INSTRUCTIONS

POROS MabCapture Select Resins

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Number	Description
82079	**POROS MabCapture A Select,** 5mL settled resin
82080	**POROS MabCapture A Select,** 15mL settled resin
82081	**POROS MabCapture A Select,** 50mL settled resin
82082	**POROS MabCapture G Select,** 5mL settled resin
82083	**POROS MabCapture G Select,** 15mL settled resin
82084	**POROS MabCapture G Select,** 50mL settled resin
82085	**POROS MabCapture A/G Select,** 5mL settled resin
82086	**POROS MabCapture A/G Select,** 15mL settled resin
82087	**POROS MabCapture A/G Select,** 50mL settled resin

Resin: Crosslinked poly(styrene-divinylbenzene)

Supplied:	POROS MabCapture A Select	50% slurry in 0.1M sodium phosphate, pH 7.0 and 20% ethanol
	POROS MabCapture G Select	50% slurry in water and 20% ethanol
	POROS MabCapture A/G Select	50% slurry in water and 20% ethanol

Storage: Upon receipt store at 2-8°C. DO NOT FREEZE. Product shipped on wet ice.

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Introduction

Thermo Scientific™ POROS™ MabCapture™ Select Resins are chromatography resins designed for rapid, high-capacity purification of antibodies using packed column flow-based chromatography. POROS MabCapture Select resins are 45µm beads consisting of rigid, polymeric crosslinked poly[styrene-divinylbenzene] beads with a unique open pore structure that allows for rapid binding of large molecules, consistently producing high dynamic binding capacities over a wide range of low- and high-flow conditions. To allow for the purification of a wide variety of antibody isotypes and species of origin, a family of POROS MabCapture Select resins are available, containing recombinant Protein A, Protein G or Protein A/G. A description of each ligand and its unique selectivities are outlined below and in Table 1.

- **Protein A:** Cell wall component produced by several strains of *Staphylococcus*. Recombinant Protein A essentially functions the same as native Protein A. The Protein A molecule contains five high-affinity binding sites ($K_a = 10^8$/M) capable of binding several different species including human and rabbit (Table 1). The Protein A molecule has greater stability than Protein G. Protein A is heat-stable and retains its native conformation when exposed to denaturing reagents such as 4M urea, 4M thiocyanate and 6M guanidine hydrochloride**.**

- **Protein G:** Bacterial cell wall protein isolated from group G *Streptococci*. Recombinant Protein G is produced in *E. coli* and has been modified to eliminate its binding to albumin. The Protein G molecule contains two binding domains. Although the tertiary structures of Protein A and Protein G are very similar, their amino acid compositions differ significantly, resulting in different binding characteristics (Table 1). Protein G has greater affinity than Protein A for most mammalian IgGs, especially for certain subclasses including human IgG_3, mouse IgG_1 and rat IgG_{2a}.

- **Protein A/G:** Functional chimeric protein produced by a gene fusion of the seven Fc-binding domains of Protein A and Protein G. Protein A/G is an excellent purification tool for most immunoglobulins as it has a broader species binding range than either Protein A or Protein G individually (Table 1). The multiple binding domains of Protein A/G can also increase capacity for many antibody isotypes compared to Protein G alone.

Table 1. Binding characteristics of Protein A, G, and A/G.

Species	Antibody Class	Protein A	Protein G	Protein A/G
Human	Total IgG	S	S	S
	IgG_1	S	S	S
	IgG_2	S	S	S
	IgG_3	W	S	S
	IgG_4	S	S	S
	IgM	W	NB	W
	IgD	NB	NB	NB
	IgA	W	NB	W
	Fab	W	W	W
	ScFv	W	NB	W
Mouse	Total IgG	S	S	S
	IgM	NB	NB	NB
	IgG_1	W	M	M
	IgG_{2a}	S	S	S
	IgG_{2b}	S	S	S
	IgG_3	S	S	S
Rat	Total IgG	W	M	M
	IgG_1	W	M	M
	IgG_{2a}	NB	S	S
	IgG_{2b}	NB	W	W
	IgG_{2c}	S	S	S
Cow	Total IgG	W	S	S
	IgG_1	W	S	S
	IgG_2	S	S	S
Goat	Total IgG	W	S	S
	IgG_1	W	S	S
	IgG_2	S	S	S
Sheep	Total IgG	W	S	S
	IgG_1	W	S	S
	IgG_2	S	S	S
Horse	Total IgG	W	S	S
	IgG(ab)	W	NB	W
	IgG(c)	W	NB	W
	IgG(T)	NB	S	S
Rabbit	Total IgG	S	S	S
Guinea Pig	Total IgG	S	W	S

Binding Strength:

W = weak
M = medium
S = strong
NB = no binding

Table 1 (continued). Binding characteristics of Protein A, G, and A/G.

Species	Antibody Class	Protein A	Protein G	Protein A/G
Hamster	Total IgG	M	M	M
Donkey	Total IgG	M	S	S
Monkey	Total IgG	S	S	S
Pig	Total IgG	S	W	S
Dog	Total IgG	S	W	S
Cat	Total IgG	S	W	S
Chicken	Total IgY	NB	NB	NB

Binding Strength:

W = weak
M = medium
S = strong
NB = no binding

Table 2. Properties of Thermo Scientific POROS MabCapture Select Resins.

Support matrix	Crosslinked poly(styrene-divinylbenzene)
Ligands	Recombinant Protein A Recombinant Protein G Recombinant Protein A/G
Dynamic binding capacity	A: ≥37mg/mL(1) G: ≥12mg/mL(2) A/G: ≥20mg/mL(2)
Shipping solvent	A: 50% slurry in phosphate buffer and 20% ethanol G: 50% slurry in water and 20% ethanol A/G: 50% slurry in water and 20% ethanol
Average particle size	45µm
Shrinking/swelling	<1% from 1-100% solvent
Mechanical resistance	100bar/1450psi/10MPa
Compatibility	
pH range	2-10
Ionic strength range	0-5M, all common salts
Buffer additives	Protein A: All common reagents including 1N sodium hydroxide, 8M urea, 2-6M guanidine hydrochloride, ethylene glycol, detergents, 0-100% alcohol, acetonitrile, and 1-2M acids Protein G: All common reagents including 8M urea, 0.1M glycine, 1M acetic acid, 1M hydrochloric acid, and 70% ethanol Protein A/G: All common reagents including 0.1M glycine, 0.1M sodium hydroxide, 8M urea, 1M acetic acid, 1M hydrochloric acid, and 70% ethanol Agents that may degrade the protein ligand are not recommended.
Solvents	Ethanol, methanol Do not expose resin to strong oxidizers (e.g., hypochlorite), oxidizing acids (e.g., nitric acid), strong reducing agents (e.g., sulfite), acetone, or benzyl alcohol.
Operating temperature	2-30° C Do not freeze

(1) 5% breakthrough of Human IgG in PBS, pH 7.5 at 300cm/hr in 0.46cm ID x 20cm length column
(2) 10% breakthrough of Human IgG in PBS, pH 7.2 at 300cm/hr in 0.50cm ID x 5cm length column

Important product information

- To aid in resin handling, adjust resin into a slurry containing 0.1M NaCl.
- For all column chromatography applications, we recommend that highly pure buffer components be used and that all buffers and samples are degassed and filtered through a 0.45µm filter before use.
- Final protein yield and purity during a chromatographic separation are dependent upon the sample complexity and concentration in load sample, as well as the buffer conditions and flow rates used. Therefore, it is important to optimize these parameters before attempting a large-scale purification.
- If you are required to stir the resin slurry over an extended period of time, use a free-standing paddle stirrer. Do not use magnetic stir bars or paddles that mate with the bottom of the container.

Resin handling (<100mL)

Below are general recommendations for handling resin, adjusting slurry percentage, and exchanging resin into new buffers.

1. Transfer the required volume of resin slurry to a bottle-top filter or sintered-glass frit.
2. Apply vacuum to remove the liquid from the top of the resin bed.
3. Resuspend the resin cake to the desired final resin slurry volume with 0.1M NaCl. Mix with a plastic or rubber spatula. Do not grind or press resin against the sintered glass material. If using a paper filter membrane, take care to not damage or rip the filter material.
4. Repeat steps 2 and 3 for a total of three exchanges. After the third exchange, recover the resin slurry into an appropriate container and store appropriately.
5. Verify the slurry concentration by transferring 10-100mL of slurry into a graduated cylinder and allow the resin to settle for ≥4 hours. If needed, adjust the slurry concentration to 50–70%.
6. Proceed with column packing (below).

Column packing for lab-scale columns (<100mL)

Note: POROS MabCapture Select resins are mechanically rigid and incompressible. They can be packed effectively in low-pressure glass columns and high-pressure stainless steel columns. For volumes greater than 100mL, please refer to the POROS column packing instructions at https://tools.thermofisher.com/content/sfs/manuals/4468731.pdf.

There is a 1.06 packing factor for POROS MabCapture Select resins to account for the difference in bed volume between a gravity-settled bed in 0.1M NaCl and a 1- to 3-bar pressure-packed bed. This factor, along with the slurry ratio, is used to determine the volume of slurry required to yield the intended final column volume (CV).

1. Determine the required slurry volume:

$$Required\ slurry\ volume = \frac{Target\ Column\ Volume}{Slurry\ ratio} \times packing\ factor$$

Example: A column of dimensions 2.5cm ID × 10.2cm length would have a packed bed of 50mL ($Volume = \pi r^2 H$)

To properly pack this volume using a 50% resin slurry of POROS MabCapture A Select resin, 106mL is needed:

$$\frac{50mL}{0.50} \times 1.06 = 106mL\ of\ slurry\ required$$

2. Ensure that the column outlet is closed and plumbed directly to waste. Do not connect the column outlet to the chromatography system. Plumbing into the system creates backpressure that fights against the inlet pressure trying to settle the bed and pack the column.

3. Ensure that the column is level and locked in place before beginning the pack. If needed, attach appropriate column packing extender to allow full resin slurry volume to be added.
4. Add 0.1M NaCl to the empty column until the solution is 5cm above the bottom frit.
5. Wet bottom frit by opening the column outlet and allow liquid to enter frit and begin to flow. This will ensure the air trapped into the frit will not enter the resin bed. Close outlet when the solution reaches 3-5mM above the bottom frit
6. Slowly deliver the required slurry volume to the column and immediately move on to the next steps. Do not allow the resin to fully gravity-settle in the column before packing.

   Note: POROS MabCapture Select resin beads typically have a density similar to water and does not settle rapidly.
7. Bring the top flow adaptor to the top of the slurry level and tighten the O-ring to until it begins to form a seal with the column. Do not push the resin up and over the O-ring.
8. Continue to lower the adaptor slowly to remove the bubbles from the top of the slurry liquid. Do not allow large air bubbles between the top adaptor and the top of the resin slurry.
9. Tighten the O-ring completely and connect the column to the system.
10. Open the column outlet.
11. Start flow and increase the flow rate to at least 50% above the maximum flow rate used during your planned purifications. For columns with a diameter of <1.0cm, a flow rate of 1000-2000cm/hr is recommended.
12. Continue flow until a stable resin bed forms. Monitor the pressure; it will gradually rise as the column packs. Do not exceed maximum pressure recommended for column or instrument hardware.

    Note: While packing the column, you may observe some turbidity in the eluent as packing begins. Turbidity will clear as packing proceeds and 1-2 bed volumes of packing buffer pass through the column.
13. After the bed is formed, remove the top adaptor and remove excess solution with a pipette. Leave 2-5cm of liquid above the resin bed.
14. Re-insert top adaptor and bring the top flow adaptor to the top of the resin bed and tighten the O-ring until it begins to make a seal. Lower the adaptor slowly to remove the bubbles from the top of the column.
15. Bring the adaptor into contact with the top of the bed and seal O-ring. POROS resin does not shrink or swell, so an open headspace is not recommended. Do not push the resin over the O-ring or use pressure to compress the resin bed.
16. Flow at the packing flow rate again for 2-5 column volumes, taking note of the bed height and pressure at the desired flow rate.
17. If running the column in a unidirectional flow, then packing is completed. If running the column in both directions, continue on to the next step to further condition the column.
18. If reversing the flow of the column during operation, condition the column in upflow. Switch column inlet and outlet position related to the pump flow for upflow and downflow. Do not invert the column.
    a. Flow 2–3 column volumes in upflow at the operating flow rate.
    b. Flow 2–3 column volumes in downflow at the operating flow rate, then adjust the adaptor if needed.
    c. Flow 2 column volumes after you adjust the adaptor.

Qualify the column

To qualify the integrity of a packed column, determine the HETP (height equivalent to a theoretical plate) and asymmetry using a non-binding analyte (a "plug"). Equation calculators are available in the Thermo Scientific™ ChromaPro™ mobile application available online. Alternatively, use the equations following the protocol.

A. Setting specifications

Qualification results depend on a number of factors including:

- Solutions and method used
- Scale
- Column hardware
- Chromatography system

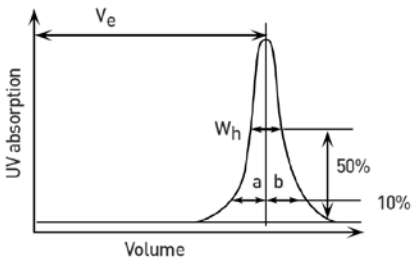
After defining a column qualification procedure or a specific system (column plus chromatography system), base the qualification acceptance criteria on historical values and ranges instead of theoretical qualification results. Performing the column qualification method consistently and reproducibly is critical to obtaining meaningful results.

B. Qualification procedure

Table 3. Recommended column qualification conditions.

Condition	Recommendation
Flow rate	Target operating flow rate (cm/hour)
Equilibration buffer	0.1M NaCl
Plug solution	1M NaCl
Plug volume	2% of column volume

1. Equilibrate the column and all buffers to working temperature. Ensure that all solutions are degassed.
2. Equilibrate the system.
3. Equilibrate the column with 5-15 column volumes of the equilibration buffer.
4. Inject your plug and continue running the system for 1.5 column volumes.
5. Record the elution volume peak shape of the plug after it passes through the column.
6. Determine HETP and Asymmetry factor.



$N = 5.54\left(\frac{V_e}{W_h}\right)^2$	$HETP = \frac{L}{N}$	$A_S = \frac{b}{a}$
N = Number of theoretical plates	HETP = Height equivalent to a theoretical plate	As = Asymmetry factor
V_e = Elution volume	L = Bed height (cm)	a = Width at 10% height, leading side
W_h = Width at peak's half height (in volume)	N = Number of theoretical plates	b = Width at 10% height, tailing side
		As > 1 = Tailing
		As < 1 = Leading

Purification

A. Standard purification conditions

Buffer composition	• Buffers used during the equilibration, binding, wash, and elution can influence the purity of your eluted antibody. To reach the desired purity, optimize the purification protocol. The conditions recommended below are provided as a general guide meant as a starting point.
Equilibration	• Use standard neutral buffers (pH 6-8), such as 10-50mM sodium phosphate or Tris. Adding 0.1-0.2M NaCl or KCl may prevent nonspecific adsorption from protein/protein interactions. **Note:** Use equilibration buffer for washes.
Wash	• Same as equilibration buffer; however, to improve wash stringency and improve purity, the wash buffer can be modified to include higher salt concentrations (up to 1M) for varying the pH higher or lower, or other additives such as detergents, glycols, organics, or low concentrations of chaotropic agents. Equilibrate resin with equilibration buffer before elution. **Note:** Some buffer additions can have a negative impact on the stability of certain antibodies.
Elution	• Use 0.1M glycine, pH 2.0. If antibody stability is compromised with short term exposure to pH 2, as an alternative, use 0.1M glycine, pH 2.8-3.0 or Thermo Scientific™ Gentle Ag/Ab Elution Buffer, pH 6.6 (Product No. 21013). Refer to Tech Tip #27 (Optimized elution conditions for immunoaffinity purifications) at thermofisher.com for more elution buffer choices. • Use a solution that has higher buffering strength greater than the equilibration buffer strength to ensure a good pH transition. Use a step elution to obtain a concentrated elution; however, if higher purity is needed, a gradient elution can be used.
Flow rate	• We recommend starting with a flow rate that allows for a 1-6 minute resident time (contact time) of a sample across the column. Flow rates can be increased or decreased significantly depending on sample concentration and complexity.

B. Standard purification protocol

1. Equilibrate the column and all buffers to working temperature. Perform purifications at 2-30°C. Ensure that all solutions are degassed.
2. Prepare the purification system by washing pumps and filling tubing with buffer.
3. Connect the column to the system.
4. Equilibrate the column with 5-10 column volumes of PBS, pH 7.2 or desired equilibration buffer.
5. Apply any sample volume that does not exceed 80% of the column-dynamic binding capacity for target protein and flow conditions.
6. Wash the resin with >5 column volumes of wash buffer or until the absorbance approaches baseline
7. Elute your antibody using 0.1M glycine, pH 2.0 or desired elution buffer.
8. Neutralize your antibody by using a high pH solution. It is recommended you neutralize your eluted antibody by placing 1M Tris, pH 8.0-8.5 in your collection tubes prior to elution.

Procedure for resin cleaning and storage

A. Column cleaning and lifetime study considerations

Resin lifetime depends on how the resin is used and cleaned. The frequency at which this cleaning procedure is performed will be dependent on your sample, column performance, and regulatory needs. The cleaning conditions specific for each purification process must be determined, particularly if long lifetime is desired. A typical lab-scale cleaning procedure for POROS MabCapture Select resins is:

1. Wash the resin with 5 column volumes of 1M NaCl.
2. Wash the resin with 5 column volumes of ultrapure water.
3. Wash the resin with 5 column volumes of 70% ethanol.
4. Wash the resin with 5 column volumes of ultrapure water.
5. Store resin in ultrapure water containing 20% ethanol at 4°C.

To further optimize cleaning procedures, test the following solutions in this order, then optimize cleaning based on results:

- 1M acetic acid (with or without 20% ethanol)
- 0.1M NaOH (POROS MabCapture A Select only)
- Elution buffer titrated to a lower pH (target pH 1.5–2.0)
- Elution buffer titrated to a lower pH plus 1–2M NaCl
- 1M hydrochloric acid

Troubleshooting

Problem	Cause	Solution
Low protein yield	Protein did not bind to the column	Adjust binding buffer, modify salt concentration, or modify pH
	Flow rate was too fast	Decrease flow rate during binding to allow for greater residence time and increased binding
Poor protein purity	Co-elution of contaminating molecules	Modify elution conditions or gradient
		Follow up with a second purification using an IEX resin
		Add an intermediate wash step
	Column was overloaded	Apply less protein to the column
	Column was dirty	Follow clean-in-place procedure to remove non-specifically bound impurities
Slow column flow	Column was dirty after multiple uses	Perform a clean-in-place procedure
High backpressure	Rapid switch in buffer type	Avoid rapid switches between organic and aqueous solutions. Switch under reduced flow rates or over a gradient
	Presence of any amount of ethanol in the slurry or column	Fully remove ethanol before packing
	Compromised flow path	Characterize the pressure of the entire chromatography system with 1) no column in place, 2) an empty column with the column outlet plumbed to the waste, and 3) an empty column with the column outlet plumbed back to the chromatography system
		Ensure the entire flow path is clear
		Change in-line filters
	Clogged frits	Change or clean frits
		Run the column in reverse for 3 column volumes
	Particle size gradient in the column caused by gravity settling	Do not gravity-settle POROS resin in the column before packing
		Repack column
	Resin was frozen	Do not freeze
Turbid column effluent after >3 column volumes during packing	Column frits were too large for the resin	Use frits with <25 micron pore size
	Defective O-ring or improperly assembled column	Disassemble column and inspect all components

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Problem	Cause	Solution
Column qualification – high asymmetry	Column was underpacked	Pack at higher flow rate/pressure
	Salt injection was not optimized	Verify the desired amount of salt is loaded by checking the peak height and width
	Column needed more post-pack conditioning to stabilize the packed bed	Equilibrate the column with 2-5 column volumes of packing solution in downflow at the operating flow rate
	System plumbing allowed for dilution of salt plug	Characterize the salt plug through the chromatography system at the qualification flow rate to understand how the plug moves through the system with no packed column in-line
	Improper slug solution used	Use the recommended column qualification conditions
Column qualification – low asymmetry	Water used as the mobile phase	Add some salt to the mobile phase to reduce the charge interaction between the salt and the resin
	Column overpacked or packed inconsistently	Repack the column following the recommended procedure
	Column not equilibrated long enough with NaCl before salt injection	Equilibrate with >4 column volumes if the packing solution is different from the qualification mobile phase
Decreased performance • Increased band spreading • Dynamic binding capacity decrease • Recovery issues • Increased pressure drop • Ghost peaks during other steps	Column fouling occurred due to precipitation of product or impurity, irreversible binding of lipid material, or other impurities	Clean the column

Related Thermo Scientific Products

A26458	**POROS MabCapture A Select,** 1L (see thermofisher.com for larger pack sizes)
82071	**POROS™ XS**, 10mL
82073	**POROS™ XQ**, 10mL
82077	**POROS™ 50 HQ**, 10mL
20333	**Pierce™ Protein A Agarose,** 5mL
20365	**Pierce™ Recombinant Protein A Agarose,** 5mL
20398	**Pierce™ Recombinant Protein G Agarose,** 2mL
20421	**Pierce™ Recombinant Protein A/G Agarose,** 3mL
20510	**Pierce™ Recombinant Protein L Agarose,** 2mL
89978	**NAb™ Protein A Plus Spin Kit**
89979	**Nab™ Protein G Spin Kit**
89980	**Nab™ Protein A/G Spin Kit**
89981	**Nab™ Protein L Spin Kit**
21013	**Gentle Ag/Ab Elution Buffer, pH 6.6**
21012	**Gentle Ag/Ab Binding Buffer, pH 8.0**
21009	**IgG Elution Buffer, pH 2.8**
21028	**IgG Elution Buffer, pH 2.0**

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | thermofisher.com