POROS™ A and G Affinity Columns and POROS™ CaptureSelect™ Affinity Columns

Pub. No. 8-0003-40-1093 **Rev.** K

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

POROS™ Affinity Columns are designed for analytical and preparative purification of antibodies and fusion proteins. The packed columns contain cross-linked poly(styrene-divinylbenzene) flow-through particles with a unique pore structure that provides rapid mass transport and enables rapid chromatography. The particle surface is coated with a cross-linked polyhydroxylated polymer. This coating is further derivatized with affinity ligands:

- **Recombinant Protein A** – Binds Fc-containing immunoglobulin proteins, except IgG3.
- **Recombinant Protein G** – Binds Fc-containing immunoglobulin proteins with species specificity different from Protein A.
- **CaptureSelect™ affinity ligands** – Single-domain monospecific antibody fragment produced in *Saccharomyces cerevisiae*, binds the antigens listed in Table 2.

POROS™ Affinity Columns are high-flow, high-pressure columns that are designed for HPLC systems. The columns can be used for the quantitation and small-scale purification of biomolecules specific to the derivatized affinity ligands.

Column packages include the following items:

- Packed column, with sealing endcaps
- Column Test Certificate
- E-Z Grip™ stainless steel fittings

Storage

Store resins at 2°C–8°C. Do not freeze.

Specifications

Table 1 POROS™ A 20 and G 20 column product characteristics and stability

Product	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Immobilized ligand	
• POROS™ A 20	Recombinant Protein A (soy-based)
• POROS™ G 20	Recombinant Protein G
Dynamic binding capacity	
• POROS™ A 20	19.0 mg/mL
• POROS™ G 20	9.0 mg/mL
Shipping solution	0.1 M sodium phosphate, pH 7.0, 0.02% sodium azide
Pressure limit	170 bar
Recommended maximum flow rate	5000 cm/hour
Stability	
pH	
• POROS™ A 20	2–10
• POROS™ G 20	2–9
Ionic strength	0–5 M, all common salts
Buffer	All common agents, including 4 M urea, 3 M guanidine hydrochloride, ethylene glycol, and detergents. Agents that may degrade the protein ligands are not recommended.
Solvent	Water, 0–100% alcohol, acetonitrile, 2 M acetic acid, 1 M HCl, other common organic solvents **Note:** Do not expose the columns to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric acid), strong reducing agents (such as sulfite), acetone, or benzyl alcohol.
Operating temperature	2–40°C **DO NOT FREEZE**

Table 2 POROS™ CaptureSelect™ column product characteristics

Product	Immobilized ligand description	Binding capacity
Antibody-based ligands		
POROS™ CaptureSelect™ CH1-XL	IgG Fab affinity ligand, CH1 domain of human IgG antibodies	26.2 mg/ml human IgG 8.8 mg/ml human Fab fragment
POROS™ CaptureSelect™ IgA	IgA affinity ligand, human-specific	7.6 mg/mL
POROS™ CaptureSelect™ FcXL	IgG Fc affinity ligand, human-specific	7.3 mg/ml
POROS™ CaptureSelect™ IgM	IgM affinity ligand, human-, mouse-, and rat-specific	4.0 mg/mL
POROS™ CaptureSelect™ KappaXL	Light Chain Kappa affinity ligand, human-specific	16.5 mg/ml
POROS™ CaptureSelect™ LC Lambda	Light Chain Lambda affinity ligand, human-specific	10.0 mg/mL IgG
Other ligands		
POROS™ CaptureSelect™ FSH	Follicle Stimulating Hormone affinity ligand, human-specific	1.5 mg/mL
POROS™ CaptureSelect™ GCSF	Granulocyte Colony-Stimulating Factor affinity ligand, human-specific	5.2 mg/mL (ligand density)
POROS™ CaptureSelect™ hCG	Chorionic Gonadotropin affinity ligand, human-specific	2.7 mg/ml
POROS™ CaptureSelect™ hGH	Growth Hormone affinity ligand, human-specific	1.7 mg/mL
POROS™ CaptureSelect™ HSA	Human Serum Albumin affinity ligand, human-specific	2.6 mg/mL

Table 3 POROS™ CaptureSelect™ column specifications

Specification	Description
Support matrix	Cross-linked poly(styrene-divinylbenzene)
Shipping solution	0.1 M sodium phosphate, pH 7.0, 0.02% sodium azide
Pressure limit	170 bar
Recommended maximum flow rate	5000 cm/hour
Stability	
pH (all ligands)	1–10
Ionic strength	0–5 M, all common salts
Buffer additive	All common agents, including 4 M urea, 3 M guanidine hydrochloride, ethylene glycol, and detergents. Agents that may degrade the protein ligands are not recommended (for example, pH > 10).
Solvent	Water, 0–100% alcohol, acetonitrile, 2 M acetic acid, 1 M HCl, other common organic solvents **Note:** Do not expose the columns to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric acid), strong reducing agents (such as sulfite), acetone, or benzyl alcohol.
Operating temperature	2–40°C **DO NOT FREEZE**

Connect and prepare the column

Connect the column

POROS™ columns are provided with E-Z Grip™ stainless steel fittings that are designed to be tightened by hand. Do not use any fittings that require tightening with a wrench. Overtightening can strip the threads of the column.

Use a pre-column filter (0.5–2 µm) to minimize column fouling.

Prepare the column

1. Before you use the column for the first time, pump 5–10 column volumes (CVs) of equilibration buffer to remove the shipping solvent.
2. Equilibrate with 10–15 CVs of starting/wash buffer.

Perform a blank run

To avoid artifactual refractive index (RI) peaks, the salinity of equilibration and elution buffers should be the same.

For maximum sensitivity, minimize the RI blank peak by using:

- 50 mM phosphate pH 7, 0.15 M NaCl as the starting/wash buffer
- 0.1% (12 mM) HCl, 0.15 M NaCl as the elution buffer

Select and prepare the starting/wash buffer

- In most cases, simple buffers such as 10–50 mM phosphate or Tris can be used.
- Use the starting/wash buffer pH listed below, but note that binding is usually strongest in the higher pH range.
  - POROS™ A 20 and G 20 column: pH 6.0–9.0
  - POROS™ CaptureSelect™ column: pH 6.0–8.0
- Add 0.1–0.2 M NaCl or KCl to prevent nonspecific adsorption due to protein/protein interactions.

Regardless of the buffer system you choose, always:

- Use buffer salts and reagents of the highest purity practical.
- Degas and filter (0.22 or 0.45 µm) all buffers prior to use.

Prepare and load the sample

Prepare samples

To ensure efficient binding and prevent resin and column-frit fouling:

- Dissolve, dilute, or exchange samples into the equilibration buffer. This is particularly important for large samples (>25% of the column volume).
- Centrifuge and filter samples (0.22 or 0.45 µm) before injection.
- Heat-treat serum samples (56°C for 30 minutes) to remove residual fibrinogen that can lead to fouling on multiple runs.
- Delipidate samples, if possible. Lipids can cause irreversible fouling.

Determine the sample load

To ensure efficient binding and prevent resin and column fouling:

- The dynamic binding capacities of POROS™ A 20 and G 20 columns are listed in Table 1. The dynamic binding capacities or ligand densities of POROS™ CaptureSelect™ columns are listed in Table 2.
- The binding capacity for other antibodies depends on the antibody source and subclass and the ligand used, but is generally lower than the capacity for IgG listed in Table 1 and Table 2.
- In analytical applications, minimum and maximum load is determined by the linearity of the standard curve.

Elution conditions

Analytical applications

For all ligands, use a 25–100 mM phosphate, pH 2.0–3.5, with or without sodium chloride up to 150 mM. Other elution buffer components that may be used include hydrochloric acid, glycine, citrate, acetate, or other components that buffer well at low pH.

Preparative applications

Column	Elution conditions
POROS™ A 20 and G 20 column	• To elute most antibodies, reduce the pH to 2–3. Common buffer systems include phosphate, acetate, hydrochloric acid, and glycine. Buffer concentrations can range from 6–100 mM or 2–20% (v/v), depending on the buffer system. • 6–12 mM HCl with 0.15 NaCl can be used to obtain the desired pH range and minimizes the refractive index affects. • Because antibodies differ by both species and subclass in their binding/elution behavior, the best elution conditions should be determined empirically. • In general, lower pH conditions are required for elution from POROS™ G columns than from POROS™ A columns.
POROS™ CaptureSelect™ column	• 0.1 M glycine pH 2.0 yields complete elution for all of the ligands. Most of the ligands also show complete elution at pH 3.0. • Because the ligands are extremely acid-stable, you can also elute with 6–12 mM HCl. • The HSA affinity column can be eluted at neutral pH with the addition of 2.0 M $MgCl_2$, or 50% propylene glycol.

Clean and regenerate the column

Columns are generally very robust; lifetimes of 3000 injections per column have been reported. With extended reuse of the column, monitor column backpressure and assess the performance of an assay control sample over time. To prolong column lifetime, periodically clean the column before backpressure increases to remove residual material from the column frits and to clean the resin.

Typical cleaning solutions include 2–6 M guanidine hydrochloride, 1 M acetic acid, 20% ethanol, 1 M acetic acid plus 20% ethanol, 20% isopropanol, elution buffer titrated to pH 1.5–2.0, and elution buffer plus 1–2 M sodium chloride.

To clean, make 2 or 3 injections of cleaning solution at a volume equal to the column bed volume, followed by 2 or 3 injections of equilibration buffer—for example, 2 × 100 µL cleaning solution, 2 × 100 µL equilibration buffer. Alternatively, you can run multiple column volumes of cleaning solution. You can reverse flow to help clean the top frit. If the system does not allow flow reversal, you can plumb the column in reverse, clean, and return to normal after cleaning. Monitor the cleaning sequence for eluted protein peaks.

Store the column

Always store columns:

- In a neutral-pH solution with a bacteriostatic agent.
- In the refrigerator, but **DO NOT FREEZE**.
- With the endcaps in place, carefully sealed to prevent drying. Drying results in decreased chromatographic efficiency.

Note: Store POROS™ CaptureSelect™ columns at 2-8°C, in 0.1 M Tris, 1.0 M NaCl, 20% (v/v) ethanol, pH 8.0.

Ordering information

Product description	Column dimension (diameter x length)	Column volume	Cat. no.
POROS™ A 20			
20 µm (stainless steel column)	2.1 mmD x 30 mmL	0.1 mL	1-5024-12
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	2-1001-00
	4.6 mmD x 50 mmL	0.8 mL	1-5022-24
	4.6 mmD x 100 mmL	1.7 mL	1-5022-26
	10 mmD x 100 mmL	7.9 mL	1-5022-46
POROS™ G 20			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	2-1002-00
	4.6 mmD x 50 mmL	0.8 mL	1-5122-24
	4.6 mmD x 100 mmL	1.7 mL	1-5122-26
	10 mmD x 100 mmL	7.9 mL	1-5122-46
POROS™ CaptureSelect™ CH1-XL			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	A37052
	4.6 mmD x 50 mmL	0.8 mL	A37053
	4.6 mmD x 100 mmL	1.7 mL	A37054
POROS™ CaptureSelect™ IgA			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4485162
	4.6 mmD x 50 mmL	0.8 mL	4485166
	4.6 mmD x 100 mmL	1.7 mL	4485170
	10 mmD x 100 mmL	7.9 mL	4485174
POROS™ CaptureSelect™ FcXL			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	A37058
	4.6 mmD x 50 mmL	0.8 mL	A37059
	4.6 mmD x 100 mmL	1.7 mL	A37060
POROS™ CaptureSelect™ IgM			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4469152
	4.6 mmD x 50 mmL	0.8 mL	4469164
	4.6 mmD x 100 mmL	1.7 mL	4469169
	10 mmD x 100 mmL	7.9 mL	4469174
POROS™ CaptureSelect™ KappaXL			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	A37061
	4.6 mmD x 50 mmL	0.8 mL	A37062
	4.6 mmD x 100 mmL	1.7 mL	A37063
POROS™ CaptureSelect™ LC Lambda			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4469150
	4.6 mmD x 50 mmL	0.8 mL	4469163
	4.6 mmD x 100 mmL	1.7 mL	4469168
	10 mmD x 100 mmL	7.9 mL	4469173

Product description	Column dimension (diameter x length)	Column volume	Cat. no.
POROS™ CaptureSelect™ FSH			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4481822
	4.6 mmD x 50 mmL	0.8 mL	4481824
	4.6 mmD x 100 mmL	1.7 mL	4481826
	10 mmD x 100 mmL	7.9 mL	4481828
POROS™ CaptureSelect™ GCSF			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4485157
	4.6 mmD x 50 mmL	0.8 mL	4485164
	4.6 mmD x 100 mmL	1.7 mL	4485168
	10 mmD x 100 mmL	7.9 mL	4485172
POROS™ CaptureSelect™ hCG			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	A37055
	4.6 mmD x 50 mmL	0.8 mL	A37056
	4.6 mmD x 100 mmL	1.7 mL	A37057
POROS™ CaptureSelect™ hGH			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4485161
	4.6 mmD x 50 mmL	0.8 mL	4485165
	4.6 mmD x 100 mmL	1.7 mL	4485169
	10 mmD x 100 mmL	7.9 mL	4485173
POROS™ CaptureSelect™ HSA			
20 µm (PEEK polymer column)	2.1 mmD x 30 mmL	0.1 mL	4469151
	4.6 mmD x 50 mmL	0.8 mL	4469165
	4.6 mmD x 100 mmL	1.7 mL	4469170
	10 mmD x 100 mmL	7.9 mL	4469175

Support

For service and technical support, go to **thermofisher.com/poros** or call toll-free in US: 1.800.831.6844.

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to **thermofisher.com/support**, or contact you local Thermo Fisher Scientific representative.

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Revision history: Pub. No. 8-0003-40-1093

Revision	Date	Description
K	14 June 2018	Removed IgGfc and LC Kappa products.
J	19 December 2017	Addition of new products.
H	28 August 2017	Change to pressure value in Specifications.
G	10 January 2017	Baseline for this revision history.

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