

# Affinity Chromatography

## **TOYOPEARL Resins for AFC**

### Activated Resins

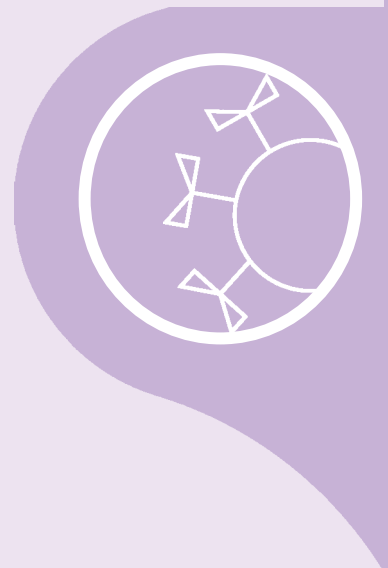
TOYOPEARL AF-Epoxy  
TOYOPEARL AF-Tresyl

### Reactive Resins

TOYOPEARL AF-Carboxy  
TOYOPEARL AF-Amino  
TOYOPEARL AF-Formyl

## **TOYOPEARL Resins with Group Specific Ligands that are ready to use**

TOYOPEARL AF-Blue-HC-650  
TOYOPEARL AF-Chelate-650  
TOYOPEARL AF-Red-650



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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 use for direct coupling of a protein or other ligand. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve 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 HW-65 resins have the following characteristics:

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

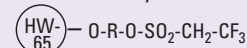
Table I

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

Figure 1

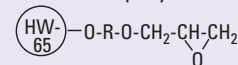
### Activated TOYOPEARL affinity resins

TOYOPEARL AF-Tresyl-650M



Ligand Density: 80~mol/g (dry)

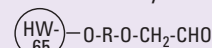
TOYOPEARL AF-Epoxy-650M



Ligand Density: 800~mol/g (dry)

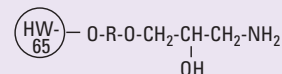
### Reactive TOYOPEARL affinity resins

TOYOPEARL AF-Formyl-650M



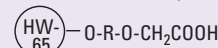
Ligand Density: 60~eq/mL

TOYOPEARL AF-Amino-650M



Ligand Density: 100~mol/mL

TOYOPEARL AF-Carboxy-650M

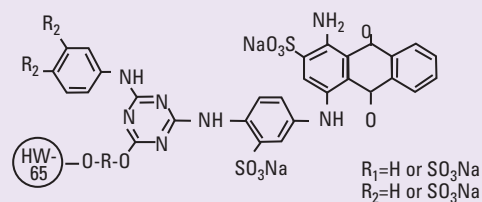


Ligand Density: 100eq/mL

Figure 2

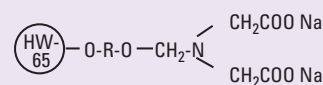
### Group-specific TOYOPEARL affinity resins

TOYOPEARL AF-Blue-HC



Ligand Density: 16~mol/mL

TOYOPEARL AF-Chelate



Ligand Density: 20~mol/mL

## Activated resins – ready for direct ligand attachment

**TOYOPEARL AF-Tresyl-650M** activated resin is highly reactive towards amine and thiol groups. It is provided in dry form, ready for combination with buffered solutions of protein or other ligand. Coupling is accomplished in neutral to slightly alkaline (pH 7 - 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 moderate 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 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 4). Glutathione and glycine have, for example, been coupled at densities  $> 100 \mu\text{mol/mL}$  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 purpose active ligands may be introduced onto this dimensionally stable, macroporous support.

## Reactive resins – requiring 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 enzymes have been immobilized on the aldehyde-bearing support for industrial applications. 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 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 3

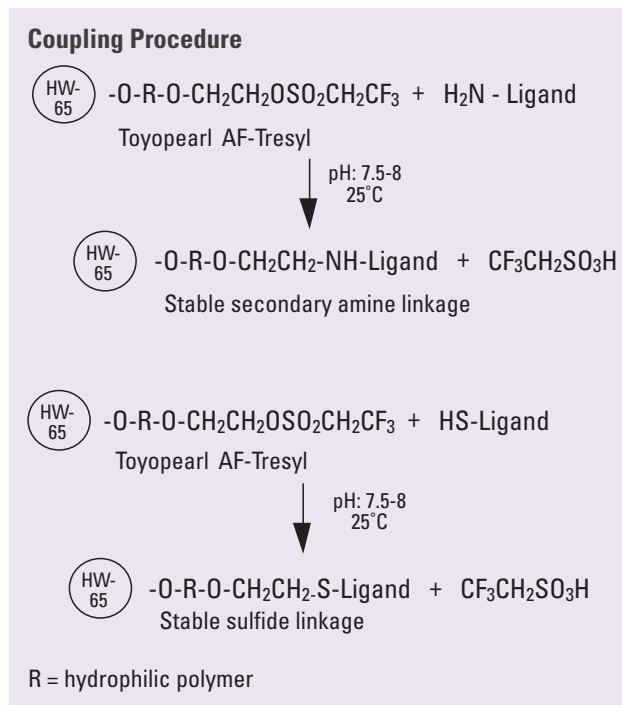
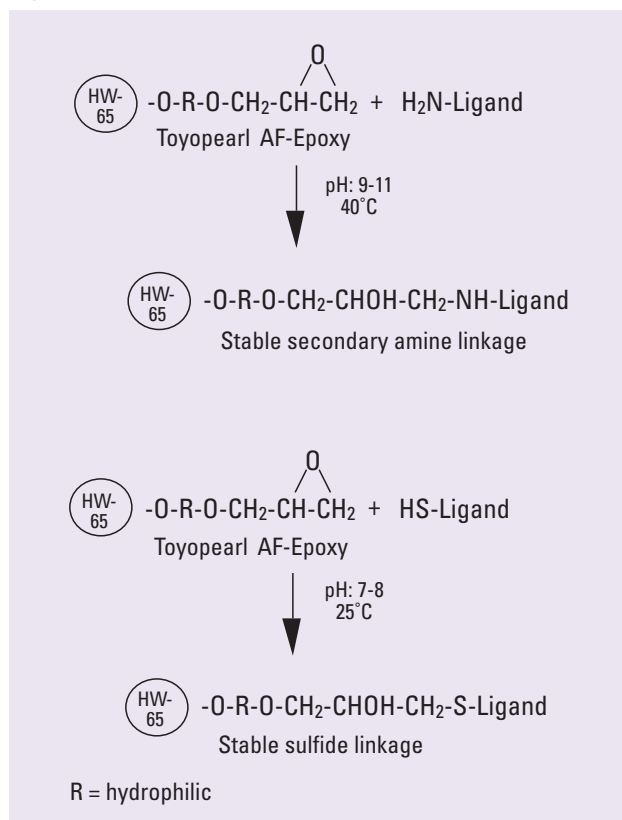
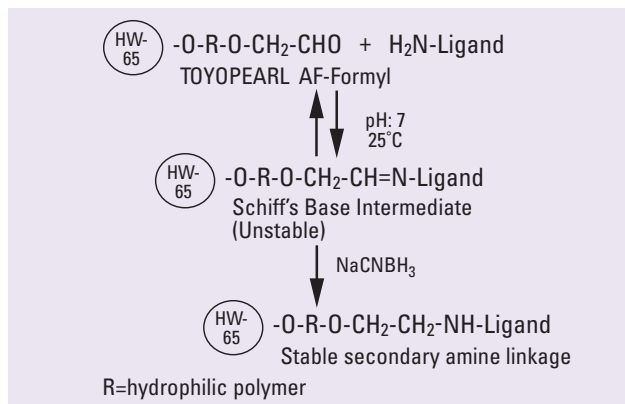


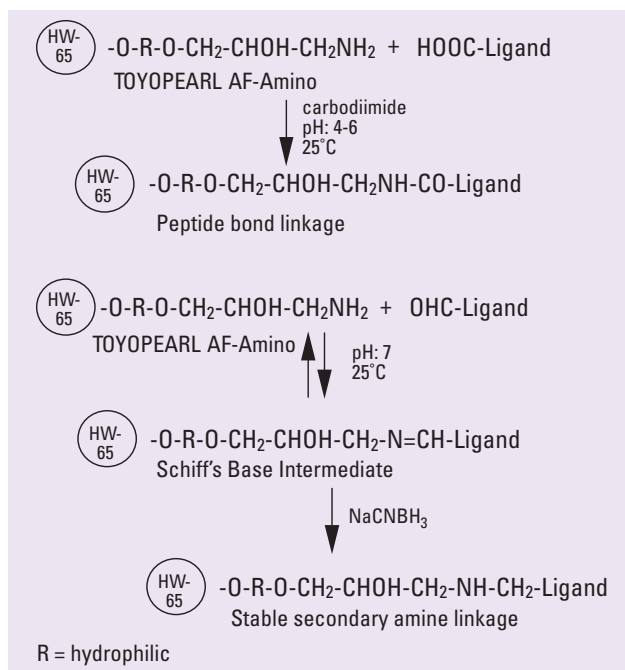
Figure 4



**Figure 5**



**Figure 6**



**Table II**

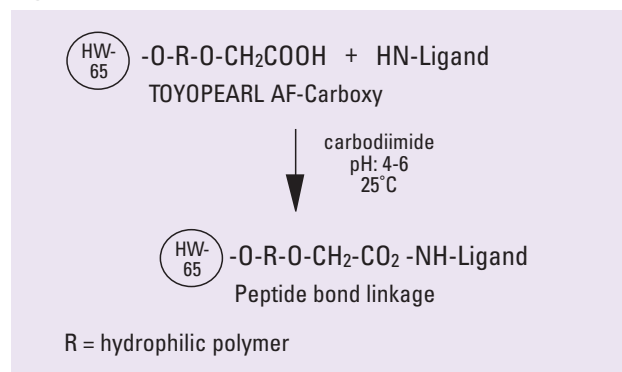
Representative coupling densities for activated and reactive TOYOPEARL media				
TOYOPEARL resin	AF-Tresyl-650M	AF-Formyl-650M	AF-Amino-650M	AF-Carboxy-650M
<i>Protein coupled (mg/mL resin)</i>				
Soybean trypsin inhibitor	16	3.5	5.8	15
Protein A	1.9	—	—	—
Concanavalin A	13	—	—	—
α1-Antitrypsin	12.3	—	—	—
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>NaCNBH<sub>3</sub></i>	<i>NaCNBH<sub>3</sub> or Carbodiimide</i>	<i>Carbodiimide</i>
<i>Optimal pH</i>	7–9	6.9–9.0	4.5–6.0	4.5–6.0

**TOYOPEARL AF-Amino-650M** resin may be used to couple ligands using their carboxylate groups (peptide bond formation) or aldehyde groups (reductive amination) (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 intermediate functional group density of TOYOPEARL AF-Amino-650M (90 μmol/mL) renders this material useful 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 μmol/mL resin. Coupling densities for various proteins are given in Table 12.

**TOYOPEARL AF-Carboxy-650M** resin provides another useful and mild 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.

**Figure 7**



## **TOYOPEARL Resins with Group Specific Ligand**

### **TOYOPEARL AF-Chelate-650M**

This resin is derivatized with iminodiacetic acid (IDA) at a concentration of *ca.* 35  $\mu\text{mol/mL}$ . In typical applications, selected metal ions, most often  $\text{Ca}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Co}^{2+}$  are bound to the support by stable tridentate chelation. The resultant metal ion-bearing resin binds to the histidine and free cysteine containing sequences of a peptide or protein. Immobilized metal ion affinity chromatography 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 high capacity and for proteins, particularly albumin. This resin displays improved stability to base treatment and reduced dye ligand leakage.

### **TOYOPEARL AF-Red 650M**

TOYOPEARL AF-Red 650M functionalized with Procion Red HE-3B, also known as Reactive Red 120. This resin is useful in 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 found in Table III.

## **Pressure/Flow Characteristics**

### **Physical/Chemical Stability**

TOYOPEARL resin remains dimensionally stable within wide extremes of pH and ionic strength. Moreover, the semi-rigid TOYOPEARL particles do not distort under flow rates generating up to 3 bar max. pressure. These properties of the resin, combined with the narrow particle size distribution, 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 deep bed (see Figure 8).

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

TOYOPEARL resins may be used routinely over the pH range 2 to 13. Sanitization or cleaning may be conducted with up to 0.5 M NaOH or 0.5 M HCl depending upon the ligand. In affinity chromatography in particular, the choice of cleaning solution will be largely dependent upon the chemical stability of the ligand, rather than that of the base resin.

**Table III**

<b>Representative binding capacities for TOYOPEARL Dye-Ligand Affinity Media</b>		
<i>Protein Coupled (mg/mL resin)</i>	<b>AF-Blue HC-650M</b>	<b>AF-Red 650M</b>
Hexokinase	3	—
Bovine serum albumin	16	—
Human serum albumin	18 $\pm$ 2.5	3.5 $\pm$ 1
Lactate dehydrogenase	27	11

## Ordering Information

### TOYOPEARL LABPAK

Part #	Product description	Contents	Particle size ( $\mu\text{m}$ )
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)	2 g	10
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\*1g yields approximately 3.5 mL.

### TOYOPEARL affinity chromatography resins:

Part #	Product description	Container size (mL)	Ligand density	Adsorption capacity
<b>Group specific resins</b>				
19688	AF-Blue HC-650M	25	>16 $\mu\text{mol/mL}$	>18 mg/mL (HSA)
19689		100		
19690		1,000		
19691		5,000		
08651	AF-Red-650ML	25	7 $\mu\text{mol/mL}$	2.5 - 4.5 mg/mL
19801		100		
42102		1,000		
14475	AF-Chelate-650M	25	25-45 $\mu\text{eq/mL}$	$\geq 60$ mg/mL
19800		100		
14907		1,000		
14908		5,000		
<b>Reactive resins</b>				
43411	AF-Amino-650M	10	70-130 $\mu\text{eq/mL}$	–
08002		25		
08039		100		
18074		1,000		
18316		5,000		
43412	AF-Carboxy-650M	10	80-120 $\mu\text{eq/mL}$	–
08006		25		
08041		100		
18827		1,000		
18828		5,000		
43413	AF-Formyl-650M	10	40-70 $\mu\text{eq/mL}$	–
08004		25		
08040		100		
17396		1,000		
17397		5,000		
<b>Activated resins</b>				
43402	AF-Epoxy-650M	5g*	600 - 1000 $\mu\text{eq/g}$	$\geq 60$ mg/g**
08000		10g*		
08038		100g*		
18315		1,000g*		
14471	AF-Tresyl-650M	5g*	80 $\mu\text{mol/mL}$	$\geq 60$ mg/g**
14472		100g*		
14905		200g*		
14906		1,000g*		

Conditions: Samples for adsorption capacity are as follows: AF-Chelate-650M – Lysozyme; AF-Red-650ML – Human Serum Albumin; AF-Tresyl-650M Soybean Trypsin Inhibitor. All TOYOPEARL affinity resins are provided at a particle size of 65  $\mu\text{m}$ . This particle size is ideal for both small and large scale separations.

\*1g yields approximately 3.5 mL. \*\*Measured as amount of test protein coupled per gram of dry gel.