Resin Type	Process Media
Activated Resins	TOYOPEARL AF-Epoxy-650 TOYOPEARL AF-Tresyl-650
Reactive Resins	TOYOPEARL AF-Carboxy-650 TOYOPEARL AF-Amino-650 TOYOPEARL AF-Formyl-650
Ready-to-Use Resins with Group Specific Ligands	TOYOPEARL AF-Blue HC-650 TOYOPEARL AF-Chelate-650 TOYOPEARL AF-Red-650 TOYOPEARL AF-Heparin HC-650

The role of Affinity Chromatography in Process Purification

Affinity chromatography (AFC) is distinctive as a chromatographic technique as it is the only mode that enables the purification of a biomolecule on the basis of functionality or unique chemical structure. Affinity chromatography works on the basis of a specific, reversible, interaction between a target molecule and a specific ligand coupled to a base chromatography bead. Affinity chromatography is inherently a high resolution media because it is highly selective for the molecule of interest, and usually high capacity as well. Because affinity resins are highly specific, post-purification product titer increases of more than 1000× are not unheard of. Purifications that would otherwise be difficult or even impractical using other modes can often be easily accomplished through the use of affinity chromatography.

TOYOPEARL Affinity Resins

There are many custom designed affinity ligands available to the chromatographer. TOYOPEARL affinity chromatography resins are functionalized with chemically active groups or with group-specific ligands. Resins with activated functional groups are ready to directly couple a protein or other ligand. The coupled ligand must preserve its specific binding affinity for the target molecule and the binding between the ligand and target molecule must be reversible to allow the target to be eluted without substantial alterations. Resins with reactive groups require carbodiimide coupling or reductive amination to achieve a stable covalent linkage.

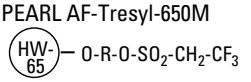
Tosoh Bioscience offers a spectrum of carefully selected TOYOPEARL affinity resins with activated or reactive groups which can be used to covalently attach almost any custom ligand. The structures of TOYOPEARL resins with activated and reactive ligands are shown in Figure 1 and structures of TOYOPEARL resins with group-specific ligands are listed in Figure 2. In general, TOYOPEARL AF-Tresyl-650M and TOYOPEARL AF-Formyl-650M resins 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 also 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.

TOYOPEARL affinity resins are composed of hydrophilic, dimensionally stable base resins that exhibit excellent pressure-flow characteristics. These resins use the TOYOPEARL HW-65 SEC resin as a base bead. The 100 nm pore diameter of the TOYOPEARL affinity resins can accommodate large globular proteins up to 5×10^6.

Figure 1: Activated and reactive TOYOPEARL affinity resins

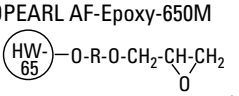
Activated TOYOPEARL affinity resins

TOYOPEARL AF-Tresyl-650M



Ligand Density: 80 µmol/g (dry)

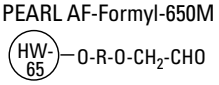
TOYOPEARL AF-Epoxy-650M



Ligand Density: 800 µmol/g (dry)

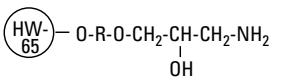
Reactive TOYOPEARL affinity resins

TOYOPEARL AF-Formyl-650M



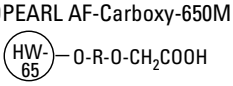
Ligand Density: 60 µeq/mL

TOYOPEARL AF-Amino-650M



Ligand Density: 100 µmol/mL

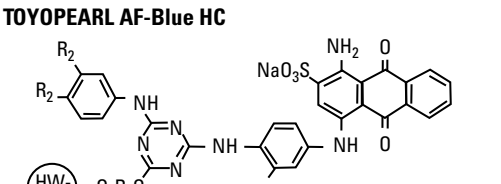
TOYOPEARL AF-Carboxy-650M



Ligand Density: 100 eq/mL

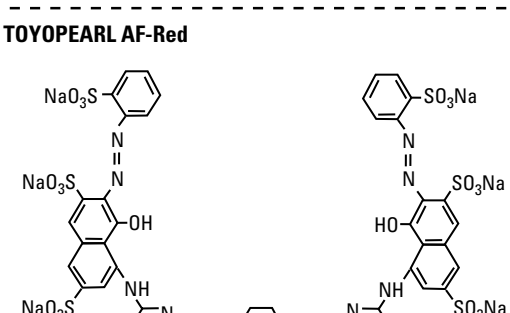
Figure 2: Group-specific TOYOPEARL affinity resins

TOYOPEARL AF-Blue HC



Ligand Density: 15 µmol/mL

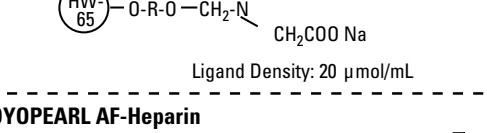
TOYOPEARL AF-Red



TOYOPEARL AF-Chelate

Ligand Density: 20 µmol/mL

TOYOPEARL AF-Heparin

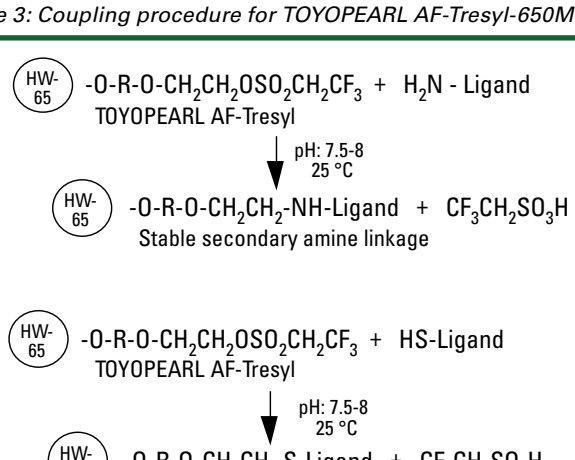


Approximate Ligand Density: 5 mg/mL

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 the ligand to be coupled. Coupling is accomplished in a neutral to slightly alkaline (pH 7 - 8) solution (Figure 3).

Figure 3: Coupling procedure for TOYOPEARL AF-Tresyl-650M



R = hydrophilic polymer

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 µmol/mL hydrated resin) is sufficient to provide substantial protein binding while avoiding excessive multi-point attachment and consequent impairment of ligand affinity and activity. Representative data are presented in Table 1.

TOYOPEARL AF-Epoxy-650M activated resin, also packaged in dry form, has a high density of epoxy-functionality (ca. 800 µmol/mL). Under appropriate reaction conditions, this may be used to immobilize 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 100 µmol/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.

Figure 4: Coupling procedure for TOYOPEARL AF-Epoxy-650M

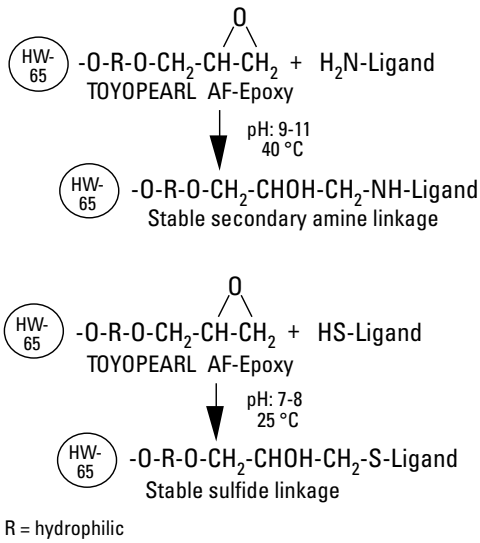


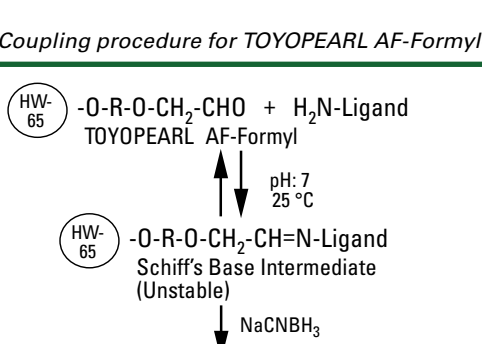
Table 1 Representative coupling densities for activated and reactive TOYOPEARL media

TOYOPEARL resin *Protein coupled (g/L resin)*	**AF-Tresyl-650M**	**AF-Formyl-650M**	**AF-Amino-650M**	**AF-Carboxy-650M**
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	—	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

Reactive resins - require activation for ligand attachment

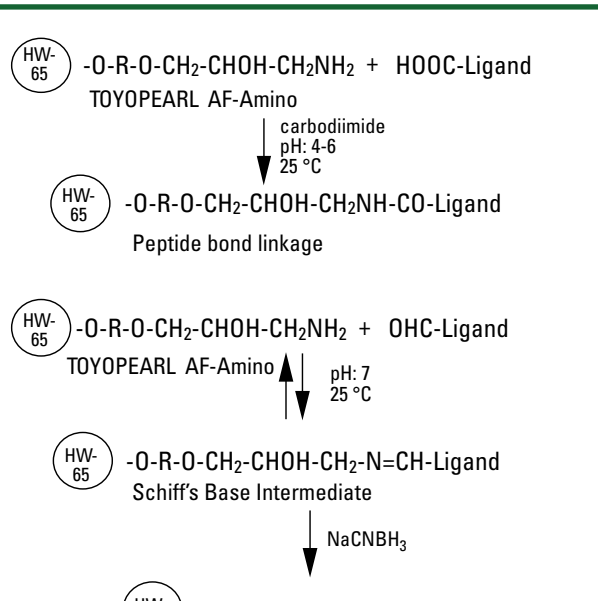
Ligands may be coupled to TOYOPEARL AF-Formyl-650M (aldehyde-bearing) resin under mild conditions exclusively using primary amines. The ligand is bound to the resin by a stable secondary amine linkage (Figure 5). A wide variety of industrial enzymes have been immobilized on aldehyde-bearing supports. 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 ensured from batch to batch.

Figure 5: Coupling procedure for TOYOPEARL AF-Formyl-650M



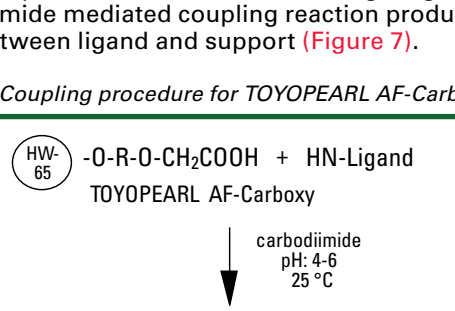
TOYOPEARL AF-Amino-650M resin may be used to couple ligands using their carboxyl groups through peptide bond formation or aldehyde groups by reductive amination as shown in Figure 6. Aldehyde groups may be present in a carbohydrate or glycoprotein ligand or may be introduced into the ligand by mild, periodate oxidation. The optimized functional group density of TOYOPEARL AF-Amino-650M (100 µmol/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 µmol/mL resin.

Figure 6: Coupling procedure for TOYOPEARL AF-Amino-650M



TOYOPEARL AF-Carboxy-650M resin provides another useful, though 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).

Figure 7: Coupling procedure for TOYOPEARL AF-Carboxy-650M



Resins with group specific ligands

TOYOPEARL AF-Chelate-650M resin is derivatized with iminodiacetic acid (IDA) at a concentration of ca. 20 µmol/mL. In typical applications, selected metal ions, most often Cu^{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, glycophorins, and whole cells.

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).

Figure 8: Comparison of human serum albumin binding capacities at various pHs of TOYOPEARL AF-Blue HC-650M and Agarose (blue functionalized agarose) resins

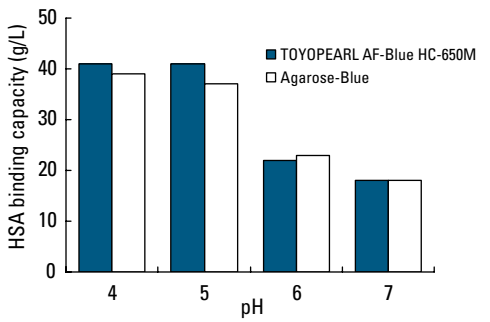
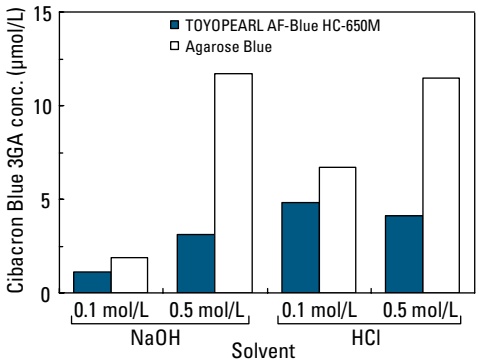


Figure 9: Comparative dye leakage study of TOYOPEARL AF-Blue HC-650M and Agarose Blue resins at 25 °C (after 24 hours)



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. Both TOYOPEARL AF-Blue HC-650M and TOYOPEARL AF-Red-650ML resins are useful for the purification of nucleotide-dependent enzymes, albumin, cell growth factors, interferons, transferases, cyclases, and polymerases. Typical binding capacities are shown in Table 2.

Table 2: Representative binding capacities for TOYOPEARL dye-ligand affinity media

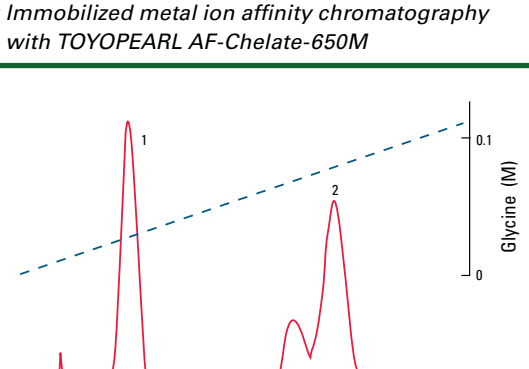
Protein (g/L)	TOYOPEARL AF-Blue HC-650M	TOYOPEARL AF-Red 650ML
hexokinase	3	
bovine serum albumin	16	
human serum albumin	18 ± 2.5	3.5 ± 1
lactate dehydrogenase	27	11

TOYOPEARL AF-Heparin HC-650M resin is a high capacity, affinity adsorbent with excellent chemical stability. The heparin ligand 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.

Separation of Two Proteins

Metal ion affinity chromatography is often used for the purification of histidine-rich or histidine-tagged proteins. For example, in the separation of two proteins, zinc ions were immobilized to the resin and salt was used in the eluent to suppress the ionic interactions between the sample and the carboxyl groups of the AF-Chelate-650M resin (Figure 10). These conditions favor chelation of the proteins by the resin-bound metal ions over potential ion exchange interactions. Typical elution gradients use imidazole (1 mmol/L to 20 mmol/L), glycine (0 to 0.2 mol/L), or a pH gradient (8.0 to 4.0).

Figure 10: Immobilized metal ion affinity chromatography with TOYOPEARL AF-Chelate-650M

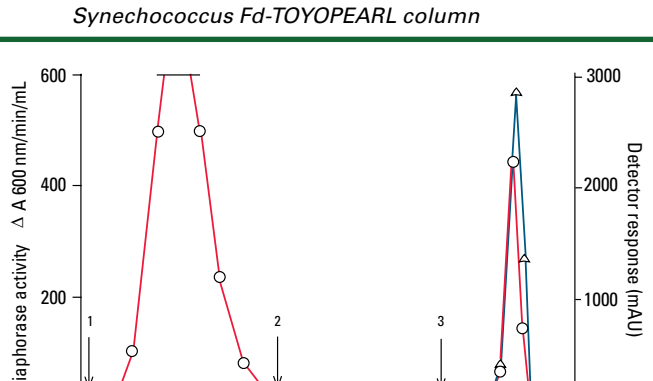


Resin:	**TOYOPEARL AF-Chelate-650M**
Column size:	8 mm ID × 7.5 cm
Metal ion:	Zn^{+2}
Mobile phase:	Buffer A: 20 mmol/L Tris-HCl, 0.5 mol/L NaCl, pH 8.0 Buffer B: buffer A + 0.2 mol/L glycine
Gradient:	0 - 100% B (120 min)
Flow rate:	60 cm/hr (0.5 mL/min)
Detection:	UV @ 280 nm
Samples:	1. ribonuclease A. 250 µg 2. transferrin, 250 µg

Purification of Ferredoxin-NADP Reductase

Synechococcus ferredoxin (Fd) was coupled to TOYOPEARL AF-Tresyl using a 0.1 mol/L $NaHCO_3$, pH 8, coupling buffer. The resulting *Synechococcus* Fd-TOYOPEARL was used to purify ferredoxin-NADP reductase, as shown in Figure 11[1]. The TOYOPEAERL AF-Tresyl was preferred by the authors over agarose-based affinity resins due to the superior flow properties of the TOYOPEARL resin.

Figure 11: Affinity chromatography of spinach FNR on a Synechococcus Fd-TOYOPEARL column



Resin:	***Synechococcus* Fd-TOYOPEARL**
Column size:	22 mm ID × 10 cm
Mobile phase:	1. Load with crude spinach FNR in 20 mmol/L Tris-HCl, pH 7.5 2. Wash with 20 mmol/L Tris-HCl, pH 7.5 3. Elute with 20 mmol/L Tris-HCl, pH 7.5 with 0.25 mol/L NaCl
Flow rate:	16 cm/hr (1 mL/min)
Detection:	UV @ 275 nm, specific activity
Sample:	spinach FNR

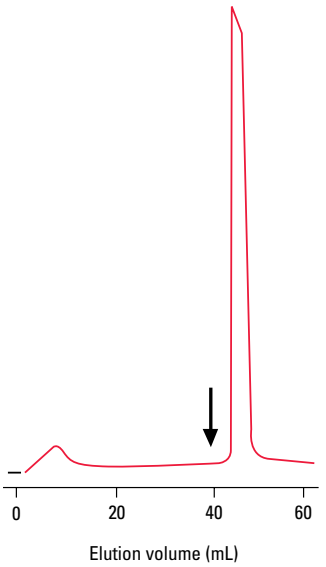
[1]Sakihama, N.; Nagai, K.; Ohmori, H.; Tomizawa, H.; Tsujita, M.; Shin, M. Immobilized ferredoxins for affinity chromatography of ferredoxin-dependent enzymes. *J. Chroma. A.* **1992**, *597*, 147-153.

Purification of Lectins

The high density of epoxy functionality is especially useful for generating specialized affinity supports with low molar mass ligands. For example, 150 mg N-acetylgalactosamine (GalNAc) was couple to 1.0 g of hydrated resin by reaction in 3 mL of 0.1 mol/L sodium hydroxide at 45 °C for 16 hours with gentle agitation[2]. The product was washed with distilled water, 1 mol/L sodium chloride, and distilled water. Residual epoxy groups were blocked by treatment with 1 mol/L ethanolamine (25 °C, 12 hours).

The TOYOPEARL AF-GalNAc resin was used to purify a lection from *Grifola frondosa* (GFL), an edible mushroom (Figure 12). A two-step affinity chromatography scheme yielded 3.2 mg of FGL with 86% of the initial activity found in 2.34 g of crude protein from an ammonium sulfate precipitation.

Figure 12: Purification of lectins with specialized supports prepared from TOYOPEARL AF-Epoxy-650M



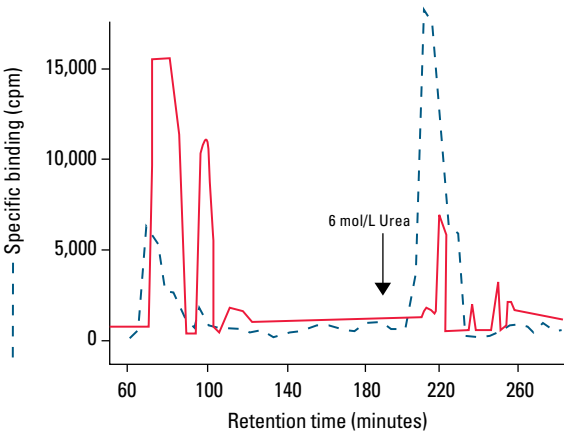
Resin:	**GalNAc-Epoxy TOYOPEARL**
Column size:	10 mm ID × 5 cm
Mobile phase:	Buffer A: 10 mmol/L phosphate-buffered saline, pH 7.4
	Buffer B: buffer A + 0.1mol/L lactose
Gradient:	equilibrate/load/wash 100% buffer A isocratic elution 100% buffer B
Detection:	UV @ 275 nm
Sample:	4.0 mg impure *Grifola frondosa* lectin

[2]Kawagishi, H.; Nomura, A.; Mizuno, T.; Kimura, A.; Chiba. S. Isolation and characterization of a lectin from Grifola frondosa fruiting bodies. Biochimica et Biophysica Acta (BBA) - General Subjects. **1990**, *1034*, (3), 247-252.

Purification of GH Receptor Protein

As shown in Figure 13, growth hormone (GH) was coupled to TOYOPEARL AF-Formyl-650M, and then was used to purify GH receptor protein[3]. A size exclusion column (TSKgel G3000SW) was directly connected to the affinity column. This approach eliminated the urea that co-eluted with the GH receptor from the affinity column, and enabled high receptor activity as denaturation was minimized. This one-step procedure provided a 1,000-fold purification, yielding 50 mg of GH receptor.

Figure 13: Growth hormone immunoaffinity support prepared with TOYOPEARL AF-Formyl-650M



Resin:	**GH-Formyl TOYOPEARL**
Column size:	4.6 mm ID × 15 cm in series with TSKgel 3000SW, 7.6 mm ID × 60 cm
Mobile phase:	Buffer A: 50 mmol/L Tris-HCl, 20 mmol/L NaCl, 10 mmol/L $MgCl_2$, 0.3 mmol/L phenylmethylsulfonyl fluoride GH receptor protein, 0.1% Triton™ X-100, pH 7.4
	Buffer B: buffer A + 6 mol/L urea
Gradient:	isocractic elution 100% buffer B
Flow rate:	36 cm/hr for 60 min, then 220 cm/hr
Detection:	UV @ 280 nm, specific binding assay
Sample:	16 mg growth hormone (GH) receptor protein in 6 mL Triton X-100

[3]Yagi, S.; Izawa, K.; Nakagawa, T.; Tanaka, H.; Yoshitake, A.; Mohri, Z. Efficient high performance liquid chromatographic system for protein purification. *J. Chroma. A.* **1989**, *493*, (1), 27-33.

A selection of screening tools are available for TOYOPEARL Affinity resins. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Affinity resins:

Part #	Product description	Container size (mL)	Typical ligand density	Typical capacity (g/L)	
TOYOPEARL Affinity Resins with Group Specific Ligands					
19688	TOYOPEARL AF-Blue HC-650M	25	15 µmol/mL	> 18 (HSA)	
19689	TOYOPEARL AF-Blue HC-650M	100	15 µmol/mL	> 18 (HSA)	
19690	TOYOPEARL AF-Blue HC-650M	1,000	15 µmol/mL	> 18 (HSA)	
19691	TOYOPEARL AF-Blue HC-650M	5,000	15 µmol/mL	> 18 (HSA)	
08651	TOYOPEARL AF-Red-650ML	25	7 µmol/mL	2.5 - 4.5 (HSA)	
19801	TOYOPEARL AF-Red-650ML	100	7 µmol/mL	2.5 - 4.5 (HSA)	
42102	TOYOPEARL AF-Red-650ML	1,000	7 µmol/mL	2.5 - 4.5 (HSA)	
14475	TOYOPEARL AF-Chelate-650M	25	25 - 45 µeq/mL	≥ 60 (lysozyme)	
19800	TOYOPEARL AF-Chelate-650M	100	25 - 45 µeq/mL	≥ 60 (lysozyme)	
14907	TOYOPEARL AF-Chelate-650M	1,000	25 - 45 µeq/mL	≥ 60 (lysozyme)	
14908	TOYOPEARL AF-Chelate-650M	5,000	25 - 45 µeq/mL	≥ 60 (lysozyme)	
20030	TOYOPEARL AF-Heparin-HC-650M	10	5 mg/mL	≥ 5 (AT III)	
20031	TOYOPEARL AF-Heparin-HC-650M	100	5 mg/mL	≥ 5 (AT III)	
20032	TOYOPEARL AF-Heparin-HC-650M	1,000	5 mg/mL	≥ 5 (AT III)	
20033	TOYOPEARL AF-Heparin-HC-650M	5,000	5 mg/mL	≥ 5 (AT III)	
TOYOPEARL Reactive Affinity Resins					
43411	TOYOPEARL AF-Amino-650M	10	70 - 130 µeq/mL		
08002	TOYOPEARL AF-Amino-650M	25	70 - 130 µeq/mL		
08039	TOYOPEARL AF-Amino-650M	100	70 - 130 µeq/mL		
18074	TOYOPEARL AF-Amino-650M	1,000	70 - 130 µeq/mL		
18316	TOYOPEARL AF-Amino-650M	5,000	70 - 130 µeq/mL		
43412	TOYOPEARL AF-Carboxy-650M	10	80 - 120 µeq/mL		
08006	TOYOPEARL AF-Carboxy-650M	25	80 - 120 µeq/mL		
08041	TOYOPEARL AF-Carboxy-650M	100	80 - 120 µeq/mL		
18827	TOYOPEARL AF-Carboxy-650M	1,000	80 - 120 µeq/mL		
18828	TOYOPEARL AF-Carboxy-650M	5,000	80 - 120 µeq/mL		
43413	TOYOPEARL AF-Formyl-650M	10	40 - 70 µeq/mL		
08004	TOYOPEARL AF-Formyl-650M	25	40 - 70 µeq/mL		
08040	TOYOPEARL AF-Formyl-650M	100	40 - 70 µeq/mL		
17396	TOYOPEARL AF-Formyl-650M	1,000	40 - 70 µeq/mL		
17397	TOYOPEARL AF-Formyl-650M	5,000	40 - 70 µeq/mL		

HSA = Human Serum Albumin

Part #	Product description	Container size (g)	Typical ligand density	Adsorption capacity (mg/g)	
TOYOPEARL Activated Affinity Resins					
43402	TOYOPEARL AF-Epoxy-650M*	5	600 - 1,000 µeq/g	> 60**	
08000	TOYOPEARL AF-Epoxy-650M*	10	600 - 1,000 µeq/g	> 60**	
08038	TOYOPEARL AF-Epoxy-650M*	100	600 - 1,000 µeq/g	> 60**	
18315	TOYOPEARL AF-Epoxy-650M*	1,000	600 - 1,000 µeq/g	> 60**	
14471	TOYOPEARL AF-Tresyl-650M*	5	80 µmol/mL	≥ 60**	
14472	TOYOPEARL AF-Tresyl-650M*	100	80 µmol/mL	≥ 60**	
14905	TOYOPEARL AF-Tresyl-650M*	200	80 µmol/mL	≥ 60**	
14906	TOYOPEARL AF-Tresyl-650M*	1,000	80 µmol/mL	≥ 60**	
18371	TOYOPEARL AF-Tresyl-650M*	5,000	80 µmol/mL	≥ 60**	

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