

AFC AFFINITY CHROMATOGRAPHY



AFC PRODUCTS

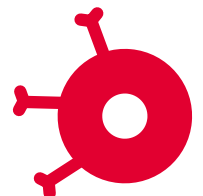
- Activated TOYOPEARL Resins for Affinity Ligand Coupling
 - TOYOPEARL AF-Tresyl-650M
 - TOYOPEARL AF-Epoxy-650M

- Reactive TOYOPEARL Resins for Affinity Ligand Coupling
 - TOYOPEARL AF-Formyl-650M
 - TOYOPEARL AF-Amino-650M
 - TOYOPEARL AF-Carboxy-650M

- Group specific TOYOPEARL Affinity resins
 - TOYOPEARL AF-Chelate-650M
 - TOYOPEARL AF-Blue-650M
 - TOYOPEARL AF-Red-650M
 - TOYOPEARL AF-Heparin HC-650M

➤ TOSOH FACT

Tosoh Bioscience GmbH offers workshops on chromatography in process development & production. These three day courses deliver a comprehensive background to bioprocess chromatographic purification as an integrated process technique. The workshops provide a balance of effective presentations and practical hands-on experience with process scale and methods development equipment under the guidance of qualified and knowledgeable Tosoh Bioscience technical experts. Every year more than 30 participants use this opportunity to broaden or refresh their chromatographic and downstream processing knowledge.





AFFINITY CHROMATOGRAPHY

TOYOPEARL AFFINITY RESINS FOR PROCESS SCALE CHROMATOGRAPHY

There are many custom designed affinity ligands available to the chromatographer. TOYOPEARL affinity chromatography resins are functionalized with chemically active groups or group-specific ligands. Resins with activated functional groups are ready to directly couple a protein or other ligand. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve a stable covalent linkage.

AFC – HOW DOES IT WORK

In affinity chromatography, the target protein is specifically and reversibly bound by a complementary ligand. The sample is applied under conditions that favor specific binding to the ligand. Unbound material is washed out of the column, and bound target protein is recovered by changing conditions to those favoring elution. Elution is performed specifically, using a competitive ligand, or nonspecifically, by changing, for example, pH, ionic strength, or polarity. The target protein is usually eluted in a purified and concentrated form.

MECHANICAL & CHEMICAL STABILITY

TOYOPEARL resins remain dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL particles do not distort under flow rates that generate up to 3 bar pressure. These properties of the resins combined with the narrow particle size distributions result in superior pressure-flow characteristics for the packed TOYOPEARL bed. Linear velocities of 300 - 500 cm/h generate a pressure of between 1 and 2 bar in a 20 cm length bed. Achievement of high linear velocities at relatively low pressure enables high throughput production scale chromatography using equipment with moderate pressure limitations. Sanitization or cleaning may be conducted with up to 0.5 mol/L NaOH or 0.5 mol/L HCl depending upon the ligand. In affinity chromatography, in particular, the choice of cleaning agent will be largely dependent upon the chemical stability of the ligand, rather than that of the base resin.

➤ **TABLE I**

Activated resin	Reactive resin	Group specific
AF-Tresyl	AF-Amino	AF-Blue HC
AF-Epoxy	AF-Carboxy	AF-Red
	AF-Formyl	AF-Chelate
		AF-Heparin HC

➤ **FEATURES**

- Active, reactive and group specific resins
- Standard 100 nm pore size
- Porous, hydrophilic polymer matrix
- High mechanical stability

➤ **BENEFITS**

- Broad range of applications
- High capacity for large biopolymers
- Suitable for laboratory and process scale purifications
- Constant bed volume over a wide range of buffer composition

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FIGURE 1

TOYOPEARL RESINS FOR AFFINITY LIGAND COUPLING

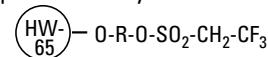
TOYOPEARL offers a spectrum of carefully selected affinity resins primed with activated or reactive groups which can be used to covalently attach almost any custom ligand. The structures of TOYOPEARL activated and reactive ligands are shown in Figure 1.

In general, TOYOPEARL AF-Tresyl-650M and TOYOPEARL AF-Formyl-650M resin are recommended for coupling proteins, while TOYOPEARL AF-Epoxy-650M resin is suited for coupling lower molecular weight ligands. TOYOPEARL AF-Amino-650M and TOYOPEARL AF-Carboxy-650M resins may be used for both.

TOYOPEARL affinity resins may be used in combinatorial chemistry or for solid phase synthesis of peptides and oligonucleotides because of their excellent stability in a variety of organic solvents and under extremes of pH.

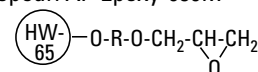
ACTIVATED TOYOPEARL AFFINITY RESINS

Toyopearl AF-Tresyl-650M



Ligand Density: 80 $\mu\text{mol/g}$ (dry)

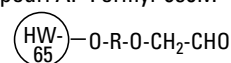
Toyopearl AF-Epoxy-650M



Ligand Density: 800 $\mu\text{mol/g}$ (dry)

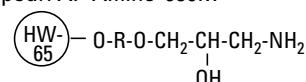
REACTIVE TOYOPEARL AFFINITY RESINS

Toyopearl AF-Formyl-650M



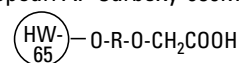
Ligand Density: 60 meq/L

Toyopearl AF-Amino-650M



Ligand Density: 100 mmol/L

Toyopearl AF-Carboxy-650M



Ligand Density: 100 meq/L

TABLE II

REPRESENTATIVE COUPLING DENSITIES FOR ACTIVATED AND REACTIVE TOYOPEARL MEDIA

TOYOPEARL resin	AF-Tresyl-650M	AF-Formyl-650M	AF-Amino-650M	AF-Carboxy-650M
Protein coupled (mg/mL resin)				
Soybean trypsin inhibitor	16	3.5	5.8	15
Protein A	1.9	-	-	-
Concanavalin A	13	-	-	-
α 1-Antitrypsin	12.3	-	-	-
α -Chymotrypsin	12.5	-	-	-
Myoglobin	12.4	-	-	-
Ovalbumin	-	2.5	6.7	0.8
Bovine serum albumin	12.4	14	19.2	3.3
Human IgG	10.0	15	6.7	11.7
Cytochrome C	-	5.8	3.3	7.5
Lysozyme	60	20	5.8	17.5
Coupling agent	not required	NaCNBH ₃	NaCNBH ₃ or Carbodiimide	Carbodiimide
Optimal pH	7.0 - 9.0	6.9 - 9.0	4.5 - 6.0	4.5 - 6.0



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ACTIVATED RESINS – READY FOR DIRECT LIGAND ATTACHMENT

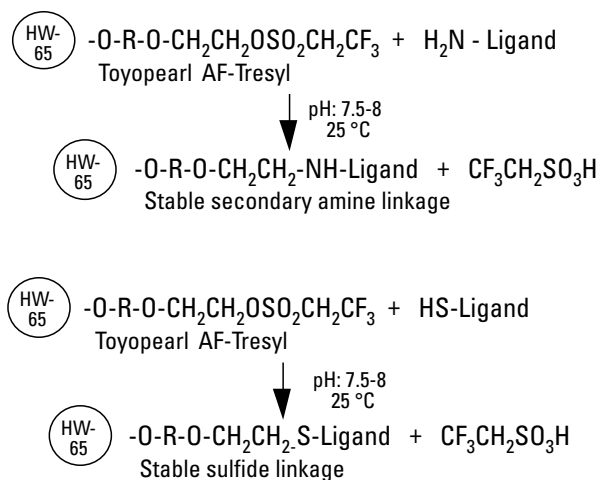
TOYOPEARL AF-Tresyl-650M activated resin is highly reactive toward amine and thiol groups. It is provided in dry form, ready for reaction in buffered solutions containing protein or other ligand. Coupling is accomplished in neutral to slightly alkaline (pH 7 - 8) solution (Figure 2).

Under such conditions, even proteins of limited stability may be successfully coupled. Coupling leads to the formation of a highly stable secondary amine or thio-ether linkage. The optimized tresyl-density (ca. 20 $\mu\text{mol/mL}$ hydrated resin) is sufficient to provide substantial protein binding while avoiding excessive multi-point attachment and consequent impairment of ligand affinity/activity. Representative data are presented in Table II.

TOYOPEARL AF-Epoxy-650M activated resin, also packaged in dry form, has a high density of epoxy-functionality (ca. 800 $\mu\text{mol/mL}$). Under appropriate reaction conditions, this may be used for immobilization of proteins or low molecular weight ligands. It is particularly useful when high densities of low molecular weight ligands must be attached (Figure 3). Glutathione and glycine have, for example, been coupled at densities greater than 100 $\mu\text{mol/mL}$ hydrated resin.

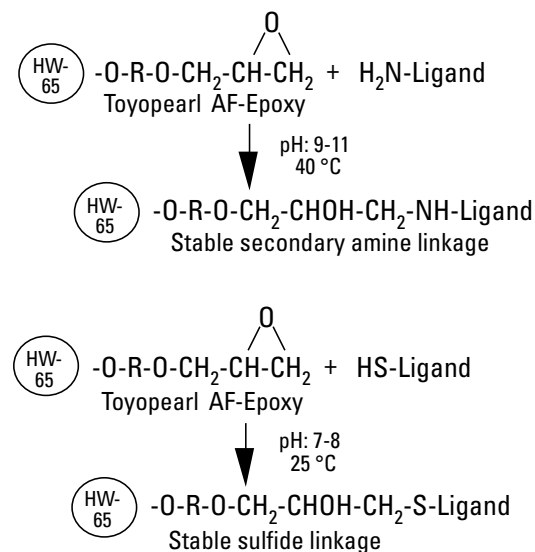
TOYOPEARL AF-Epoxy-650M resin is a highly versatile starting material for conversion to other chemically active functional groups required in special applications. This resin may be readily activated to hydrazide-bearing materials. This is particularly useful for immobilization of carbohydrates or glycoproteins. Using the reaction sequences described, special ligands may be introduced onto this dimensionally stable, macroporous support.

FIGURE 2
TOYOPEARL AF-TRESYL COUPLING PROCEDURE



R = hydrophilic polymer

FIGURE 3
TOYOPEARL AF-EPOXY COUPLING PROCEDURE



R = hydrophilic

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REACTIVE RESINS - REQUIRE ACTIVATION FOR LIGAND ATTACHMENT

Ligands may be coupled to **TOYOPEARL AF-Formyl-650M** (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 4). Representative coupling capacities are shown in Table II.

A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports. Typically, these supports have been synthesized by industrial users by partial oxidation of polysaccharide supports (e.g. cellulose and agarose) or partial hydrolysis of polyacetals. In contrast, TOYOPEARL AF-Formyl-650M resin is a ready-to-use aldehyde support formulated from a dimensionally stable, macroporous matrix. Consistent aldehyde content and physical properties are assured from batch to batch.

TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups (peptide bond formation) or aldehyde groups (reductive amination) as shown in Figure 5. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate oxidation.

The optimized functional group density of TOYOPEARL AF-Amino-650M (100 mmol/L) is ideal for coupling of either proteins or low molecular weight ligands. For example, lactose was coupled by reductive alkylation to yield a ligand density of ca. 30 $\mu\text{mol/mL}$ resin. Coupling densities for various proteins are given in Table II.

TOYOPEARL AF-Carboxy-650M resin provides another useful and milder approach for coupling to amino groups of proteins or low molecular weight ligands. The carbodiimide mediated coupling reaction produces an amide bond between ligand and support (Figure 6). Representative coupling densities are given in Table II.

FIGURE 4
TOYOPEARL AF-FORMYL COUPLING PROCEDURE

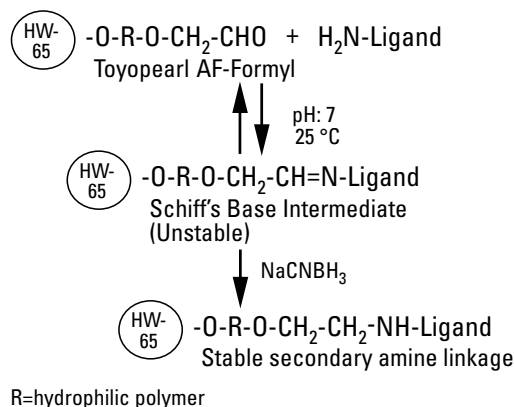


FIGURE 5
TOYOPEARL AF-AMINO COUPLING PROCEDURE

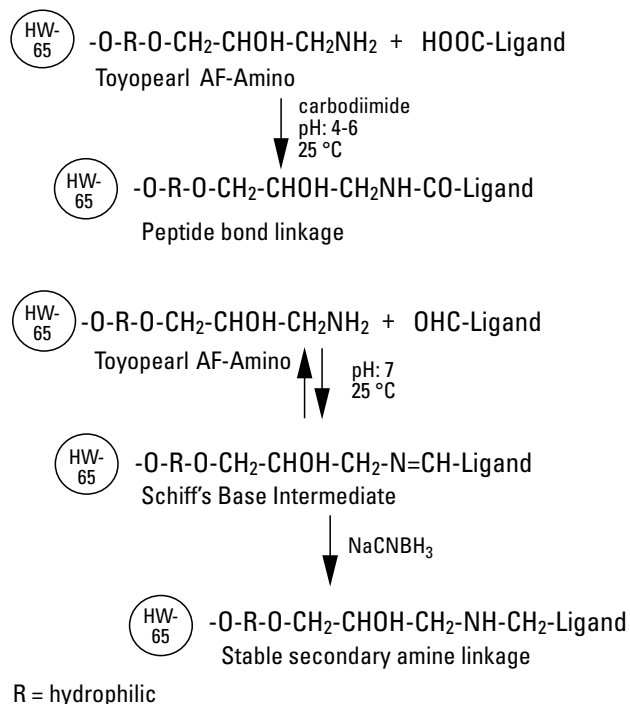
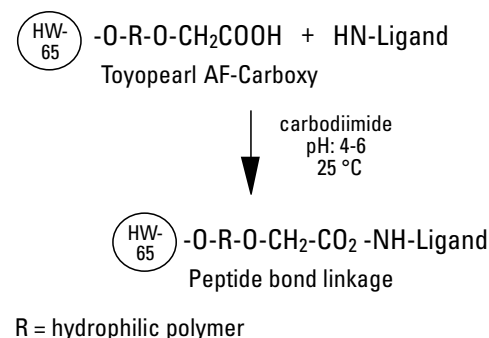


FIGURE 6
TOYOPEARL AF-CARBOXY COUPLING PROCEDURE





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TOYOPEARL RESINS WITH GROUP SPECIFIC LIGANDS

The structures of TOYOPEARL group specific ligands are shown in Figure 7.

TOYOPEARL AF-Chelate-650M

This resin is derivatized with iminodiacetic acid (IDA) at a concentration of ca. 20 $\mu\text{mol/mL}$. In typical applications, selected metal ions, most often Ca^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} and Cu^{2+} are bound to the support by stable chelation. The resultant metal ion-bearing resin binds to histidine and free cysteine containing sequences of a peptide or protein. Immobilized metal ion affinity chromatography (IMAC) has been used for purification of recombinant human growth factor, tissue plasminogen activator, glycoporphins, and whole cells.

TOYOPEARL AF-Blue HC-650M

Functionalized with Cibachron Blue F3G-A, TOYOPEARL AF-Blue HC-650M resin has excellent capacity for proteins, particularly albumin (Figure 8). In addition, this high capacity resin has improved caustic stability, reduced dye ligand leakage, and superior pressure-flow characteristics relative to more traditional agarose materials (Figure 9).

TOYOPEARL AF-Red-650ML

Toyoscreen and TOYOPEARL AF-Red-650ML resins are functionalized with Procion Red HE-3B, (also known as Reactive Red 120). This resin is useful for the purification of nucleotide dependent enzymes, lipoproteins, plasminogen, peptides, hormones and cytotoxins.

These two dye-ligand resins are useful in binding/purification of nucleotide-dependent enzymes, albumin, cell growth factors, interferons, transferases, cyclases, and polymerases. Typical binding capacities are shown in Table III.

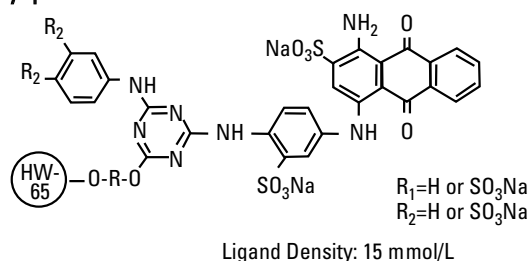
TOYOPEARL AF-Heparin HC-650M

Heparin is a linear and highly sulfated glycosaminoglycan which has anti-coagulant properties. Due to its polyanionic nature, heparin interacts with a wide range of biomolecules including plasma components, lipoprotein lipase, collagenase, and DNA polymerase.

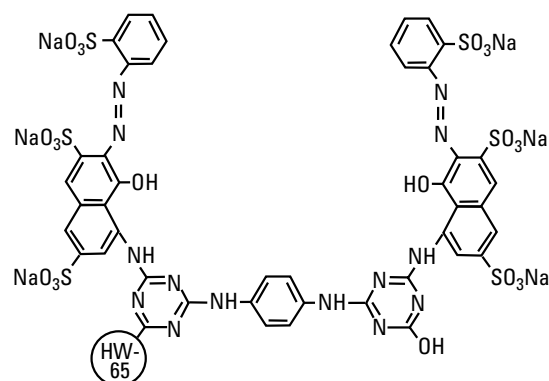
Immobilized heparin is widely used as an adsorbent in affinity chromatography for the purification of biological substances. TOYOPEARL AF-Heparin HC-650M resin is a high capacity, affinity adsorbent with excellent chemical stability.

FIGURE 7
GROUP-SPECIFIC TOYOPEARL AFFINITY RESIN

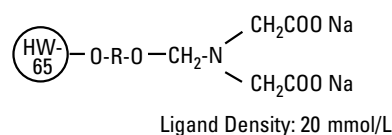
Toyopearl AF-Blue HC



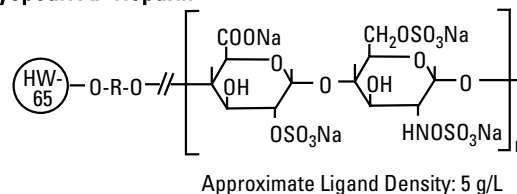
Toyopearl AF-Red



Toyopearl AF-Chelate



Toyopearl AF-Heparin



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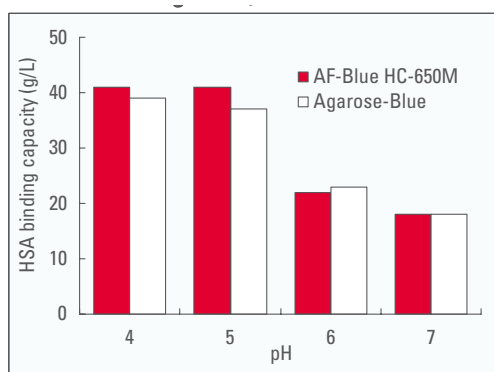
TABLE III

REPRESENTATIVE BINDING CAPACITIES FOR TOYOPEARL DYE-LIGAND AFFINITY MEDIA

Protein (mg/mL res±in)	AF-Blue HC-650M	AF-Red-650ML
Hexokinase	3	-
Bovine serum albumin	16	-
Human serum albumin	18 ±2.5	3.5 ±1
Lactate dehydrogenase	27	11

FIGURE 8

COMPARISON OF HUMAN SERUM ALBUMIN BINDING CAPACITIES AT VARIOUS pH'S OF AF-BLUE HC-650M AND AGAROSE (BLUE FUNCTIONALIZED AGAROSE)

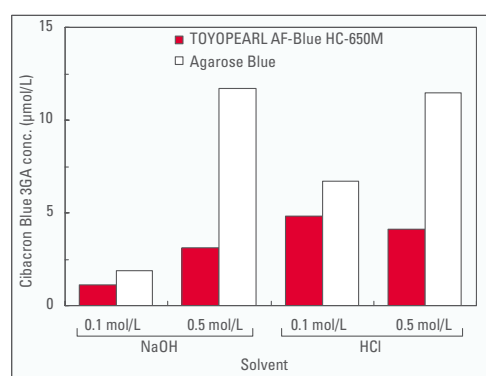


Conditions

- A 1.0 mL of adsorbent was washed with 10 mL of equilibration buffer (pH 4.0 and 5.0; 0.1 mol/L sodium acetate buffer, pH 6.0 and 7.0; 0.1 mol/L sodium phosphate buffer).
- A 5.0 mL of 1 % solution of human albumin dissolved in each equilibration buffer was charged onto the column.
- After 10 min, unbound albumin was eluted and the column was washed with 10 mL of each equilibration buffer.
- Adsorbed human albumin was eluted with 0.1 mol/L sodium phosphate buffer at pH 7.0 containing 2.0 mol/L sodium chloride (desorption buffer) and 10 mL fractions were collected.
- Human albumin content was measured spectrophotometrically by using E 0.1 % at 280 nm = 0.55.

FIGURE 9

COMPARATIVE DYE LEAKAGE STUDY OF AF-BLUE HC-650M AND COMPETITOR BLUE @ 25 °C (AFTER 24 HOURS)



Conditions:

- 200 mg of each material was suspended in 4 mL of solvent and incubated at 25 °C and shaking for 24 h. The absorption at 620 nm of the supernatants were measured after appropriate adjustment to neutrality with known volume of acid or base. Dye concentrations were estimated assuming a molar extinction coefficient of 12,750 (L/M cm).



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► ORDERING INFORMATION

ToyoScreen PROCESS DEVELOPMENT COLUMNS FOR AFC

PART #	PRODUCT DESCRIPTION	PACKAGE
0021384	ToyoScreen AF-Chelate-650M	1 mL x 6 each
0021385	ToyoScreen AF-Chelate-650M	5 mL x 6 each
0021386	ToyoScreen AF-Blue HC-650M	1 mL x 6 each
0021387	ToyoScreen AF-Blue HC-650M	5 mL x 6 each
0021388	ToyoScreen AF-Red-650ML	1 mL x 6 each
0021389	ToyoScreen AF-Red-650ML	5 mL x 6 each
0021390	ToyoScreen AF-Heparin HC-650M	1 mL x 6 each
0021391	ToyoScreen AF-Heparin HC-650M	5 mL x 6 each

ToyoScreen COLUMN ACCESSORIES

PART #	PRODUCT DESCRIPTION
0021400	ToyoScreen Column Holder

TOYOPEARL LABPAK

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0043400	AFFIPAK ACT (AF-Epoxy-, AF-Tresyl-650M)	2 x 5 g	65
0043410	AFFIPAK (AF-Amino-, AF-Carboxy-, AF-Formyl-650M)	3 x 10 mL	65

TSKgel RESIN

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	PARTICLE SIZE (µm)
0016208	Tresyl-5PW (10)	2 g	10

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ORDERING INFORMATION

TOYOPEARL AFFINITY CHROMATOGRAPHY RESIN

GROUP SPECIFIC RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0019688	TOYOPEARL AF-Blue HC-650M	25	15 mmol/L	minimum 18 g/L
0019689		100		
0019690		1,000		
0019691		5,000		
0008651	TOYOPEARL AF-Red-650ML	25	5 mmol/L	2.5 - 4.5 g/L (HSA)
0019801		100		
0042102		1,000		
0014475	TOYOPEARL AF-Chelate-650M	25	25-45 meq/L	-
0019800		100		
0014907		1,000		
0014908		5,000		
0020030	TOYOPEARL AF-Heparin HC-650M	10	-	5 g/L (AT III)
0020031		100		
0020032		1,000		
0020033		5,000		

REACTIVE RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0043411	TOYOPEARL AF-Amino-650M	10	70-130 meq/L	-
0008002		25		
0008039		100		
0018074		1,000		
0018316		5,000		
0043412	TOYOPEARL AF-Carboxy-650M	10	80-120 meq/L	-
0008006		25		
0008041		100		
0018827		1,000		
0018828		5,000		
0043413	TOYOPEARL AF-Formyl-650M	10	40-70 meq/L	-
0008004		25		
0008040		100		
0017396		1,000		
0017397		5,000		

ACTIVATED RESINS

PART #	PRODUCT DESCRIPTION	CONTAINER SIZE (mL)	TYPICAL LIGAND DENSITY	TYPICAL CAPACITY
0043402	TOYOPEARL AF-Epoxy-650M	5 g*	600 - 1000 µeq/g	-
0008000		10 g*		
0008038		100 g*		
0018315		1,000 g*		
0014471	TOYOPEARL AF-Tresyl-650M	5 g*	80 mmol/L	-
0014472		100 g*		
0014906		1,000 g*		

Conditions: All TOYOPEARL affinity resins are provided at a particle size of 65 µm. This particle size is ideal for both small and large scale separations.

*1 g yields approximately 3.5 mL of hydrated resin.