

# Affinity Chromatography

A successful affinity separation requires a biospecific ligand covalently attached to a chromatographic bed material, the matrix. Properties of affinity matrices are presented in the table below. These properties will differ with the ligand bound. For example, the Toyopearl® matrix is stable at pH 2-12, but the specific ligand chemistry may limit the usable range. Toyopearl Epoxy media are used at pH 9-11, 40 °C to make a stable secondary amine linkage, but at pH 7-8, 25 °C for a stable sulfide linkage.

## Affinity Matrices

Matrix	PH Range	Max. Operating Pressure & Flow	Properties/Limitations
<b>Acrylic Beads</b>			
150 µm	cleaning: 0-12 operating: 5-9	0.2 MPa, 100 mL/min (2 x 28 cm column)	Macroporous; stable in organics; hydrophilic, neutral; no shrinking/swelling with changes in ionic strength; mechanically stable; high binding capacity
<b>Agarose, Beaded</b>			
2%: 60-200 µm 4%: 45-165 µm 6%: 45-165 µm	4-9	2%: 0.04 MPa, 0.8 mL/min 4%: 0.08 MPa, 1.0 mL/min 6%: 0.20 MPa, 1.2 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10 <sup>7</sup> MW; temperature to 40 °C; sterilizable (chemical); insoluble in all common solvents; should not be used with oxidizing agents or chaotropic salts
<b>Cellulose</b>			
fibrous tubes, 30-60 µm diam., 6%: 45-165 µm	2-10	0.07 MPa, 2.4 mL/min (2.5 cm column diameter)	Low resolution; pliable material
<b>Sephacrose®</b>			
2B: 60-200 µm 4B: 45-165 µm 6B: 45-165 µm	4-9	2B: 0.04 MPa, 0.8 mL/min 4B: 0.08 MPa, 1.0 mL/min 6B: 0.20 MPa, 1.2 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10 <sup>7</sup> MW; temperature to 40 °C; sterilizable (chemical); insoluble in all common solvents; should not be used with oxidizing agents or chaotropic salts
<b>Sephacrose CL</b>			
2B: 60-200 µm 4B: 45-165 µm 6B: 45-165 µm	3-14	CL-2B: 0.05 MPa, 1.2 mL/min CL-4B: 0.12 MPa, 2.2 mL/min CL-6B: 0.20 MPa, 2.5 mL/min (2.5 x 30 cm column)	Analytes up to 4 x 10 <sup>7</sup> MW; high flow rates; sterilizable (autoclave/chemical); insoluble in all common solvents; can be used in dissociating media, high concentrations of chaotropic salts
<b>Toyopearl</b>			
40-90 µm	operating: 2-12 cleaning: 1-13	0.7 MPa	Analytes up to 1 x 10 <sup>6</sup> MW; 1000 Å pores; hydrophilic, neutral; compatible with solvents; chemically resistant; volume stable to changes in pH or ionic strength

80 cm H<sub>2</sub>O = 0.8 bar = 11.6 psi = 0.08 MPa.





## Affinity Ligands

The ligand should exhibit specific and reversible binding affinity for the substance to be purified. In addition, it should have chemically modifiable groups that allow it to be attached to the matrix without destroying its ligand-binding activity. The dissociation constant ( $K_d$ ) for the ligand-binding substance complex should ideally be in the range of  $10^{-4}$  to  $10^{-8}$  M in free solution. If no information is available on the strength of the binding complex, use a trial and error approach. It is important to consider the region of the ligand that will be used for attachment to the matrix. If several functional groups are available, the ligand should be coupled via the group least likely to be involved in the specific interaction with the molecule to be isolated.

## Spacer Arms

The active site on a biological molecule is often located deep within the molecule. Adsorbents prepared by coupling small ligands directly to the matrix can exhibit low capacities, due to steric hindrance. To prevent this, a spacer arm can be used between the matrix and ligand, to facilitate effective binding. Alternatively, if the spacer arm is too long, nonspecific effects become pronounced and reduce the selectivity of the separation.

## Affinity Media

*Group-specific media* have affinity for a group of related compounds, rather than for a single substance, thus enabling the analyst to use the same general ligand to purify several substances (e.g., a class of enzymes). Within the group there is either a structural or functional similarity. For example, heparin-bearing affinity media recognize  $\beta$ -pleated sheet domains. Thus, heparin media are useful for purifying coagulation factors, lipoproteins and lipoprotein lipases, growth factors, and enzymes active in nucleic acid metabolism.

*Activated media* are resins with activated functional groups ready for direct coupling of a protein or other ligand. The cyanogen bromide-activated matrices are typical examples. The reaction of cyanogen bromide with the matrix activates the product to which proteins, nucleic acids, or other biopolymers then can be coupled, under mild conditions, via primary amino or similar nucleophilic groups.

Resins with *reactive groups* employ carbodiimide coupling or reductive amination to achieve covalent bonding. For example, the Toyopearl® AF-Amino-650M matrix can be used to couple ligands via their carboxylate groups (peptide bond formation) or aldehyde groups (reductive amination). Aldehyde groups may be present in a carbohydrate or glycoprotein ligand, or can be introduced into the intended ligand by mild periodate oxidation. This reactive matrix is used for coupling either proteins or low molecular weight ligands (e.g., lactose).

## Applications for Affinity Media

Affinity Class	Potential Applications
Activated	Functional spacer; support matrix; eliminates handling of toxic reagents.
Amino Acid	Serum proteins; proteins; peptides; enzymes; rRNA; dsDNA.
Avidin & Biotin	Purification of biotin/avidin & derivatives; biotinylated substances. Biotin derivatives dissociate under non-denaturing conditions.
Carbohydrate	Glycoproteins; lectins; other carbohydrate metabolite proteins. Proper selection can ensure one-step purification.
Dye	Nonspecific interaction. Mimic biological substances (substrates, cofactors, effectors); proteins. Optimize purification protocol with different dyes.
Glutathione	Detoxification enzymes; glutathione S-transferase and fusion proteins; glutathione peroxidase and glyoxalase.
Hydrophobic	Couple ligands containing free carboxyl groups; proteins.
Immunochemical	Removal of antibodies from antisera or serum proteins. Solid phase second antibodies.
Lectin	Soluble glycoproteins; other carbohydrate-containing substances.
Nucleotide/Coenzyme	Dehydrogenases; kinases; transaminases. Reliable adsorbents.
Nucleic Acid	mRNA; DNA; rRNA; other nucleic acids and oligonucleotides.
Specialty	Purification of specific classes or types of proteins, coenzymes, or physiological partners.

### Toyopearl® Resins

Group Specific	Large pore diameter; medium pressure. Nucleotide-dependent enzymes; bind histidine and free cysteines of peptides or proteins; purify coagulation factors; lipoproteins; enzymes active in nucleic acid metabolism.
Activated	Large pore diameter; medium pressure; optimal pH 7-9; highly reactive to amine/thiol groups. Immobilize protein or low MW ligands.
Reactive	Large pore diameter; medium pressure; pH range 6.9-9 or 4.5-6. Primary amines; carboxylate, aldehyde, or amino groups.





## Recommended Reading

### Affinity Chromatography: Principles & Methods

This handbook serves as an introduction to affinity chromatography and a practical guide to the media developed by Pharmacia Biotech. The text covers ligands, spacers, and coupling gels, and describes general experimental methods ..... **23499**

# Selective!



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# Products for Affinity Chromatography

Description	Ligand	Adsorption Capacity (per mL gel)	Typical Uses	Cat. No.
<b>TSK-GEL® Affinity Chromatography Columns</b>				
<b>ABA-5PW</b> 7.5 cm × 7.5 mm I.D., 10 µm particle size	<i>p</i> -aminobenzamidine	3-4 mg trypsin	trypsin, bovine thrombin, urokinase, enterokinase, plasminogen activator	813067
<b>Boronate-5PW</b> 7.5 cm × 7.5 mm I.D., 10 µm particle size	<i>m</i> -aminophenylboronic acid	40 µmol sorbitol	glycoproteins, nucleases, nucleotides, catecholamines, carbohydrates, transfer RNAs	813066
<b>Chelate-5PW</b> 7.5 cm × 7.5 mm I.D., 10 µm particle size	iminodiacetic acid	~20 µmol Cu <sup>2+</sup> or Zn <sup>2+</sup>	serum proteins, interferon, collagenase, granule protein, plasminogen activator, lactoferrin	808645
<b>Guard Column Kit: Chelate-5PW</b> 1 cm × 6.0 mm I.D., 20 µm particle size	na	na	Guard column	808647

Description	Cat. No.
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## Media for Epitope Tag Purification

### GST

Glutathione Agarose	G4510
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### FLAG®

FLAG Immunoprecipitation Kit	FLAGIPT1
ANTI-FLAG® M1 Agarose Affinity Gel	A4596
ANTI-FLAG M2 Affinity Gel	A2220
EZview™ Red ANTI-FLAG M2 Affinity Gel	F2426

### HISTIDINE

HIS-Select® Cobalt Affinity Gel	H8162
HIS-Select Nickel Affinity Gel	P6611
HIS-Select HF Nickel Affinity Gel	H0537
EZview Red HIS-Select HC Nickel Affinity Gel	E3528
Monoclonal Anti-polyHistidine-Agarose antibody produced in mouse	A5713

Description	Cat. No.
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### Pre-packed columns

HIS-Select Cartridge 1.25 mL Columns	H8286
HIS-Select Elution Buffer	H5413
HIS-Select High Flow Cartridge volume 6.4 mL	H7163
HIS-Select Spin Columns	H7787
HIS-Select Wash Buffer	H5288
HIS-Select iLAP® 5 mL Column	H9913

### 96-well plates

HIS-Select Filter Plate	H0413
HIS-Select High Capacity (HC) Nickel coated plates, clear, polystyrene	S5563
HIS-Select High Sensitivity (HS) Nickel Coated Plates clear strip-well plate	S5688
HIS-Select iLAP HC Nickel Coated Plate, Clear	H9412

### HA

Monoclonal Anti-HA Agarose antibody produced in mouse	A2095
Anti-HA Immunoprecipitation Kit	IP0010
EZview Red Anti-HA Affinity Gel	E6779



Description	Cat. No.
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**HSV**

Anti-HSV Agarose Affinity Gel	A7220
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**c-Myc**

Anti-c-Myc Agarose Affinity Gel antibody produced in rabbit	A7470
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EZview™ Red Anti-c-Myc Affinity Gel	E6654
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Anti-c-Myc Immunoprecipitation Kit	IP0020
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**PROTEIN A AND G**

EZview Red Protein A Affinity Gel	P6486
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Protein A–Agarose	P2545
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Protein A–Agarose, 2.5 mL column	PA1
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Protein A Antibody Purification Kit	PURE1A
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EZview Red Protein G Affinity Gel	E3403
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Protein G, immobilized on crosslinked 4% agarose	83219
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Protein G Immunoprecipitation Kit	IP50
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**BIOTIN/STREPTAVIDIN**

EZview Red Streptavidin Affinity Gel	E5529
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Biotin–Agarose	B0519
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Streptavidin–Agarose from <i>Streptomyces avidinii</i>	S1638
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Streptavidin, immobilized on Agarose CL-4B	85881
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**THIOREDOXIN**

Anti-Thioredoxin–Agarose antibody produced in rabbit	A2582
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**V5**

Anti-V5 Agarose Affinity Gel antibody produced in mouse	A7345
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**VSV**

Monoclonal Anti-VSV-Glycoprotein-Agarose antibody produced in mouse	A1970
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Description	Cat. No.
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**Other Affinity Media**

Heparin, immobilized on Agarose 4B, from porcine intestinal mucosa, BioChemika, for affinity chromatography	51546
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Lectin, immobilized on Agarose CL-4B from <i>Triticum vulgare</i> , BioChemika, for affinity chromatography	61768
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Oligo dT-Cellulose, BioChemika, for affinity chromatography	75349
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$\omega$ -Aminohexyl–Agarose, BioChemika, for affinity chromatography, activated with cyanogen bromide	08055
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