

TOYOPEARL HW-40

TOYOPEARL HW-50

TOYOPEARL HW-55

TOYOPEARL HW-65

TOYOPEARL HW-75

The Role of Size Exclusion Chromatography in Process Purification

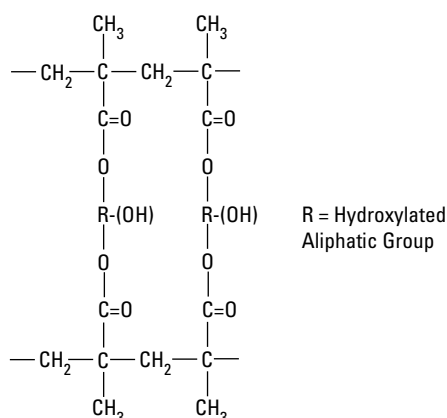
Size exclusion chromatography (SEC), also known as gel filtration chromatography, separates molecules in an aqueous mobile phase according to their hydrodynamic radius in solution as they pass through a porous structure. Molecules with a diameter greater than the largest pores within the resin are unable to enter the particle. Because they are excluded from the pores they travel quickly through the column and elute first. Smaller molecules, which are able to access pores within the resin particles, permeate a larger accessible volume within the column and are eluted later, in order of decreasing molar mass.

Because SEC has no adsorption capacity and its separation mechanism dilutes the sample during elution, it is not normally used in the capture or intermediate steps of manufacturing processes. It is most often used as a final polishing step where a target protein is being separated from its aggregates or other significantly different molar mass impurities. Another related application would be the desalting of the purified target protein in lieu of a more traditional diafiltration step.

TOYOPEARL HW SEC Resins

Tosoh Bioscience offers a number of TOYOPEARL HW resins for size exclusion chromatography (H = hydrophilic, W = water-compatible). TOYOPEARL HW size exclusion resins are hydroxylated polymethacrylic polymer beads (Figure 1). Surface hydroxyl groups render these resins very hydrophilic, therefore minimal non-specific adsorption occurs, making the TOYOPEARL HW resins useful for protein separations. The semi-rigid polymeric nature and the narrow particle size distribution of these resins give them better pressure-flow properties than softer materials such as agarose. In addition, good mechanical stability of the TOYOPEARL HW resins produces excellent flow characteristics in large industrial size columns (up to 0.3 MPa).

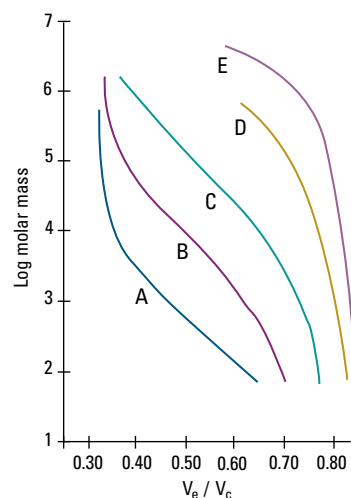
Figure 1: Resin Chemistry of TOYOPEARL HW resins (Hydroxylated Acrylic)



TOYOPEARL HW resins are chemically stable from pH 2-14. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. Also, these resins can be run at elevated temperatures (4 - 60 °C) and are autoclavable at 121 °C.

Commercial TOYOPEARL HW size exclusion resins are available in five pore sizes covering five different fractionation ranges, though there is some overlap among the listed ranges. The choice of TOYOPEARL HW resin depends on the molar mass of the feedstock components. Tables 1 and 2 show this information for proteins, dextrans and PEG (polyethylene glycol) polymers. The TOYOPEARL HW resin molar mass ranges span peptide and protein sizes between 100 to 5 x 10⁷ daltons. Each TOYOPEARL HW resin exhibits a typical calibration curve and exclusion limit for globular proteins (Figure 2).

Figure 2: Calibration curves for globular proteins on TOYOPEARL resins



Resins:	A. TOYOPEARL HW-40 B. TOYOPEARL HW-50 C. TOYOPEARL HW-55 D. TOYOPEARL HW-65 E. TOYOPEARL HW-75
Column size:	22 mm ID x 30 cm
Mobile phase:	0.06 mol/L phosphate buffer, pH 7.0, + 0.06 mol/L KCl
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	protein standards
Legend:	V _e = elution volume V _c = column volume

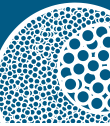
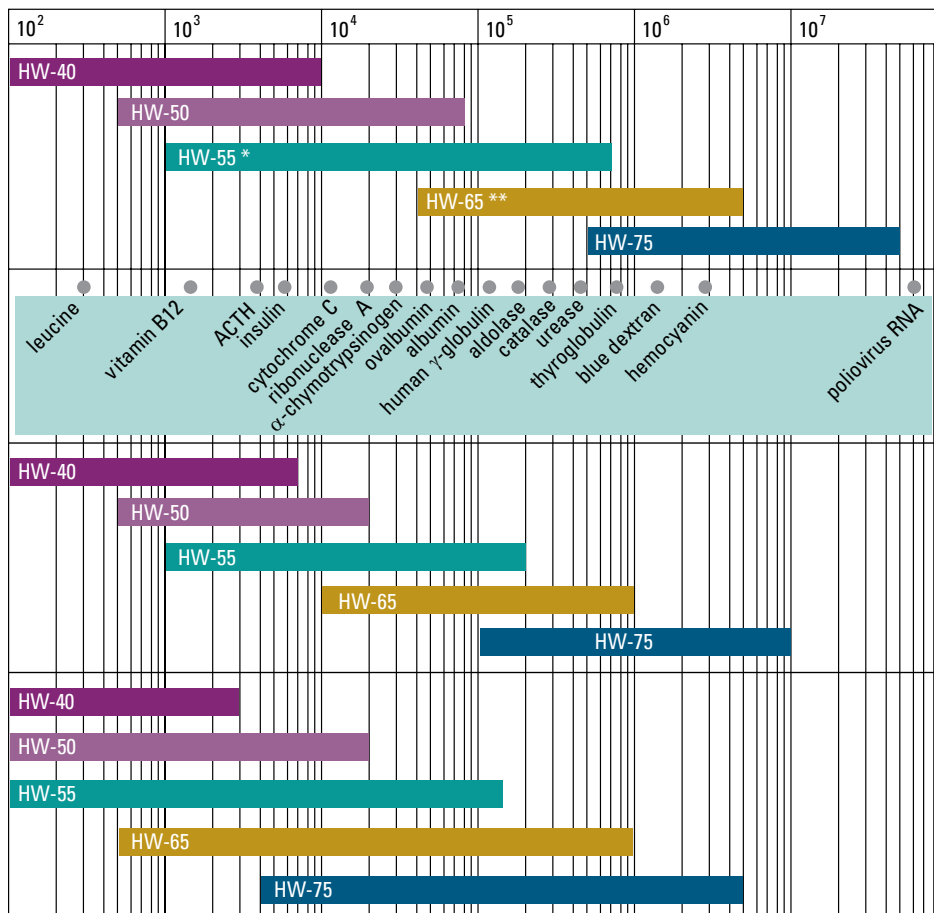


Table 1: Properties and molar mass separation ranges of TOYOPEARL HW resins

TOYOPEARL resin	Particle size (µm)	Pore size (nm)	Molar mass of sample (Da)		
			Polyethylene glycols and oxides	Dextrans	Globular proteins
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	5	100 - 3,000	100 - 7,000	100 - 1 × 10 ⁴
HW-50S HW-50F	20 - 40 30 - 60	12.5	100 - 1.8 × 10 ⁴	500 - 2 × 10 ⁴	500 - 8 × 10 ⁴
HW-55S HW-55F	20 - 40 30 - 60	50	100 - 1.5 × 10 ⁵	1,000 - 2 × 10 ⁵	1,000 - 7 × 10 ⁵
HW-65S HW-65F	20 - 40 30 - 60	100	500 - 1 × 10 ⁶	1 × 10 ⁴ - 1 × 10 ⁶	4 × 10 ⁴ - 5 × 10 ⁶
HW-75S HW-75F	20 - 40 30 - 60	>100	4,000 - 5 × 10 ⁶	1 × 10 ⁵ - 1 × 10 ⁷	5 × 10 ⁵ - 5 × 10 ⁷

Table 2: Molar mass separation ranges for TOYOPEARL HW resins

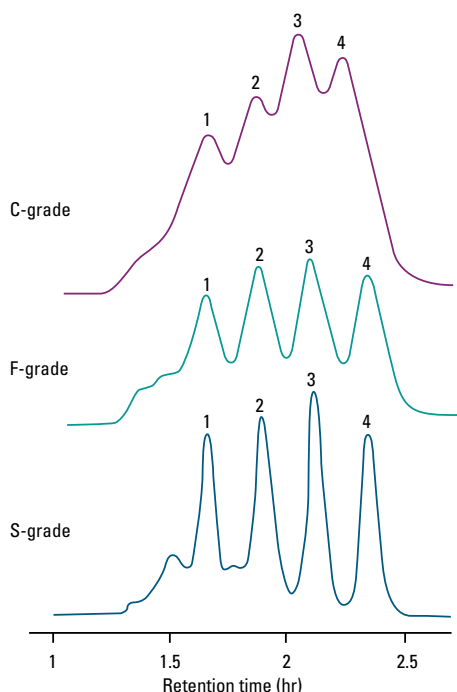


Resolution increases with decreasing particle size (Figure 3). Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks. Most TOYOPEARL HW resins are available in three particle size ranges:

- S-grade = 20 - 40 μm (Superfine)
- F-grade = 30 - 60 μm (Fine)
- C-grade = 50 - 100 μm (Coarse)

When the highest resolution is needed, the smaller S and F grade beads are preferred for process SEC. For desalting, where the resin is used in a filtration mode to remove the target from a buffer, the C grade is primarily employed because of its better flow dynamics at lower operating pressures. TOYOPEARL HW-40 is manufactured in an “EC-grade” (extra coarse) with a 100 - 300 μm bead.

Figure 3: Comparison of resolution on different particle sizes of TOYOPEARL HW-55 resin



Resin: **TOYOPEARL HW-55**
 Column size: 26 mm ID x 70 cm
 Mobile phase: 33.3 mmol/L phosphate buffer, pH 7.0, 0.2 mol/L NaCl
 Flow rate: 20 cm/hr (1.77 mL/min)
 Detection: UV @ 280 nm
 Temperature: 25 °C
 Injection vol.: 1 mL
 Samples:
 1. thyroglobulin (0.3%)
 2. γ-globulin (0.3%)
 3. β-lactoglobulin (0.3%)
 4. cytochrome C (0.1%)

General properties of TOYOPEARL HW resins in aqueous eluents are detailed in Table 3. TOYOPEARL HW resins can be used in organic solvents or mixtures of organic solvents and water. Bed volumes may swell or shrink relative to water depending on the solvent as shown in Tables 4 and 5. DMSO can be used for SEC of oligosaccharides and polyethylene glycols. The compatibility of DMF with TOYOPEARL also permits SEC separation of hydrophobic substances such as polystyrenes.

Table 3: Properties of TOYOPEARL HW resins in aqueous eluents

High mechanical stability	All TOYOPEARL resins can be operated at pressures up to 3 bar without deformation.
Minimum change in gel bed volume	Changes in the column bed volume under operational salt conditions are negligible. TOYOPEARL does not shrink or swell even in high concentrations of strong denaturing agents such as urea or guanidine hydrochloride.
Chemical stability	TOYOPEARL is stable from pH 2-13 and can tolerate levels outside of that range (pH 0-14) for short periods of time. Biomolecules which are only soluble at extreme pH values can be readily separated.
Sharp chromatographic peaks	TOYOPEARL's narrow particle size distribution (min. 80% – within declared limits) results in better peak shapes and higher elution target concentrations than other SEC materials.
Temperature stability	TOYOPEARL HW SEC resins are thermally stable and do not degrade or denature even in boiling water. TOYOPEARL resins can be sterilized by autoclaving at 121 °C.
Microorganism resistance	TOYOPEARL is an organosynthetic material and is resistant to degradation by microorganisms.
Suitability for enzyme immobilization	TOYOPEARL resins contain numerous hydroxyl groups on the external and internal bead surfaces. These, in combination with the chemical stability of the polymer, make the resin well suited for the covalent bonding of enzymes or other ligands. Please see the affinity chromatography section for more information.

Table 4: Swelling properties in various solvents

Mobile phase	TOYOPEARL resin				
	HW-40	HW-50	HW-55	HW-65	HW-75
Water	100	100	100	100	100
0.2 mol/L KCl	100	100	100	100	100
MeOH	100	100	100	100	105
EtOH	100	100	100	100	110
DMF	110	110	105	105	120
Acetone	80	80	85	90	110
Toluene	65	70	70	75	90

Table 5: Additional swelling data for TOYOPEARL HW-40 resin

TOYOPEARL resin	Mobile phase				
	DMSO	Ethyl Acetate	Benzene	CHCl ₃	CHCl ₃ /MeOH (1:1)
HW-40	140	80	70	105	120

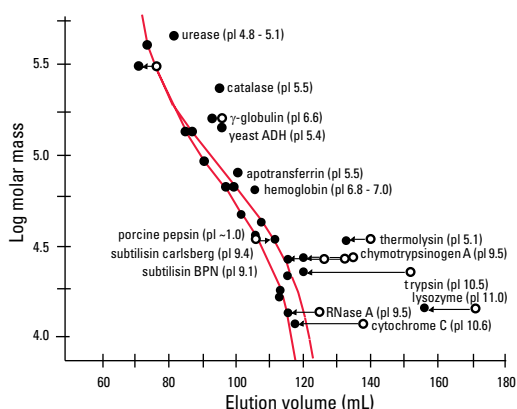
Mobile phase components, such as salts, can affect SEC separations. The presence or absence of sodium chloride influences the elution volume of proteins. This is demonstrated in Figure 4, in which a mixture of various proteins was separated on a column packed with TOYOPEARL HW-55F resin. Salt concentrations can change the hydrodynamic radius of proteins and either increase or decrease their molar mass as a function of salt strength.

TOYOPEARL HW resins are commonly used in size exclusion chromatography and desalting applications though they can be used for other important functions, such as:

- Removal of surfactants such as Triton® X-100 from biological solutions by an adsorption mechanism
- Use in hydrophobic interaction chromatography (HIC) for the separation of very hydrophobic molecules
- Use in HIC separations as a guard column for hydrophobic impurities
- Possible use as a stationary phase for either normal or reversed phase separations depending on solvent system selected

All of the physical and chemical properties discussed for the TOYOPEARL HW SEC resins make them an excellent choice for use as the base beads for the ion exchange, hydrophobic interaction, mixed-mode, and affinity chromatographic resins discussed in the later sections of this catalog.

Figure 4: Comparison of the elution volumes of proteins in presence and absence of NaCl

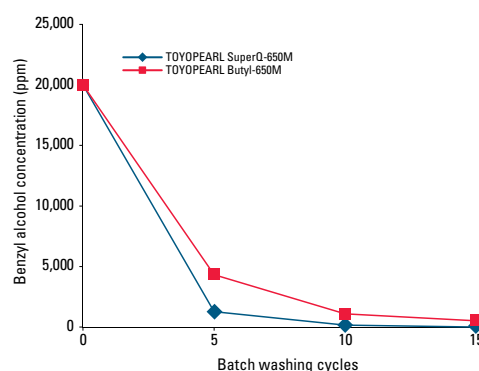


Resin: **TOYOPEARL HW-55F**
 Column size: 22 mm ID × 50 cm
 Mobile phase: 25 mmol/L Tris-HCl with (•) or without (○) 0.5 mol/L NaCl, pH 7.5
 Flow rate: 16 cm/hr (1.0 mL/min)
 Detection: UV @ 280 nm, 420 nm for heme proteins, 200 nm for proteins without aromatic amino acids
 Temperature: 5-10 °C

A 2% solution of benzyl alcohol in water has been identified as a suitable alternative to 20% ethanol as a preservative in resin storage solutions. Samples of TOYOPEARL SuperQ-650M and Butyl-650M resin (which serve as a representative sample of all TOYOPEARL resins, including the TOYOPEARL HW SEC resins) were prepared by adding 100 mL of aqueous 2% benzyl alcohol to 100 mL of suction filtered resin. A 100 mL aliquot of DI water was added to the filtered resin and stirred to make a slurry. This resin/ DI water slurry was allowed to stand for 5 minutes and was then suction filtered to remove the supernatant. This procedure was repeated 14 more times, for a total of 15 washes.

Samples of the filtered supernatant from the TOYOPEARL SuperQ-650M and Butyl-650M resin were taken after the 5th, 10th, and 15th washes and analyzed for benzyl alcohol concentration (Figure 5). As demonstrated in the figure, a 2% benzyl alcohol solution can be effectively removed from the TOYOPEARL SuperQ-650M and Butyl-650M resin by thorough washing with DI water.

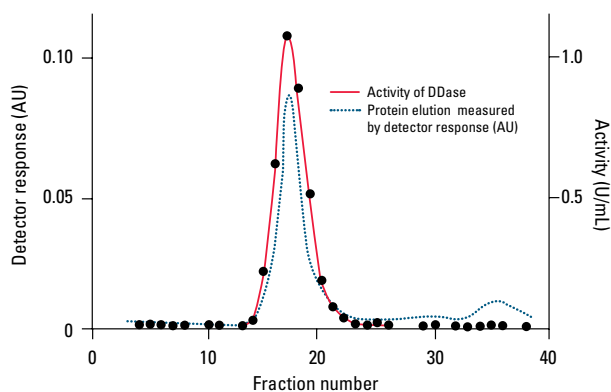
Figure 5: Concentration of benzyl alcohol in resin supernatant (batch wash)



Polishing Step for Enzyme Purification

TOYOPEARL HW SEC resins are an excellent choice when looking for a polishing step for enzyme purification. Dextrin dextranase, a 300 kDa enzyme, was purified from *Acetobacter capsulatus* using a two-step process consisting of TOYOPEARL Phenyl-650M and TOYOPEARL HW-65S. The elution profile of Dextrin dextranase is shown in (Figure 6). Due to the hydrophobic nature of the enzyme, it aggregates in 100% aqueous mobile phases, thus it was necessary to add 40% ethylene glycol to the mobile phase.

Figure 6: Elution profile of protein and activity on TOYOPEARL HW-65S

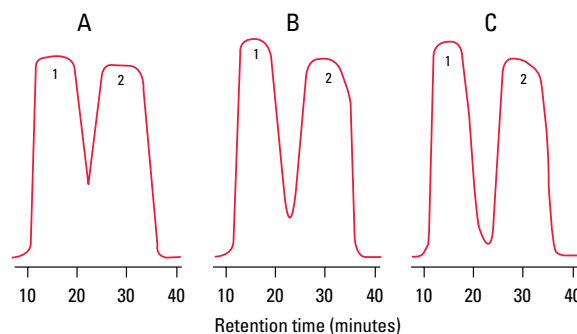


Resin: **TOYOPEARL HW-65S**
 Column size: 16 mm ID × 80 cm
 Mobile phase: 20 mol/L acetate buffer with 40% ethylene glycol, pH 4.8
 Detection: UV @ 280 nm
 Temperature: ambient
 Sample: concentrated active fractions from TOYOPEARL Phenyl-650 column

Desalting Step for Proteins

Though SEC is typically used as the polishing step in a purification process, it can also be used as an ideal desalting step for proteins that may be sensitive to membrane concentration and diafiltration steps. TOYOPEARL HW-40F allows for high total protein and activity recovery, allowing the operator to use it as a desalting resin. Figure 7 demonstrates the effect of the ionic strength of a volatile salt on the desalting of bovine serum albumin (BSA) from sodium acetate. It is important to note that the loading volumes for a desalting application are much higher than for regular SEC purifications. As much as 25% of the bed volume can be loaded for desalting steps, compared with 1% to 5% of the bed volume for normal SEC purifications.

Figure 7: Desalting of bovine serum albumin



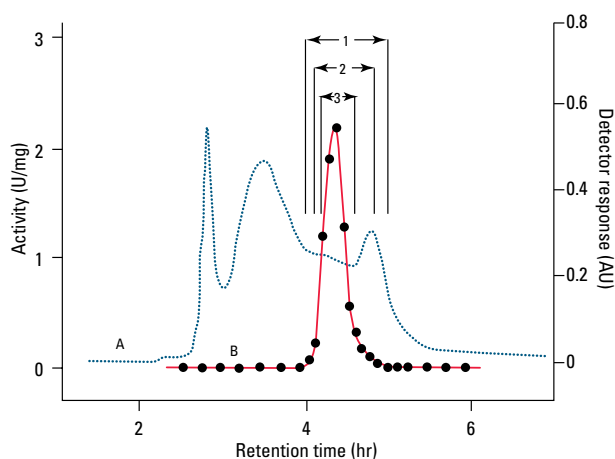
Resin: **TOYOPEARL HW-40F**
 Column size: 7.5 mm ID × 30 cm
 Mobile phase:
 A. distilled water
 B. 10 mmol/L ammonium formate
 C. 50 mmol/L ammonium formate
 Flow rate: 48 cm/hr (0.4 mL/min)
 Detection: UV @ 220 nm
 Temperature: 25 °C
 Samples: 2.5 mL of:
 1. bovine serum albumin (0.1%)
 2. sodium acetate (10%)

Recovery of Activity

Recovery of activity is a very important consideration when purifying an enzyme. As shown in **Figure 8**, crude β -galactosidase has been purified using TOYOPEARL HW-55F with excellent recovery yields (**Table 6**).

The 1, 2, and 3 brackets are representative of the pooling of fractions and what the yield and recoveries would be and are listed in **Table 6**. The widest pool sample corresponds to the greatest recovery.

Figure 8: Purification of crude β -galactosidase on TOYOPEARL HW-55F



Resin: **TOYOPEARL HW-55F**
 Column size: 25 mm ID \times 55 cm \times 2
 Mobile phase: 0.2 mol/L phosphate, pH 6.7
 Flow rate: 12 cm/hr (1.0 mL/min)
 Detection: A. UV @ 280 nm
 B. enzymatic activity
 Temperature: 22 °C
 Sample: 3 mL containing 45 mg of crude β -galactosidase

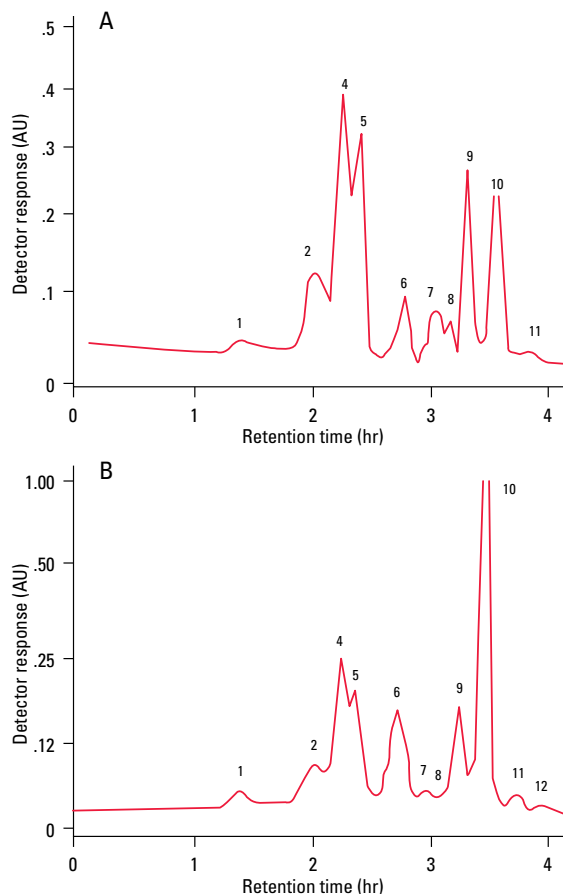
Table 6: Purification of crude β -galactosidase on TOYOPEARL HW-55F

Fraction	Yield (%)	Specific activity (units/mg)	Degree of purification
Original sample		0.95	
1	94	2.8	2.9 \times
2	93	3.7	3.9 \times
3	83	6.4	6.7 \times

Antibody Separation

Antibodies have been separated from bovine colostrum whey and human serum using TOYOPEARL HW-55F resin. **Figure 9** shows the separation of colostrum whey on TOYOPEARL HW-55F after centrifugation. Peak #2 is IgG₁, and the chromatogram shows both the 254 and 280 nm absorbance profiles.

Figure 9: Elution profiles of colostrum whey

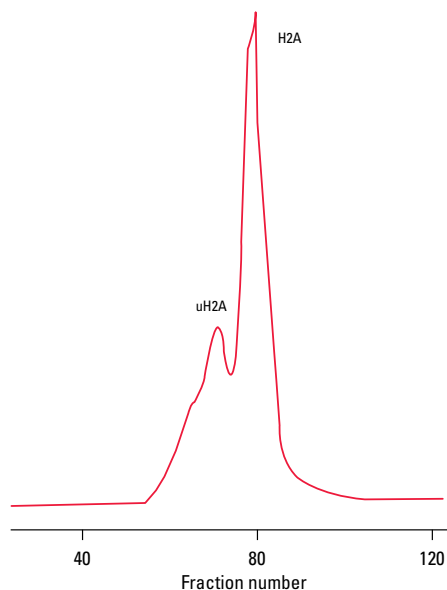


Resin: **TOYOPEARL HW-55F**
 Column size: 17 mm ID \times 64 cm
 Mobile phase: 25 mmol/L Tris-HCl with 0.3 mol/L NaCl, pH 7.5
 Flow rate: 18 cm/hr (0.7 mL/min)
 Detection: A. UV @ 280 nm
 B. UV @ 254 nm
 Temperature: 23 °C
 Samples: 1. unknown
 2. IgG₁
 3. serum albumin
 4. β -lactoglobulin (dimer)
 5. α -lactalbumin
 6 - 12. unknown

Isolation Based on Polypeptide Difference

TOYOPEARL HW-50S can help to isolate the ubiquitin-histone conjugate uH2A from the unicellular protozoan *Tetrahymena pyriformis*. Figure 10 shows the separation of uH2A from the histone, H2A. The sole difference between these two components is a small polypeptide, ubiquitin (approximately 8,500 Da).

Figure 10: Isolation of a complex protein conjugate on TOYOPEARL HW-50S

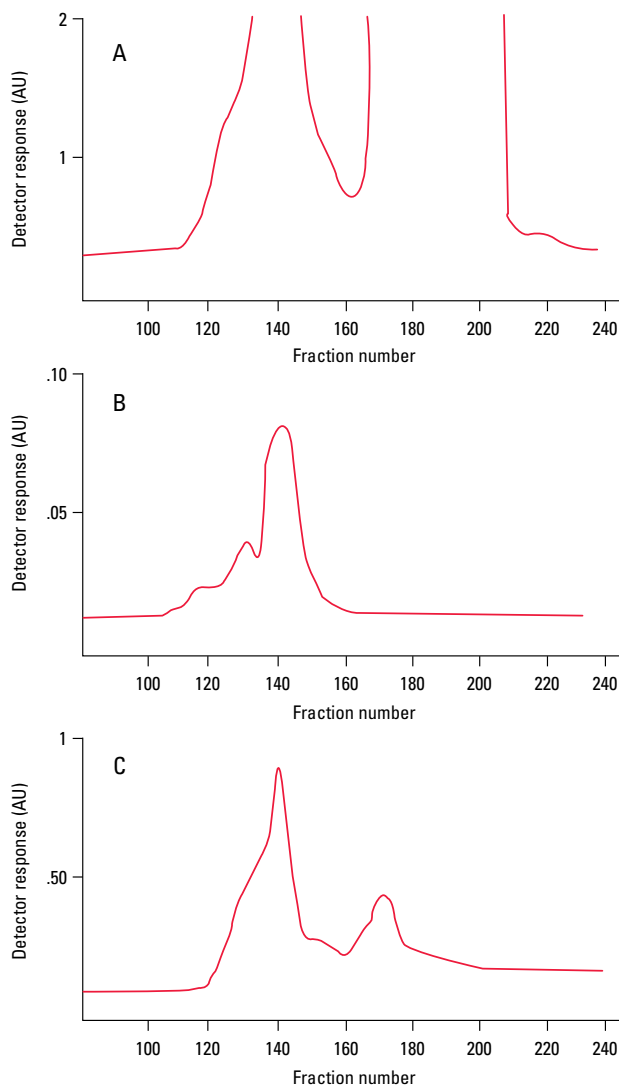


Resin: **TOYOPEARL HW-50S**
 Column size: 22 mm ID × 83 cm
 Mobile phase: 10 mmol/L HCl
 Flow rate: 1.6 cm/hr (0.1 mL/min)
 Detection: UV @ 230 nm
 Sample: fraction of crude *Tetrahymena* H2A containing the ubiquitin-histone conjugate uH2A

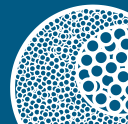
Amylose Isolation

TOYOPEARL HW-75F resins, with pores larger than 100 nm, has been used in place of ultra-centrifugation steps for purification of plasmid DNA. Ultra-centrifugation is a time-consuming process and requires expensive chemicals, such as cesium chloride. TOYOPEARL HW-75F resin provides superior separation performance for plasmid DNA, and also provides high yields. Figure 11 shows the separation of crude pBR322 DNA from contaminating RNA species using TOYOPEARL HW-75F.

Figure 11: Separation of pBR322 DNA



Resin: **TOYOPEARL HW-75F**
 Column size: 16 mm ID × 130 cm
 Mobile phase: 10 mmol/L Tris-HCl, 1 mmol/L EDTA, 0.2 mol/L NaCl, pH 8
 Flow rate: 7 cm/hr (0.23 mL/min)
 Detection: UV @ 260 nm
 Temperature: ambient
 Samples:
 A. crude plasmid DNA extract
 B. DNA from A
 C. DNA from hydroxyapatite chromatography



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

Ordering Information

TOYOPEARL SEC resins:

Conditions: Exclusion limits are +/- 30% and are determined using PEG, PEO, or dextran standards, as appropriate.

Part #	Product description	Container size (mL)	Bead diameter (µm)	Exclusion limit (Da)
19809	TOYOPEARL HW-40S	150	20-40	3 × 10 ³
07451	TOYOPEARL HW-40S	250	20-40	3 × 10 ³
07447	TOYOPEARL HW-40S	500	20-40	3 × 10 ³
14681	TOYOPEARL HW-40S	1,000	20-40	3 × 10 ³
07967	TOYOPEARL HW-40S	5,000	20-40	3 × 10 ³
19808	TOYOPEARL HW-40F	150	30-60	3 × 10 ³
07448	TOYOPEARL HW-40F	500	30-60	3 × 10 ³
14682	TOYOPEARL HW-40F	1,000	30-60	3 × 10 ³
07968	TOYOPEARL HW-40F	5,000	30-60	3 × 10 ³
19807	TOYOPEARL HW-40C	150	50-100	3 × 10 ³
07449	TOYOPEARL HW-40C	500	50-100	3 × 10 ³
14683	TOYOPEARL HW-40C	1,000	50-100	3 × 10 ³
07969	TOYOPEARL HW-40C	5,000	50-100	3 × 10 ³
21484	TOYOPEARL HW-40C	50,000	50-100	3 × 10 ³
07450	TOYOPEARL HW-40EC	500	100-300	3 × 10 ³
07970	TOYOPEARL HW-40EC	5,000	100-300	3 × 10 ³
19811	TOYOPEARL HW-50S	150	20-40	1.8 × 10 ⁴
07455	TOYOPEARL HW-50S	250	20-40	1.8 × 10 ⁴
07452	TOYOPEARL HW-50S	500	20-40	1.8 × 10 ⁴
14684	TOYOPEARL HW-50S	1,000	20-40	1.8 × 10 ⁴
08059	TOYOPEARL HW-50S	5,000	20-40	1.8 × 10 ⁴
19810	TOYOPEARL HW-50F	150	30-60	1.8 × 10 ⁴
07453	TOYOPEARL HW-50F	500	30-60	1.8 × 10 ⁴
14685	TOYOPEARL HW-50F	1,000	30-60	1.8 × 10 ⁴
08060	TOYOPEARL HW-50F	5,000	30-60	1.8 × 10 ⁴
18368	TOYOPEARL HW-50F	50,000	30-60	1.8 × 10 ⁴
19813	TOYOPEARL HW-55S	150	20-40	1.5 × 10 ⁵
07459	TOYOPEARL HW-55S	250	20-40	1.5 × 10 ⁵
07456	TOYOPEARL HW-55S	500	20-40	1.5 × 10 ⁵
14686	TOYOPEARL HW-55S	1,000	20-40	1.5 × 10 ⁵
08062	TOYOPEARL HW-55S	5,000	20-40	1.5 × 10 ⁵

Part #	Product description	Container size (mL)	Bead diameter (µm)	Exclusion limit (Da)
19812	TOYOPEARL HW-55F	150	30-60	1.5 × 10 ⁵
07457	TOYOPEARL HW-55F	500	30-60	1.5 × 10 ⁵
14687	TOYOPEARL HW-55F	1,000	30-60	1.5 × 10 ⁵
08063	TOYOPEARL HW-55F	5,000	30-60	1.5 × 10 ⁵
21918	TOYOPEARL HW-55F	50,000	30-60	1.5 × 10 ⁵
19815	TOYOPEARL HW-65S	150	20-40	1 × 10 ⁶
07467	TOYOPEARL HW-65S	250	20-40	1 × 10 ⁶
07464	TOYOPEARL HW-65S	500	20-40	1 × 10 ⁶
14688	TOYOPEARL HW-65S	1,000	20-40	1 × 10 ⁶
08068	TOYOPEARL HW-65S	5,000	20-40	1 × 10 ⁶
18377	TOYOPEARL HW-65S	50,000	20-40	1 × 10 ⁶
19814	TOYOPEARL HW-65F	150	30-60	1 × 10 ⁶
07465	TOYOPEARL HW-65F	500	30-60	1 × 10 ⁶
14689	TOYOPEARL HW-65F	1,000	30-60	1 × 10 ⁶
08069	TOYOPEARL HW-65F	5,000	30-60	1 × 10 ⁶
21852	TOYOPEARL HW-65F	50,000	30-60	1 × 10 ⁶
21481	TOYOPEARL HW-65C	150	50-100	1 × 10 ⁶
07466	TOYOPEARL HW-65C	500	50-100	1 × 10 ⁶
14690	TOYOPEARL HW-65C	1,000	50-100	1 × 10 ⁶
08070	TOYOPEARL HW-65C	5,000	50-100	1 × 10 ⁶
21482	TOYOPEARL HW-65C	50,000	50-100	1 × 10 ⁶
07471	TOYOPEARL HW-75S	250	20-40	8.25 × 10 ⁶
07468	TOYOPEARL HW-75S	500	20-40	8.25 × 10 ⁶
08071	TOYOPEARL HW-75S	5,000	20-40	8.25 × 10 ⁶
19816	TOYOPEARL HW-75F	150	30-60	8.25 × 10 ⁶
07469	TOYOPEARL HW-75F	500	30-60	8.25 × 10 ⁶
14691	TOYOPEARL HW-75F	1,000	30-60	8.25 × 10 ⁶
08072	TOYOPEARL HW-75F	5,000	30-60	8.25 × 10 ⁶