



TOSOH BIOSCIENCE

TOYOPEARL® ORIENTATION SHEET

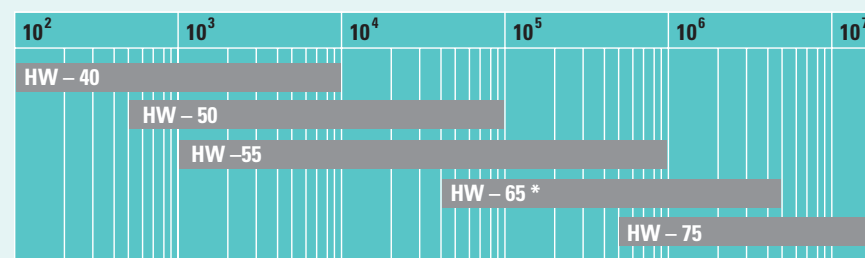
Size Exclusion Chromatography

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 glycosylated 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 SP-550 (EC), 300 Å
 S = 35 µm, M = 65 µm, C = 100 µm, EC = 200 µm

Batch adsorption capacity

| Resin | QAE-550C | DEAE-650 | SP-550C | SP-650 | CM-650 | SuperQ 650 |
|-------------------|----------|----------|---------|--------|--------|------------|
| Trypsin Inhibitor | 95 | - | - | - | - | - |
| BSA | 70 | 30 | - | - | 45 | 143 |
| Ferritin | 25 | 15 | - | - | - | 7 |
| Thyroglobulin | 9 | 12 | - | - | - | - |
| Cytochrome C | - | - | 120 | - | - | - |
| Lysozyme | - | - | 110 | 50 | 38 | - |
| Hemoglobin | - | - | 111 | 42 | 50 | - |
| STI | - | - | - | - | - | 115 |

Structure of Toyopearl IEC resins

| | | | |
|---|--|-------------------------------|--|
| DEAE-650S DEAE-650M DEAE-650C | HW-65 -O-CH ₂ -CH ₂ -NH ⁺ (C ₂ H ₅) ₂ anion exchanger | CM-650S CM-650M CM-650C | HW-65 -O-CH ₂ -COOH weak cation exchanger |
| SuperQ-650S SuperQ-650M SuperQ-650C | HW-65 -O-R'-N ⁺ -(CH ₃) ₃ strong anion exchanger | SP-650S SP-650M SP-650C | HW-65 -O-CH ₂ -CH ₂ -CH ₂ -SO ₃ ⁻ strong cation exchanger |
| QAE-550C | HW-55 -O-CH ₂ -CH ₂ -N ⁺ (C ₂ H ₅) ₃ strong anion exchanger | SP-550C MegaCap SP-550EC | HW-55 -O-CH ₂ -CH ₂ -CH ₂ -SO ₃ ⁻ 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. MegaCap SP-550EC, SP-550C, QAE-550C 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-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 capacity¹⁾/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⁾ | |

* DBC = dynamic binding capacity

Hydrophobic strength

Ether
 PPG
 Phenyl
 Butyl
 Hexyl

↓
Increasing

Structure of TSK-GEL HIC resins

TSKgel Ether-5PW 5000PW (O-CH₂CH₂)_n-OHTSKgel Phenyl-5PW 5000PW (O-C₆H₅)

Structure of Toyopearl HIC resins

Toyopearl Ether-650 (HW-65) (O-CH₂CH₂)_n-OHToyopearl PPG-600 (HW-60) (O-CH(CH₃)-CH₂)_n-OH

Structure of Toyopearl HIC resins

Toyopearl Phenyl-650 (HW-65) (O-C₆H₅)Toyopearl Butyl-650 (HW-65) (O-CH₂-CH₂-CH₂-CH₃)Toyopearl Butyl-600 (HW-60) (O-CH₂-CH₂-CH₂-CH₃)Toyopearl SuperButyl-550 (HW-55) (O-CH₂-CH₂-CH₂-CH₃)Toyopearl Hexyl-650 (HW-65) (O-CH₂-CH₂-CH₂-CH₂-CH₂-CH₃)

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 and PPG-600 are optimized for mAb purification.

Affinity Chromatography

TOYOPEARL REACTIVE RESINS

AF-Amino-650M, 1,000 Å
 AF-Carboxy-650M, 1,000 Å
 AF-Formyl-650M, 1,000 Å

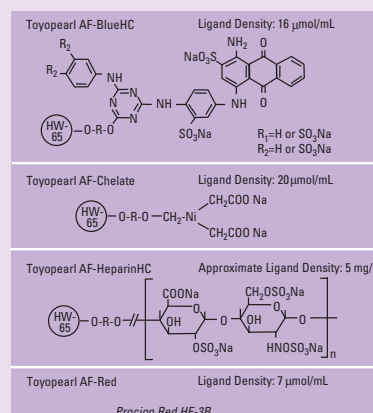
TOYOPEARL ACTIVATED RESINS

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-ethylcarbodiimide

Toyopearl Affinity Resins are mechanically stable and provide good pH stability 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 swollen 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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