

Affinity Chromatography

Toyopearl Resins for AFC

Activated Resins

Toyopearl AF-Epoxy-650
Toyopearl AF-Tresyl-650

Reactive Resins

Toyopearl AF-Carboxy-650
Toyopearl AF-Amino-650
Toyopearl AF-Formyl-650

Toyopearl Resins with Group Specific Ligands that are ready to use

Toyopearl AF-Blue HC-650
Toyopearl AF-Chelate-650
Toyopearl AF-Red-650
Toyopearl AF-Heparin HC-650

ToyoScreen Process Development Columns for AFC

Denmark

MD Scientific is authorized distributor in
Denmark for Tosoh Bioscience

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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.

Tosoh Bioscience 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 and structures of Toyopearl group-specific ligands are listed in Figure 2.

How to select an activated or reactive resin for coupling your ligand

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

Use of Toyopearl affinity resins as a resin support

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.

All Toyopearl affinity resins have the following characteristics:

- ❖ hydrophilic, dimensionally stable base resin with excellent pressure-flow characteristics
- ❖ large (1000Å) pores to accommodate the largest proteins
- ❖ M grade (40-90µm) particle size, suited to laboratory and production scale affinity applications

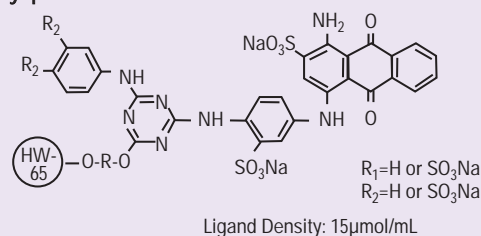
Table 1

Activated Resin	Reactive	Group Specific
AF-Tresyl	AF-Amino	AF-Blue HC
AF-Epoxy	AF-Carboxy	AF-Red
	AF-Formyl	AF-Chelate
		AF-Heparin HC

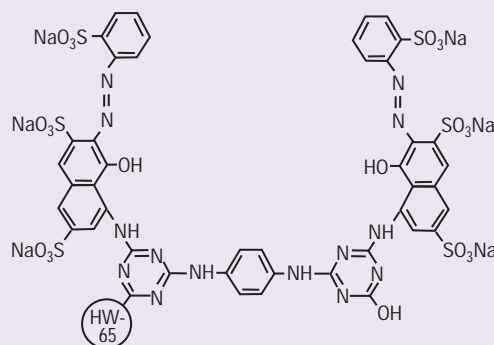
Figure 2

Group-specific Toyopearl affinity resins

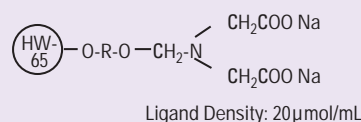
Toyopearl AF-Blue HC



Toyopearl AF-Red



Toyopearl AF-Chelate



Toyopearl AF-Heparin

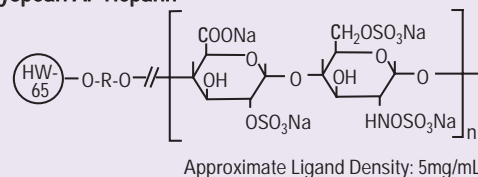
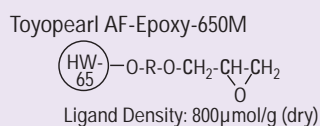
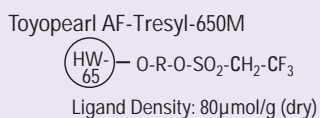
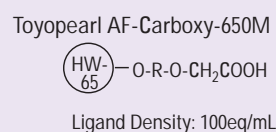
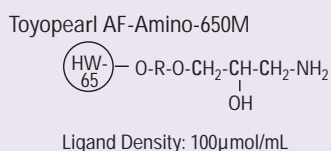
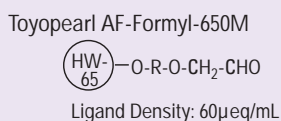


Figure 1

Activated Toyopearl affinity resins



Reactive Toyopearl affinity resins



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 (pH7 - 8) solution (Figure 3).

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. 20µmol/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. 800µmol/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 4). Glutathione and glycine have, for example, been coupled at densities greater than 100µmol/mL hydrated resin.

Toyopearl AF-Epoxy-650M 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.

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 5). 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

Figure 3

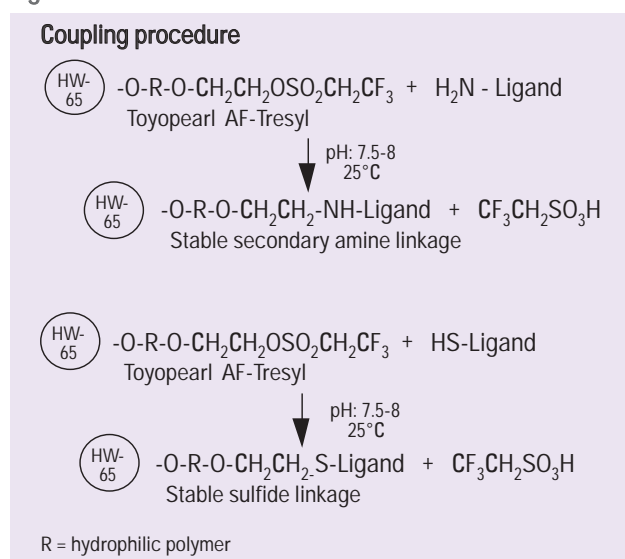


Figure 4

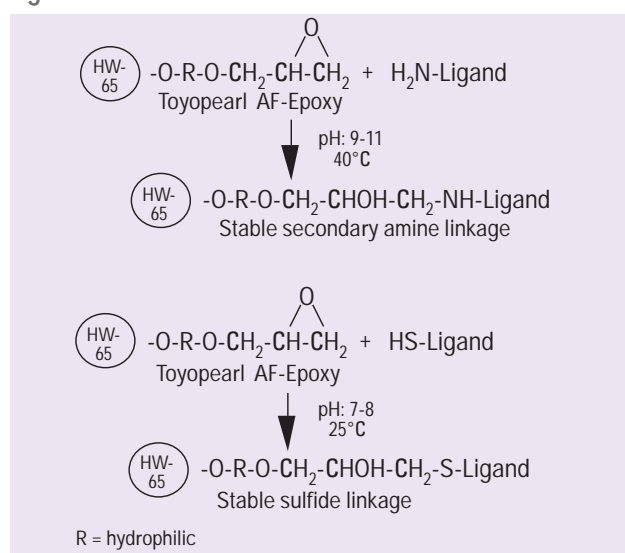


Table II

Representative coupling densities for activated and reactive Toyopearl media				
Toyopearl resin	AF-Tresyl-650M	AF-Formyl-650M	AF-Amino-650M	AF-Carboxy-650M
<i>Protein coupled (mg/mL resin)</i>				
Soybean trypsin inhibitor	16	3.5	5.8	15
Protein A	1.9	—	—	—
Concanavalin A	13	—	—	—
α1-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
<i>Coupling agent</i>	<i>not required</i>	<i>NaCNBH₃</i>	<i>NaCNBH₃ or Carbodiimide</i>	<i>Carbodiimide</i>
<i>Optimal pH</i>	7–9	6.9–9.0	4.5–6.0	4.5–6.0

supports (e.g. cellulose and agarose) or partial hydrolysis of polyacetals. In contrast, Toyopearl AF-Formyl-650M 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.

Figure 5

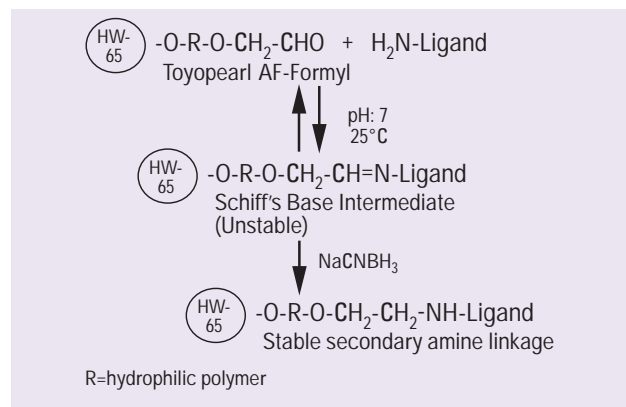


Figure 6

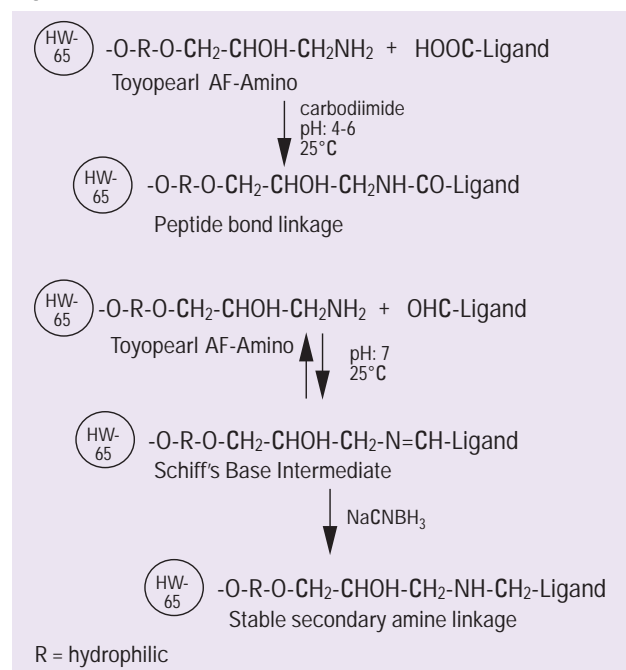
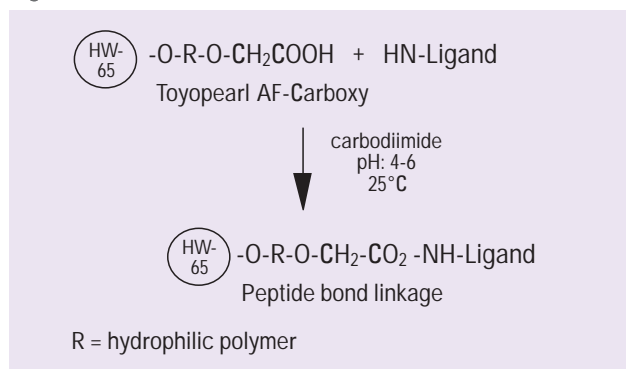


Figure 7



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 6*. 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 $\mu\text{mol/mL}$) 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 7*). Representative coupling densities are given in *Table II*.

Toyopearl resins with group specific ligands

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+} and Co^{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

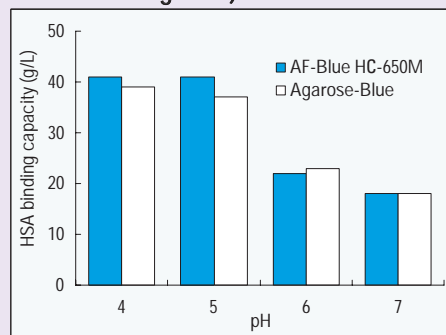
Functionalized with Cibachron Blue F3G-A, Toyopearl AF-Blue HC-650M has excellent capacity for proteins, particularly albumin (*Figure 8*). In addition, this high capacity resin has improved caustic stability, reduced dye ligand leakage, and superior pressure-flow characteristics relative to more traditional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

Toyopearl AF-Red-650ML

Toyoscreen AF-Red-650M Toyopearl AF-Red-650ML 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.

Figure 8

Comparison of human serum albumin binding capacities at various pHs of AF-Blue HC-650M and Agarose (blue functionalized agarose)



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 is a high capacity, affinity adsorbent with excellent chemical stability.

Pressure-flow characteristics and physical/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 - 500cm/h generate a pressure of between 1 and 2 bar in a 20cm length bed.

Achievement of high linear velocities at relatively low pressure enables high throughput production scale chromatography using equipment with moderate pressure limitations.

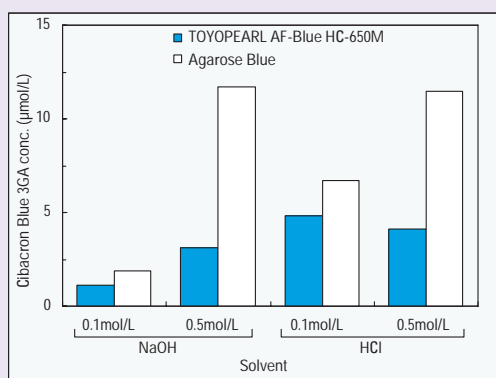
Sanitization or cleaning may be conducted with up to 0.5mol/L NaOH or 0.5mol/L HCl depending upon the ligand. In affinity chromatography, in particular, the choice of cleaning agent will be largely dependent upon the chemical stability of the ligand, rather than that of the base resin.

Toyoscreen prepacked columns for process development

Toyoscreen columns are available in 1mL and 5mL resin volumes of the following Toyopearl AFC products: Toyopearl AF-Chelate 650M, Toyopearl AF-Heparin HC-650M, Toyopearl AF-Blue HC-650M and Toyopearl AF-Red-650M. They provide a convenient way to perform early resin screening for both target retention and recovery. Multiple columns can be connected in series for additional separation. Please see the ordering information at the end of this section or contact us to request more information on our Toyoscreen offerings.

Figure 9

Comparative dye leakage study of AF-Blue HC-650M and Agarose Blue @ 25°C (after 24 hours)



Ordering Information

Toyoscreen process development columns for IEC:

Part #	Product description	Package
21384	Toyoscreen AF-Chelate-650M, 1mL	1mL x 6 each
21385	Toyoscreen AF-Chelate-650M, 5mL	5mL x 6 each
21386	Toyoscreen AF-Blue HC-650M, 1mL	1mL x 6 each
21387	Toyoscreen AF-Blue HC-650M, 5mL	5mL x 6 each
21388	Toyoscreen AF-Red-650ML, 1mL	1mL x 6 each
21389	Toyoscreen AF-Red-650ML, 5mL	5mL x 6 each
21390	Toyoscreen AF-Heparin HC-650M, 1mL	1mL x 6 each
21391	Toyoscreen AF-Heparin HC-650M, 5mL	5mL x 6 each

Toyoscreen column accessories

Part #	Product description	Comment
21400	Toyoscreen Column Holder	
42194	Toyoscreen Holder w/ Fittings	Includes 21400, 42195 and 42196 (qty. 2)
42195	Column to Column Connector	
42196	Adaptor, M6 female to 10-32 male, PEEK	
42197	Adaptor, 1/4-28 female to 10-32 male, PEEK	

continued...

Table III

Representative binding capacities for Toyopearl dye-ligand affinity media

Protein (mg/mL resin)	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

Ordering Information

Toyopearl LabPak

Part #	Product description	Contents	Particle size (μm)
43400	AFFIPAK ACT (AF-Epoxy-, AF-Tresyl-650M)	2 x 5g*	65
43410	AFFIPAK (AF-Amino-, AF-Carboxy-, AF-Formyl-650M)	3 x 10mL	65

TSK-GEL resin

16208	Tresyl-5PW (10)	2g	10
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Toyopearl affinity chromatography resin

Part #	Product description	Container size (mL)	Typical Ligand density	Typical capacity
<i>Group specific resins</i>				
19688	Toyopearl AF-Blue HC-650M	25	15 $\mu\text{mol/mL}$	minimum 18mg/mL
19689		100		
19690		1,000		
19691		5,000		
08651	Toyopearl AF-Red-650ML	25	5 $\mu\text{mol/mL}$	2.5 - 4.5mg/mL (HSA)
19801		100		
42102		1,000		
14475	Toyopearl AF-Chelate-650M	25	25-45 $\mu\text{eq/mL}$	
19800		100		
14907		1,000		
14908		5,000		
20030	Toyopearl AF-Heparin HC-650M	10		5mg/mL (AT III)
20031		100		
20032		1000		
20033		5000		

Reactive resins

43411	Toyopearl AF-Amino-650M	10	70-130 $\mu\text{eq/mL}$	–
08002		25		
08039		100		
18074		1,000		
18316		5,000		
43412	Toyopearl AF-Carboxy-650M	10	80-120 $\mu\text{eq/mL}$	–
08006		25		
08041		100		
18827		1,000		
18828		5,000		
43413	Toyopearl AF-Formyl-650M	10	40-70 $\mu\text{eq/mL}$	–
08004		25		
08040		100		
17396		1,000		
17397		5,000		

Activated resins

43402	Toyopearl AF-Epoxy-650M	5g*	600 - 1000 $\mu\text{eq/g}$	–
08000		10g*		
08038		100g*		
18315		1,000g*		
14471	Toyopearl AF-Tresyl-650M	5g*	80 $\mu\text{mol/mL}$	–
14472		100g*		
14905		200g*		
14906		1,000g*		

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.

*1g yields approximately 3.5mL.