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# TOYOPEARL® ORIENTATION SHEET

## HIC

| RESINS | Pore Size |
|---|---|
| Ether-650 (S, M) | 1,000 Å |
| PPG-600M | 750 Å |
| Phenyl-650 (S, M, C) | 1,000 Å |
| Phenyl-600M | 750 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

**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.

### Adsorption Capacity

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

[1] Static [2] Dynamic at 10 % Breakthrough

### Structure of TSK-GEL HIC Resins

TSKgel Ether-5PW — 5000PW — $(O\text{-}CH_2CH_2)_n\text{-}OH$

TSKgel Phenyl-5PW — 5000PW — O — ⬡

### Structure of Toyopearl HIC Resins

Toyopearl Ether-650 — HW 65 — $(O\text{-}CH_2CH_2)_n\text{-}OH$

Toyopearl PPG-600 — HW 60 — $(O\text{-}CH(CH_3)\text{-}CH_2)_n\text{-}OH$

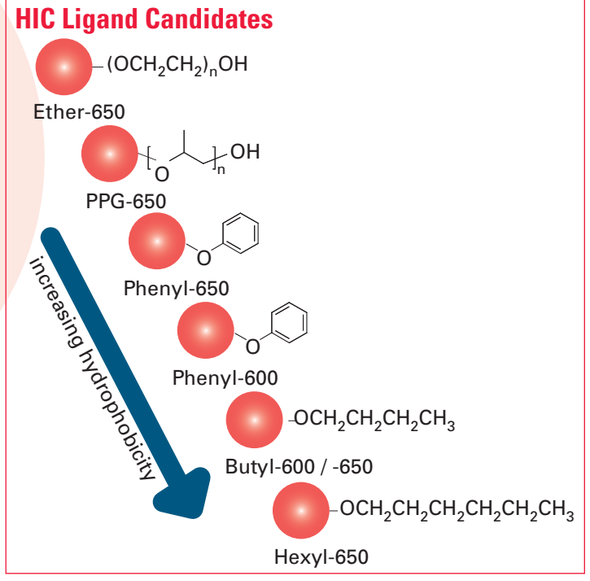
Toyopearl Phenyl-600 / -650 — HW 60/65 — O — ⬡

Toyopearl Butyl-600 / -650 — HW 60/65 — $O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$

Toyopearl SuperButyl-550 — HW 55 — $O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$

Toyopearl Hexyl-650 — HW 65 — $O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$

### HIC Ligand Candidates




## IEC

| ANION EXCHANGERS | Pore Size |
|---|---|
| GigaCap Q-650M | |
| SuperQ-650 (S, M, C) | 400 Å |
| Q-600C AR | 750 Å |
| QAE-550C | 500 Å |
| DEAE-650 (S, M, C) | 1,000 Å |

S = 35 µm, M = 65 µm, C = 100 µm

| CATION EXCHANGERS | |
|---|---|
| GigaCap S-650M | |
| GigaCap CM-650M | |
| CM-650 (S, M, C) | 1,000 Å |
| SP-650 (S, M, C) | 1,000 Å |
| SP-550C | 500 Å |
| MegaCap II SP-550EC | 500 Å |

S = 35 µm, M = 65 µm (GigaCap M = 75 µm), C = 100 µm, EC = 200 µm

### Structure of Toyopearl IEC Resins

DEAE-650S, DEAE-650M, DEAE-650C — HW-65 — $-O\text{-}CH_2\text{-}CH_2\text{-}HN^+\text{-}(C_2H_5)_2$ weak anion exchanger

GigaCap Q-650M, SuperQ-650S, SuperQ-650M, SuperQ-650C — HW-65 — $-O\text{-}R'\text{-}N^+\text{-}(CH_3)_3$ strong anion exchanger

Q-600C AR — HW-60 — $-O\text{-}R'\text{-}N^+\text{-}(CH_3)_3$ strong anion exchanger

QAE-550C — HW-55 — $-O\text{-}CH_2\text{-}CH_2\text{-}N^+\text{-}(CH_3)_3$ strong anion exchanger

GigaCap CM-650M, CM-650S, CM-650M, CM-650C — HW-65 — $-O\text{-}CH_2\text{-}COO^-$ weak cation exchanger

GigaCap S-650M — HW-65 — $-O\text{-}R'\text{-}SO_3^-$ strong cation exchanger

SP-650S, SP-650M, SP-650C — HW-65 — $-O\text{-}R'\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_3^-$ strong cation exchanger

SP-550C, MegaCap II SP-550EC — HW-55 — $-O\text{-}R'\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}SO_3^-$ strong cation exchanger

### Adsorption Capacity

| | GigaCap Q-650 | Super Q-650 | DEAE-650 | QAE-550 | Q-600C AR | GigaCap S-650 | MegaCap II SP-550 | SP-550 | SP-650 | GigaCap CM-650 | CM-650 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BSA | >160[1]; >150[2] | ≈145[2] | ≈25[2] | 70[1]; 30[2] | >120[1]; ≈100[2] | - | - | - | - | - | - |
| Lysozyme | - | - | - | - | - | >200[1] | ≈70[2] | 110[1]; 70[2] | 50[1]; 35[2] | 38[1]; 35[2] | 35[2] |
| mAb | 108[2] | 13[2] | ≈31[2] | 32[2] | 90[2] | 150[1]; 145[2] | - | 14[2] | 12[2] | >110[1]; ≈100[2] | - |
| Insulin | - | - | - | - | - | ≈140[2] | >100[1]; ≈80[2] | ≈80[2] | ≈50[2] | - | - |

[1] Static [2] Dynamic at 10 % Breakthrough

**Toyopearl Ion Exchange Resins.** IEC is the most common liquid chromatographic method used in manufacturing of biopharmaceuticals. 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 II, SP-550, Q-600C AR and especially GigaCap S, Q and CM have very high dynamic capacities and enable high throughput for concentrating dilute process streams.

## SEC

| RESINS | Pore Size |
|---|---|
| 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

**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.

### 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 |

## AFC

| REACTIVE RESINS | Pore Size |
|---|---|
| AF-Amino-650M | 1,000 Å |
| AF-Carboxy-650M | 1,000 Å |
| AF-Formyl-650M | 1,000 Å |
| **ACTIVATED RESINS** | |
| AF-Epoxy-650M | 1,000 Å |
| AF-Tresyl-650M | 1,000 Å |
| **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

**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 and EPO, 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, Factor VIII and IX.

| Resin | Target Ligand | Coupling Cond. | Coupling Agent | Human IgG* | Lysozyme* |
|---|---|---|---|---|---|
| Epoxy | $R\text{-}NH_2$ | 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 | $R\text{-}NH_2$, 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**/$NaCNBH_3$ | 6.7 | 5.8 |
| Carboxy | $R\text{-}NH_2$ | pH: 4.0-6.0, 25 °C | EDC** | 11.7 | 17.5 |
| Formyl | $R\text{-}NH_2$ | pH: 7.0, 25 °C | $NaCNBH_3$ | 15.0 | 20.0 |

* Coupling Densities (mg/ml) ** N'-(3-Dimethylaminopropyl)-N-ethylcarbodiimid

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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