

## DESCRIPTION

Octave MabCapture A Columns are designed for rapid Protein A affinity purification of monoclonal antibodies. The POROS® support consists of rigid cross-linked poly(styrene-divinylbenzene) flow-through particles with pore structure optimized for very rapid mass transport. The particle surface is coated with a cross-linked polyhydroxylated polymer. This coating is further derivatized by covalent immobilization of a recombinant Protein A.

Specifications	
Support	Cross-linked poly(styrene-divinylbenzene)
Ligand	Recombinant Protein A
Dynamic Binding Capacity @300 cm/h in 20 cm bed length	Human IgG, pH 7.5, >45 mg/ml
Bead size	45 µm
Maximum flow rate, single column	1-ml column: 20 ml/min 5-ml column: 80 ml/min
Maximum operating pressure	Resin: 100 bar Column housing: 5 bar
Media backpressure	<1 bar at 300 cm/h
Protein A leaching	<50 ppm
Ionic strength range	0-5 M, all common salts
pH range (routine use)	pH 2-10
Cleaning agents	0.1 N NaOH; 20% (v/v) acetic acid + 0.3 M MgCl <sub>2</sub>
Column housing material	Polypropylene
Column dimensions, 1 ml	6.7 mm ID x 28 mm L
Column dimensions, 5 ml	14.7 mm ID x 29.8 mm L
Column fittings	Inlet: Female 10-32 Outlet: Male M6
Chemical incompatibilities	Do not expose to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), or strong reducing agents (such as sulfite)

Octave MabCapture A Columns are a perfect match with the Octave Chromatography System for ultimate efficiency and performance in preparative monoclonal antibody capture and purification. The columns are quickly and easily connected to the Octave System using 10-32 F to M6 F adapters (available separately). The columns can also be used for automated purification on single-column liquid chromatography systems and for manual purification using a syringe.

## SAMPLE PREPARATION AND LOADING

1. Filter all buffers and samples (0.45 µm) prior to use.
2. Delipidate samples if possible.
3. In most cases, loading/wash buffer is 100 mM PBS, pH 7.0-7.5. For murine IgG<sub>1</sub> or other antibodies with low affinity for Protein A in low ionic strength buffers, using 3 M NaCl, 100 mM glycine, pH 8.5-9.0 can improve binding.
4. Determine the amount and flow rate of sample to load based on type of antibody, concentration, and capacity of the columns being used. The capacity of the beads depends on the antibody source and subclass, but is generally lower than the capacity for human IgG.
5. Flow rates up to 15 and 60 ml/min may be used with the 1-ml and 5-ml columns, respectively, on the Octave System configured in Step mode.

## WASH AND ELUTION

1. Wash unbound material from the columns with the starting/wash buffer. Generally 5 to 10 column volumes are sufficient to remove unbound proteins. Samples with high impurity levels may require a longer wash.
2. Elute the bound antibody using 2 to 5 volumes of an appropriate buffer. In most cases elution is accomplished at a low pH, e.g. 2–20% acetic acid, pH 2.0-3.0; 0.1 M glycine, pH 2.0-3.0, or 0.1 M citrate, pH 3.0. Antibodies differ by species and subclass in their binding and elution behavior, so the best elution conditions are determined experimentally.
3. Immediately neutralize the eluted antibody to prevent denaturation if it is susceptible to low pH.

## CLEANING AND STORAGE

If an increase in back pressure or significant contamination of the resin is observed, a cleaning-in-place (CIP) procedure can be performed.

1. Wash the columns with 3-5 column volumes 20% (v/v) acetic acid, 0.3 M MgCl<sub>2</sub> to remove any bound antibody that may remain. Add 20% ethanol to remove lipids.
2. (Optional) If sanitation is desired, rinse the columns with 5 volumes 0.1 N NaOH at a low flow rate for up to 30 min.
3. Re-equilibrate with 10 column volumes 100 mM PBS, pH 7.5.

Octave MabCapture A columns should be stored at 2-8°C. Do not freeze! Columns can be stored up to 1 week in PBS. Before storing for longer than 1 week, put the columns through a CIP cycle as above. Equilibrate and store the columns in 100 mM PBS, pH 7.5 in 20% ethanol or 0.02% sodium azide. Use appropriate safety precautions when handling sodium azide.