TOSOH BIOSCIENCE

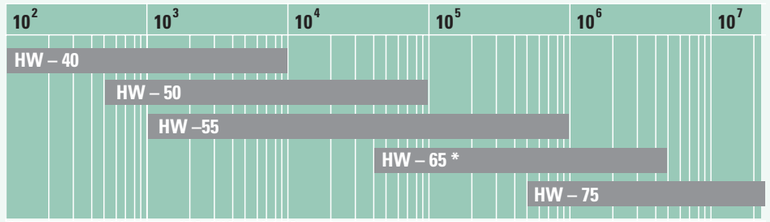
TOYOPEARL® ORIENTATION SHEET

Size Exclusion Chromatography

TOYOPEARL RESINS
HW-40 (S, F, C), 50 Å
HW-50 (S, F), 125 Å
HW-55 (S, F), 500 Å
HW-65 (S, F, C), 1.000 Å
HW-75 (F), >1.000 Å
S = 30 µm, F = 45 µm, C = 75 µm

Molecular weight operating ranges of SEC media:

Resin type	Polyethyleneglycols	Dextrans	Globular proteins
HW-40	100 - 3,000	100 - 7,000	100 - 10,000
HW-50	100 - 18,000	500 - 20,000	500 - 80,000
HW-55	100 - 150,000	1,000 - 200,000	1,000 - 700,000
HW-65	500 - 1,000,000	10,000 - 1,000,000	40,000 - 5,000,000
HW-75	4,000 - 5,000,000	100,000 - 10,000,000	500,000 - 50,000,000



* base material for most IEC, HIC and AFC products

Toyopearl Size Exclusion Resins separate molecules according to their physical size. They are ideal for fractionation of complex samples, buffer change or the final polishing step of a purification protocol.
Suggested use: HW-40: fraction of low MW compounds or buffer exchange; HW-50: for peptide fragments; HW-55, HW-65: for proteins; HW-75: for large nucleic acids or plasmid DNA.
HW resins are excellently suited for the separation of oligosaccharide or glycosilated proteins.

Ion Exchange Chromatography

ANION-EXCHANGERS
SuperQ-650 (S, M, C), 400 Å
QAE-550C, 500 Å
DEAE-650 (S, M, C), 1.000 Å
S = 35 µm, M = 65 µm, C = 100 µm

CATION-EXCHANGERS
CM-650 (S, M, C), 1.000 Å
SP-650 (S, M, C), 1.000 Å
SP-550C, 500 Å
MegaCap II SP-550EC, 300 Å
GigaCap S-650M 1.000 Å, 75 µm
S = 35 µm, M = 65 µm, C = 100 µm, EC= 200 µm

Batch adsorption capacity

Resin	QAE-550	DEAE-650	SP-550	SP-650	CM-650	Super Q-650	MegaCap II SP-550	GigaCapS -650
Trypsin Inhibitor	95	-	-	-	-	-	-	-
BSA	70	30	-	-	45	143	-	-
Ferritin	25	15	-	-	-	7	-	-
Thyroglobulin	9	12	-	-	-	-	-	-
Cytochrome C	-	-	120	-	-	-	-	-
Lysozyme	-	-	110	50	38	-	-	>200
Hemoglobin	-	-	111	42	50	-	-	-
STI	-	-	-	-	-	115	-	-
mAb	-	-	-	-	-	-	-	>150
Insulin	-	-	-	-	-	-	>100	-

Structure of Toyopearl IEC resins

DEAE-650S, DEAE-650M, DEAE-650C — HW-65 — -O-CH_2-CH_2-NH^+-(C_2H_5)$_2$ anion exchanger
SuperQ-650S, SuperQ-650M, SuperQ-650C — HW-65 — -O-R'-N^+-(CH_3)$_3$ strong anion exchanger
QAE-550C — HW-55 — -O-CH_2-CH_2-N^+-(C_2H_5)$_3$ strong anion exchanger
CM-650S, CM-650M, CM-650C — HW-65 — -O-CH_2-COOH weak cation exchanger
SP-650S, SP-650M, SP-650C — HW-65 — -O-CH_2-CH_2-CH_2-SO_3^- strong cation exchanger
SP-550C, MegaCap II, SP-550EC — HW-55 — -O-CH_2-CH_2-CH_2-SO_3^- strong cation exchanger
GigaCapS-650M — graft type, HW-65 based strong cation exchanger

Toyopearl Ion Exchange Resins. IEC is the most common liquid chromatographic method used in manufacturing of biological therapeutics. Toyopearl IEC resins have the advantages of the HW-65 or HW-55 base matrices. For example, high permeability enables rapid pH or ionic strength equilibration, often within only three column volumes.
Suggested use: all functionalities for recombinant and membrane proteins. SP-650, CM-650 for mAbs, DEAE-650 for blood products and nucleic acids, SuperQ optimized for proteins <50 kDa. MegaCapII, SP-550, QAE-550 and especially GigaCap S have very high dynamic capacities and enable high throughput for concentrating dilute process streams.

Hydrophobic Interaction Chromatography

TOYOPEARL RESINS
Ether-650 (S, M), 1.000 Å
PPG-600M , 750 Å
Phenyl-600M, 750 Å
Phenyl-650 (S, M, C), 1.000 Å
Butyl-650 (S, M, C), 1.000 Å
Butyl-600M, 750 Å
SuperButyl-550C, 500 Å
Hexyl-650C, 1.000 Å
S = 35 µm, M = 65 µm, C = 100 µm

Batch adsorption capacity[1]/DBC* (10% leakage)[2]

Resins	Protein Lysozyme (mg/ml resin)	Other (mg/ml)
Ether-650 (S, M)	10-30[1]	
PPG-600M	45-55[1]	mAb (Anti-LH): 38[2]
Phenyl-650 (S, M, C)	30-50[1]	mAb (Anti-LH): 30[2]
Butyl-600M		mAb (Anti-Lh): 54[2]
Butyl-650 (S, M , C)	40[1], 40[2]	
SuperButyl-550C	30-50[1], 61[2]	
Hexyl-650C	30-50[1]	
Phenyl-600M	60[1]	humanized mAb >50[1]

Hydrophobic strength
Ether
PPG
Phenyl
Butyl
Hexyl
increasing

* DBC =dynamic binding capacity

Structure of TSK-GEL HIC resins

TSKgel Ether-5PW — 5000PW — (O-CH_2CH_2)$_n$-OH
TSKgel Phenyl-5PW — 5000PW — O — ⬡

Structure of Toyopearl HIC resins

Toyopearl Ether-650 — HW-65 — (O-CH_2CH_2)$_n$-OH
Toyopearl PPG-600 — HW-60 — (O-$CH(CH_3)$-CH_2)$_n$-OH
Toyopearl Phenyl-650, Toyopearl Phenyl-600 — HW-65 — O — ⬡
Toyopearl Butyl-650 — HW-65 — O-CH_2-CH_2-CH_2-CH_3
Toyopearl Butyl-600 — HW-60 — O-CH_2-CH_2-CH_2-CH_3
Toyopearl SuperButyl-550 — HW-55 — O-CH_2-CH_2-CH_2-CH_3
Toyopearl Hexyl-650 — HW-65 — O-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3

Toyopearl Hydrophobic Interaction Resins provide exceptional selectivities of proteins by recognition of their surface hydrophobicities. The gentle, non-denaturing characteristics of HIC retains high levels of protein activity. Five different ligands provide a complete range of selectivities for optimization of retention, resolution, and recovery. Various pore sizes allow for optimization of dynamic capacity for individual target proteins.
Suggested Use: The protein with the lowest hydrophobicity should be bound to the most hydrophobic media and vice versa (see retention bar chart). Butyl-600, PPG-600 and Phenyl-600 are optimized for mAb purification by combining very high DBC with high recovery.

Affinity Chromatography

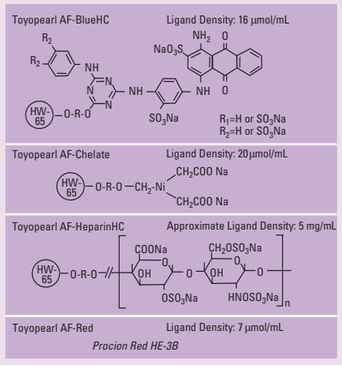
TOYOPEARL REACTIVE RESINS
AF-Amino-650M, 1.000 Å
AF-Carboxy-650M, 1.000 Å
AF-Formyl-650M, 1.000 Å

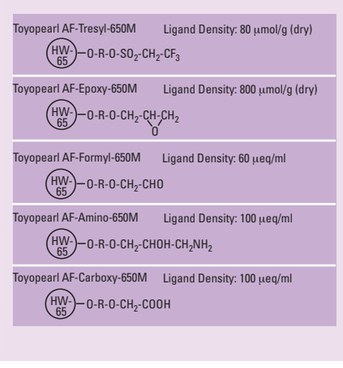
TOYOPEARL ACTIVATED RESINS
AF-Epoxy-650M, 1.000 Å
AF-Tresyl-650M, 1.000 Å

TOYOPEARL READY TO USE RESINS
AF-BlueHC-650M, 1.000 Å
AF-Chelate-650M, 1.000 Å
AF-HeparinHC-650M, 1.000 Å
AF-Red-650ML, 1.000 Å
M= 65 µm, ML =65 µm

Resin	Target ligand	Coupling cond.	Coupling agent	Human IgG	Lysozyme
Epoxy	R-NH_2	pH: 9.0-11.0, 40 °C	none		
	R-SH	pH: 7.0-8.0, 25 °C	none		
	R-OH	0.1N NaOH, 40 °C	none		
Tresyl	R-NH_2, R-SH	pH: 7.5-8.0, 25 °C	none	10.0	60.0
Amino	R-COOH, R-CHO	pH: 4.0-6.0/7.0, 25 °C	EDC*/$NaCNBH_4$	6.7	5.8
Carboxy	R-NH_2	pH: 4.0-6.0, 25 °C	EDC*	11.7	17.5
Formyl	R-NH_2	pH: 7.0, 25 °C	$NaCNBH_4$	15.0	20.0

* N'-(3-Dimethylaminopropyl)-N-ethylcarbodiimid





Toyopearl Affinity Resins are mechanical stable and provide good pH stablility and high ligand capacities. Activated resins are supplied as freeze-dried powders (1g/4ml gel), while reactive and ready to use resins are supplied as preswollen gels.
Suggested Use: Epoxy-650 readily forms a stable ether linkage to small ligands, Tresyl-650 is recommended for direct coupling of proteins with mild conditions. The reactive Amino, Carboxy, or Formyl-650 immobilize either proteins or small ligands by the carbodiimide or reductive alkylation coupling methods. BlueHC-650 is mainly used for purification of recombinant HSA, whereas Red-650 is useful in the purification of nucleotide-dependent enzymes. Chelate-650 will be used in the IMAC-mode for the purification of HIS-tagged proteins. HeparinHC-650 interacts with a wide range of plasma components, e.g. DNA polymerase.

Toyopearl resins are hydrophilic, macroporous, bulk bioprocessing media, made exclusively for large-scale chromatographic applications. Because of their polymeric backbone structure, the rigid Toyopearl packings assure excellent pressure/flow characteristics (1000 cm/h, 5 bar). The media are stable over the pH 2.0 - 13.0 range for normal operating conditions and pH 2.0 - 14.0 range for cleaning conditions. The particle sizes are 20-50 µm superfine grade for the highest performance, 40-90 µm medium grade for economical purification, and 50-150 µm coarse grade for capture chromatography. The large pore size insures high capacity for high molecular weight molecules, and faster separation and recycling times. Toyopearl media are available for Size Exclusion, Ion Exchange, Hydrophobic Interaction, and Affinity Chromatography in large-scale processes. For most resins small pre-packed columns, the ToyoScreen® columns are offered for fast and easy resin screening.

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