24 mg human IgG, 23 mg cow IgG, 19 mg goat IgG, 17 mg guinea pig IgG, 10 mg mouse IgG, 7 mg rat IgG	45–165	400 cm/h	0.1 MPa, 1 bar	3–9	2*–10	MabSelect is used for capturing monoclonal antibodies from large volumes of feed by packed bed chromatography.
17-6002-35	Immunoprecipitation Starter Pack	–	See Protein A Sepharose 4 FF See Protein G Sepharose 4 FF	45–165	400 cm/h	0.1 MPa, 1 bar	3–9 3–9	2*–10 2*–10	*pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyse at very low pH.
17-5199-01	MabSelect™ <sup>8</sup>	25 ml <sup>7</sup>	30 mg human IgG	40–130	500 cm/h*	0.2 MPa, 2 bar**	3–10	2*–11	**at large scale, see Data File 18-1149-94.

HiTrap columns are ready to use 1 ml and 5 ml columns in a convenient format for preparative purifications. They can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™ design or FPLC™ System.

HP = Sepharose High Performance  
FF = Sepharose Fast Flow

1) Maximum linear operating flow rate is calculated from measurement in packed columns with a bed height of 10 cm and i.d. of 5 cm.

2) The ranges given are estimates based on our knowledge and experience. Please note the following:

i) pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

ii) pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

iii) Protein A and protein G may hydrolyse at low pH. Complete data on the stability of protein A and protein G as a function of pH are not available.

3) Data refer to the coupled product, provided that the ligand can withstand the pH.

## Media and prepacked columns for recombinant fusion proteins

Ordering information	Product	Binding capacity per ml media (approx.)	Particle diameter $\mu\text{m}$	Maximum operating flow rate <sup>1</sup>	Maximum operating pressure	pH stability <sup>2</sup>		Application areas	
Code No.	Prepacked columns	Column size				Long term	Short term		
17-5130-02 17-5130-01 17-5131-01	GSTrap FF	2 x 1 ml 5 x 1 ml 1 x 5 ml		10 mg recombinant GST or 11 mg GST fusion protein (M <sub>r</sub> 43 000)	45–165	4 ml/min 15 ml/min	0.3 MPa, 3 bar	3–12 3–12	Glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
17-5234-01	GSTPrep FF 16/10	20 ml		See above	45–165	10 ml/min	0.15 MPa, 1.5 bar	3–12 3–12	See above.
17-0408-01 17-0409-01	HiTrap Chelating HP	5 x 1 ml 1 x 5 ml		12 mg (His) <sub>6</sub> fusion protein	24–44	4 ml/min 20 ml/min	0.3 MPa, 3 bar	3–13 2–14	Isolation and purification of proteins and peptides containing exposed histidine residues e.g. histidine-tagged proteins.
17-5112-01	HiTrap Streptavidin HP	5 x 1 ml		Biotin >300 nmol, 6 mg biotinylated BSA	24–44	4 ml/min	0.3 MPa, 3 bar	4–9 2–10.5	Biotinylated substances, such as biotin-tagged proteins.
Code No.	Kits (including buffers)	Included column							
17-1880-01	HisTrap™ Kit	3x HiTrap Chelating HP, 1 ml		12 mg (His) <sub>6</sub> fusion protein	24–44	4 ml/min	0.3 MPa, 3 bar	3–13 2–14	See HiTrap Chelating HP.
17-1362-01	RPAS Purification Module	HiTrap Anti-E Tag, 5 ml		0.7 mg ScFv	24–44	20 ml/min	0.3 MPa, 3 bar	3–9 3–10	See Media and prepacked columns for isolation and purification of immunoglobulins.
Code No.	Media	Pack size							
17-5132-01 17-5132-02 17-5132-03	Glutathione Sepharose 4 FF	25 ml 100 ml 500 ml		10 mg recombinant GST or 11 mg GST fusion protein	45–165	450 cm/h	0.1 MPa, 1 bar	3–12 3–12	Glutathione S-transferase (GST) fusion proteins produced using the pGEX series of expression vectors, other glutathione S-transferases and glutathione-dependent proteins.
17-0756-01	Glutathione Sepharose 4B	10 ml		5 mg horse liver GST	45–165	75 cm/h	0.02 MPa, 0.2 bar	3–13 3–13	See Glutathione Sepharose 4 FF.
17-0575-01	Chelating Sepharose FF <sup>®</sup>	50 ml <sup>7</sup>		24–30 $\mu\text{mole Zn}^{2+}$	45–165	700 cm/h	0.3 MPa, 3 bar	3–13 2–14	See Media and prepacked columns for metal chelate chromatography.
17-0529-01	Calmodulin Sepharose 4B	10 ml		Ligand concentration 1 mg/ml	45–165	75 cm/h	0.02 MPa, 0.2 bar	4–9 4–9	ATPases, protein kinases, phosphodiesterases, neurotransmitters, interferon, calmodulin-binding peptide (CBP) fusion protein.
17-0969-01	IgG Sepharose 6 FF <sup>®</sup>	10 ml <sup>7</sup>		2 mg protein A	45–165	400 cm/h	0.1 MPa, 1 bar	3–10 3–10	Recombinant fusion proteins containing a protein A tag.
17-5113-01	Streptavidin Sepharose HP	5 ml		Biotin >300 nmol, 6 mg biotinylated BSA	24–44	150 cm/h	0.3 MPa, 3 bar	4–9 2–10.5	Biotinylated substances, such as biotin-tagged proteins.

## Media and prepacked columns for coupling ligands

Ordering information	Product	Particle diameter $\mu\text{m}$	Capacity/substitution per ml media	Coupling conditions	Maximum operating flow rate <sup>1</sup>	Maximum operating pressure	pH stability <sup>3</sup>		Spacer <sup>6</sup>	Group to be coupled
Code No.	Prepacked columns	Column size					Long term	Short term		
17-0716-01 17-0717-01	HiTrap NHS-activated HP	5 x 1 ml 1 x 5 ml		10 $\mu\text{mole NHS groups}$	pH 6.5–9, 15–30 min, +4 °C-room temperature	4 ml/min 20 ml/min	0.3 MPa, 3 bar	3–12 3–12	10-atom	-NH <sub>2</sub>
Code No.	Media	Pack size								
17-0906-01	NHS-activated Sepharose 4 FF <sup>®</sup>	25 ml		16–23 $\mu\text{mole NHS groups}$	pH 6–8, 2–16 hours, +4 °C-room temperature	400 cm/h	0.1 MPa, 1 bar	3–13 3–13	10-atom	-NH <sub>2</sub>
17-0981-01	CNBr-activated Sepharose 4 FF <sup>®</sup>	10 g <sup>7</sup>		13–26 mg $\alpha$ -chymotrypsinogen	pH 7–9, 2–16 hours, +4 °C-room temperature	400 cm/h	0.1 MPa, 1 bar	3–11 3–11	None	-NH <sub>2</sub>
17-0430-01	CNBr-activated Sepharose 4B	15 g <sup>7</sup>		25–60 mg $\alpha$ -chymotrypsinogen	pH 8–10, 2–16 hours, +4 °C-room temperature	75 cm/h	0.02 MPa, 0.2 bar	2–11 2–11	None	-NH <sub>2</sub>
17-0490-01	Activated CH Sepharose 4B	15 g		9–16 $\mu\text{mole glycyl-leucine}$	pH 5–10, 1–4 hours, +4 °C-room temperature	75 cm/h	0.02 MPa, 0.2 bar	2–11 2–11	6-atom	-NH <sub>2</sub>
17-0571-01	ECH Sepharose 4B	50 ml		12–16 $\mu\text{mole carboxyl groups}$	pH 4.5, 1.5–24 hours, +4 °C-room temperature	75 cm/h	0.02 MPa, 0.2 bar	3–14 3–14	10-atom	-NH <sub>2</sub>
17-0480-01	Epoxy-activated Sepharose 6B	15 g		19–40 $\mu\text{mole epoxy groups}$	pH 9–13, 16 hours-several days, +20 - 40 °C	75 cm/h	0.03 MPa, 0.3 bar	2–14 2–14	12-atom -OH -SH	-NH <sub>2</sub>
17-0569-01	EAH Sepharose 4B	50 ml		7–11 $\mu\text{mole amino groups}$	pH 4.5, 1.5–24 hours, +4 °C-room temperature	75 cm/h	0.02 MPa, 0.2 bar	3–14 3–14	11-atom	-COOH
17-0640-01	Activated Thiol Sepharose 4B	15 g		1 $\mu\text{mole activated thiol groups}$	pH 4–8, 3–16 hours, +4 °C-room temperature	75 cm/h	0.02 MPa, 0.2 bar	2–8 2–8	10-atom	-SH
17-0420-01	Thiopropyl Sepharose 6B	15 g		18–31 $\mu\text{mole activated thiol groups}$	pH 4–8, 3–16 hours, +4 °C-room temperature	75 cm/h	0.03 MPa, 0.3 bar	2–8 2–8	4-atom	-SH

## Media and prepacked columns for metal chelate chromatography

Ordering information	Product	Binding capacity per ml media (approx.)	Particle diameter $\mu\text{m}$	Maximum operating flow rate <sup>1</sup>	Maximum operating pressure	pH stability <sup>2</sup>		Application areas	
Code No.	Prepacked columns	Column size				Long term	Short term		
17-0408-01 17-0409-01	HiTrap Chelating HP	5 x 1 ml 1 x 5 ml		23 $\mu\text{mole Cu}^{2+}$	24–44	4 ml/min 20 ml/min	0.3 MPa, 3 bar	3–13 2–14	Isolation and purification of proteins and peptides containing exposed His, Cys, Trp amino acids e.g. $\alpha_2$ -macroglobulin, and interferon, histidine-fusion recombinant proteins. Chelating Sepharose FF is ideal for scale up applications. Chelating Sepharose HP is ideal for high resolution purifications.
Code No.	Kit (including buffers)	Included column							
17-1880-01	HisTrap Kit	3x HiTrap Chelating HP, 1 ml		12 mg (His) <sub>6</sub> fusion protein	24–44	4 ml/min	0.3 MPa, 3 bar	3–13 2–14	
Code No.	Medium	Pack size							
17-0575-01	Chelating Sepharose FF <sup>®</sup>	50 ml <sup>7</sup>		24–30 $\mu\text{mole Zn}^{2+}$	45–165	700 cm/h	0.3 MPa, 3 bar	3–13 2–14	

4) The binding capacity values listed above are typical for the given species. However, there might be considerable deviations in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.

5) Recombinant protein A is also available on the Expanded Bed Adsorption media - STREAMLINE™ rProtein A, please contact Amersham Biosciences for further information.

6) Spacer arms are used when coupling small molecules (M<sub>r</sub> <1 000). Spacer arms are generally not used for larger molecules (M<sub>r</sub> >5 000).

7) Process scale quantities are available. Please contact Amersham Biosciences for further information.

8) BioProcess Media - Media made for bioprocessing.

### Secure Supply

Large capacity production integrated with clear ordering and delivery routines mean BioProcess™ Media are available in the right quantity, at the right place, at the right time. We can assure future supplies of BioProcess Media, making them a safe investment for your long term production.



### Validated Manufacture

Produced following validated methods and tested under strict control, BioProcess Media fulfil high performance specifications. A certificate of analysis is available with each order.

### Regulatory Support

Regulatory Support Files contain details of performance, stability, extractable compounds and analytical methods available. The essential information in these files is an invaluable starting point for process validation, as well as support for clinical and marketing applications submitted to regulatory authorities.

### From capture to polishing

Specific BioProcess Media have been designed for each chromatographic stage in a process from Capture to Polishing. Using BioProcess Media for every stage results in an easily validated economic process.

### High productivity

High flow rates, high capacity and high recovery contribute to the overall economy of an industrial process.

### Sanitization/CIP

All BioProcess Media can be cleaned and sanitized in place.

### Scalability

Packing methods are established for a wide range of scales. You can use the same BioProcess Media for development work, pilot studies, and routine production.

## Ordering Information

Columns	Pack size	Code No.
HiTrap rProtein A FF	5 × 1 ml	17-5079-01
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
HiTrap rProtein A FF	1 × 5 ml	17-5080-01
HiTrap Protein A HP	5 × 1 ml	17-0402-01
HiTrap Protein A HP	2 × 1 ml	17-0402-03
HiTrap Protein A HP	1 × 5 ml	17-0403-01
HiTrap Protein G HP	5 × 1 ml	17-0404-01
HiTrap Protein G HP	2 × 1 ml	17-0404-03
HiTrap Protein G HP	1 × 5 ml	17-0405-01
HiTrap Blue HP	5 × 1 ml	17-0412-01
HiTrap Blue HP	1 × 5 ml	17-0413-01
HiTrap Heparin HP	5 × 1 ml	17-0406-01
HiTrap Heparin HP	1 × 5 ml	17-0407-01
HiPrep 16/10 Heparin FF	1 × 20 ml	17-5189-01
HiTrap Benzamidine FF (high sub)	5 × 1 ml	17-5143-01
HiTrap Benzamidine FF (high sub)	2 × 1 ml	17-5143-02
HiTrap Benzamidine FF (high sub)	1 × 5 ml	17-5144-01
HiTrap NHS-activated HP	5 × 1 ml	17-0716-01
HiTrap NHS-activated HP	1 × 5 ml	17-0717-01
HiTrap Chelating HP	5 × 1 ml	17-0408-01
HiTrap Chelating HP	1 × 5 ml	17-0409-01
HiTrap Streptavidin HP	5 × 1 ml	17-5112-01
HiTrap IgM Purification HP	5 × 1 ml	17-5110-01
HiTrap IgY Purification HP	1 × 5 ml	17-5111-01
GSTrap FF	5 × 1 ml	17-5130-01
GSTrap FF	2 × 1 ml	17-5130-02
GSTrap FF	1 × 5 ml	17-5131-01
GSTPrep FF 16/10	1 × 20 ml	17-5234-01
Kits (including buffers)		Code No.
MABTrap Kit		17-1128-01
HisTrap Kit		17-1880-01
RPAS Purification Module		17-1362-01
Technical information*		Code No.
Handbooks and guide with detailed technical information:		
Affinity chromatography Principles and Methods		18-1022-29
Antibody Purification Handbook		18-1037-46
The Recombinant Protein Handbook Expression, Amplification and Simple Purification		18-1142-75
Convenient Protein Purification HiTrap Column Guide		18-1129-81
* Technical information can be downloaded from <a href="http://www.chromatography.amershambiosciences.com">www.chromatography.amershambiosciences.com</a>		

Media	Pack size	Code No.
Protein A Sepharose CL-4B	1.5 g	17-0780-01
Protein A Sepharose CL-4B	25 ml	17-0963-03
Protein A Sepharose 4 FF	5 ml	17-0974-01
Protein A Sepharose 4 FF	25 ml	17-0974-04
rProtein A Sepharose FF	5 ml	17-1279-01
rProtein A Sepharose FF	25 ml	17-1279-02
Protein G Sepharose 4 FF	5 ml	17-0618-01
Protein G Sepharose 4 FF	25 ml	17-0618-02
MabSelect	25 ml	17-5199-01
Immunoprecipitation Starter Pack	2 x 2 ml	17-6002-35
Protein A Sepharose 4 FF		
Protein G Sepharose 4 FF		
2',5' ADP Sepharose 4B	5 g	17-0700-01
5' AMP Sepharose 4B	5 g	17-0620-01
Agarose Wheat Germ Lectin	5 ml	27-3608-02
Arginine Sepharose 4B	25 ml	17-0524-01
Benzamidine Sepharose 6B	25 ml	17-0568-01
Benzamidine Sepharose FF (high sub)	25 ml	17-5123-01
Blue Sepharose 6 FF	50 ml	17-0948-01
Calmodulin Sepharose 4B	10 ml	17-0529-01
Chelating Sepharose FF	50 ml	17-0575-01
Con A Sepharose 4B	5 ml	17-0440-03
Con A Sepharose 4B	100 ml	17-0440-01
Gelatin Sepharose 4B	25 ml	17-0956-01
Glutathione Sepharose 4 FF	25 ml	17-5132-01
Glutathione Sepharose 4 FF	100 ml	17-5132-02
Glutathione Sepharose 4 FF	500 ml	17-5132-03
Glutathione Sepharose 4B	10 ml	17-0756-01
Heparin Sepharose 6 FF	50 ml	17-0998-01
Heparin Sepharose CL-6B	10 g	17-0467-01
IgG Sepharose 6 FF	10 ml	17-0969-01
Lentil Lectin Sepharose 4B	25 ml	17-0444-01
Lysine Sepharose 4B	15 g	17-0690-01
Red Sepharose CL-6B	10 g	17-0528-01
Streptavidin Sepharose HP	5 ml	17-5113-01
Activated CH Sepharose	15 g	17-0490-01
CNBr-activated Sepharose 4B	15 g	17-0430-01
CNBr-activated Sepharose 4 FF	10 g	17-0981-01
EAH Sepharose 4B	50 ml	17-0569-01
ECH Sepharose 4B	50 ml	17-0571-01
Epoxy-activated Sepharose 6B	15 g	17-0480-01
NHS-activated Sepharose 4 FF	25 ml	17-0906-01
Activated Thiol Sepharose 4B	15 g	17-0640-01
Thiopropyl Sepharose 6B	15 g	17-0420-01

FOR MORE INFORMATION ABOUT OUR  
CHROMATOGRAPHY **columns**  
AND **media** RANGE,

SEE OUR HOMEPAGE:

[www.chromatography.amershambiosciences.com](http://www.chromatography.amershambiosciences.com)

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