Features and Benefits of Rhinophase®-AB

1. New Cost-Effective Alternative to Protein A and Protein G Affinity Chromatographic Media
2. Particles are Made from Inert Zirconium Dioxide with A Non-Animal Source Stationary Phase
3. Particles are Chemically Stable in Acid and Base Solutions, which Allows for Depyrogenation and Cleaning of Particles
4. Rigid Particles Allow for Use of High Linear Velocities for Unparalleled Product Throughput
5. Useful for a Variety of Immunoglobulins Including IgGs, IgA and IgMs
6. Does Not Bind Serum Proteins that can "Foul" Protein A and Protein G Media
7. Tunable Selectivity for Different Immunoglobulins Using pH and Ionic Strength as the Main Variables
8. Antibody Purity Levels As Good or Better than Protein A and Protein G Media
9. Extended Media Lifetime Compared to Protein A and Protein G Media
10. Comparable Binding Capacity for Immunoglobulins to Protein A and Protein G Media

Rhinophase®-AB Product Specifications

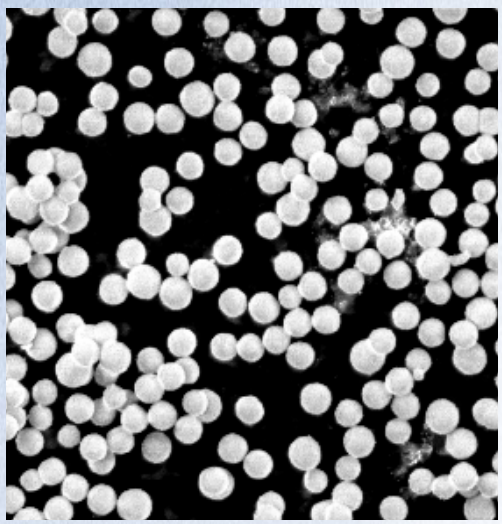


Figure 1:
SEM of 3 micron
Rhinophase®-AB

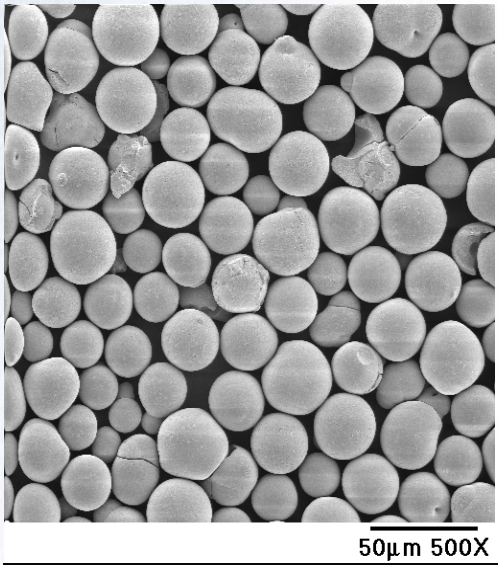
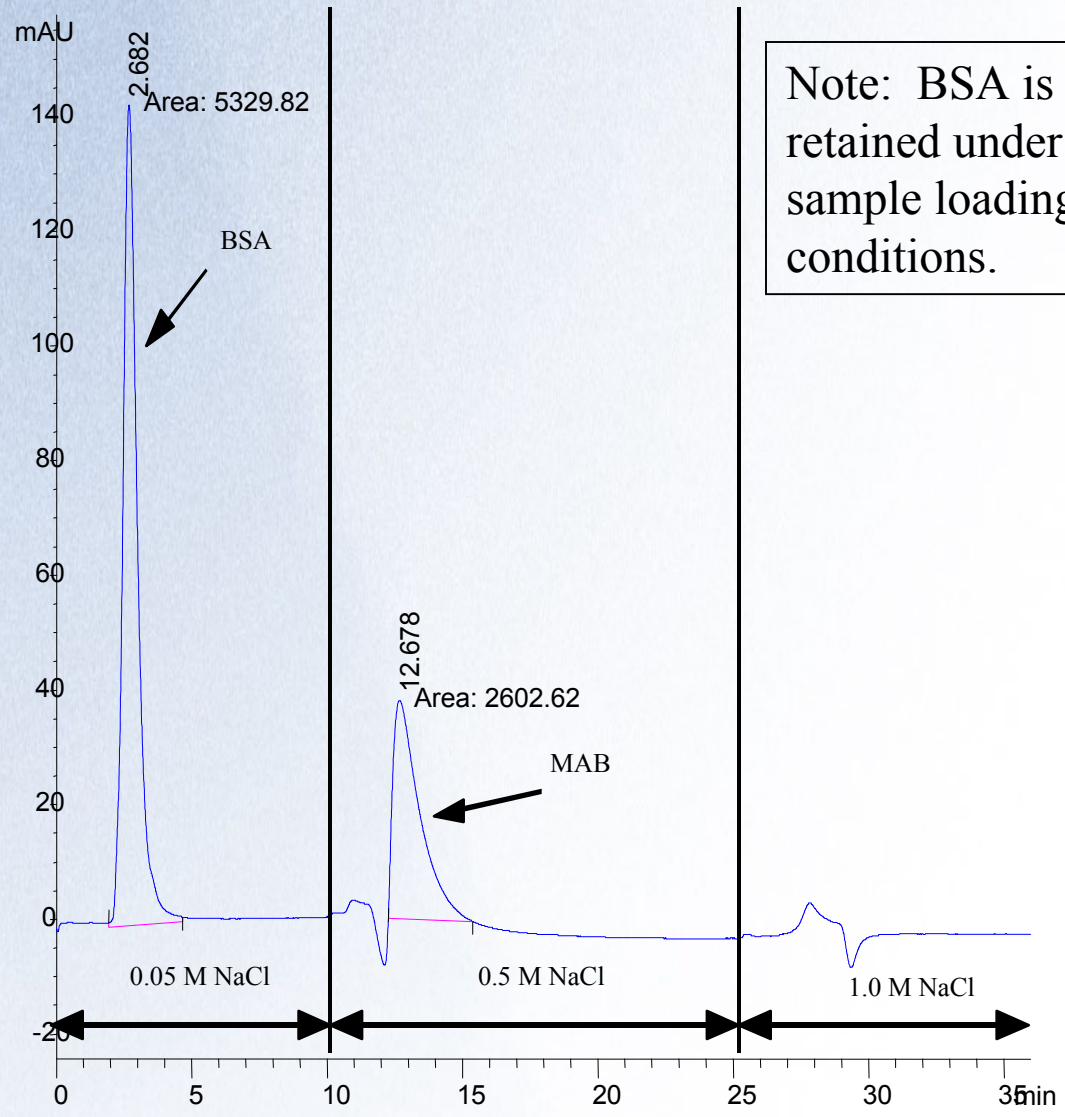


Figure 2:
SEM of 25 micron
Rhinophase®-AB

Particle Size (µm)	Surface Area (m^2/gram)	Est. Binding Capacity for IgGs (mg/mL)	Pore Size (Angstroms)	pH Range
3	15	5-10	300-500	1-13
25	15	5-10	300-500	1-13

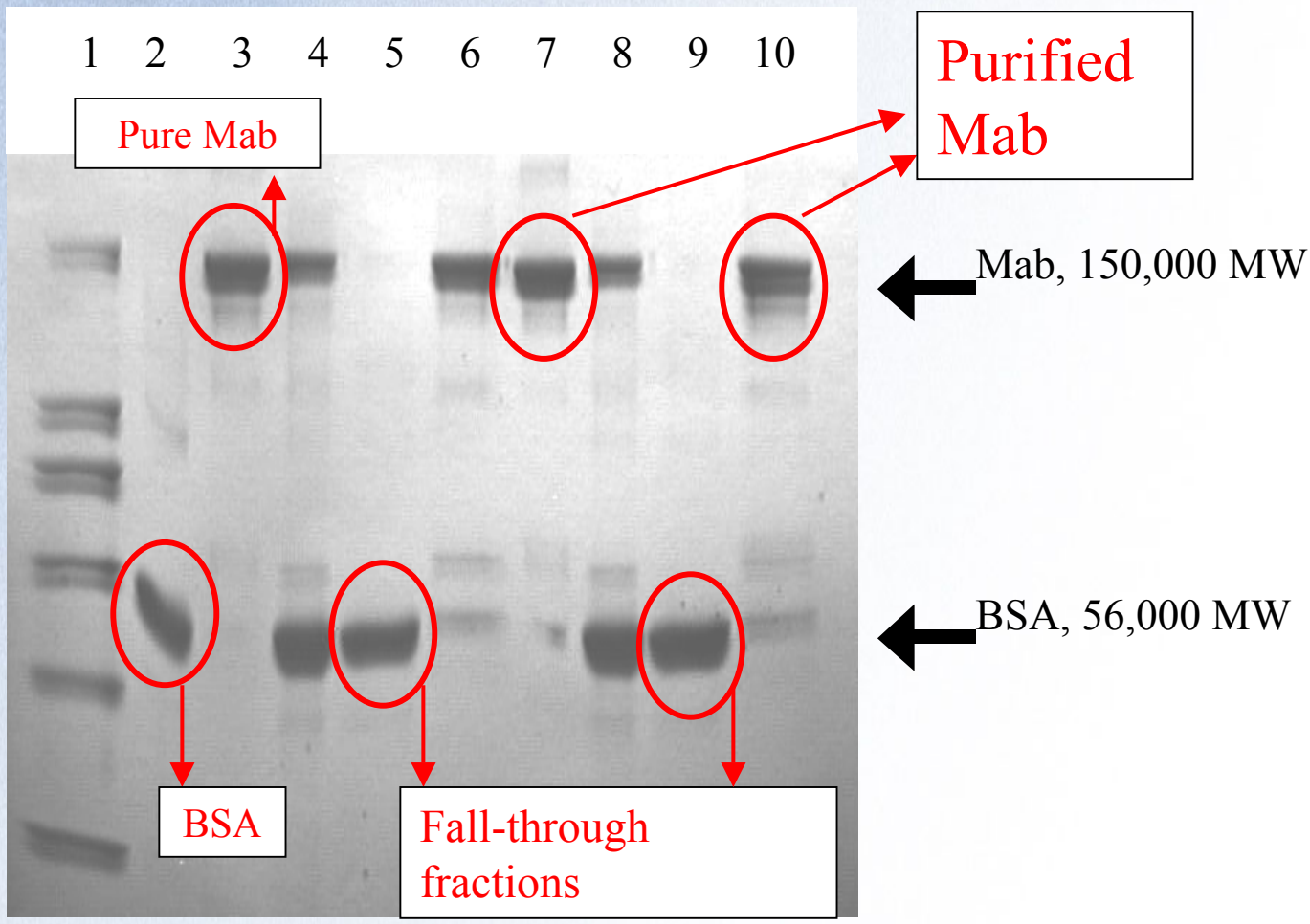
Rhinophase®-AB Does Not Bind Serum Proteins



Note: BSA is not retained under sample loading conditions.

LC Conditions: 100 *u*l injection of BSA (6.0 mg/ml) contaminated MAB (1.0 mg/ml) eluted by salt step gradient. Mobile phase: 20 mM MES, 4 mM EDTPA, 0.05 M-to-1.0 M NaCl pH=5.5. Flow rate: 2.0 ml/min. Temperature: 30°C. Detection: 280 nm.

3 Micron Rhinophase®-AB Does Not Bind Serum Proteins



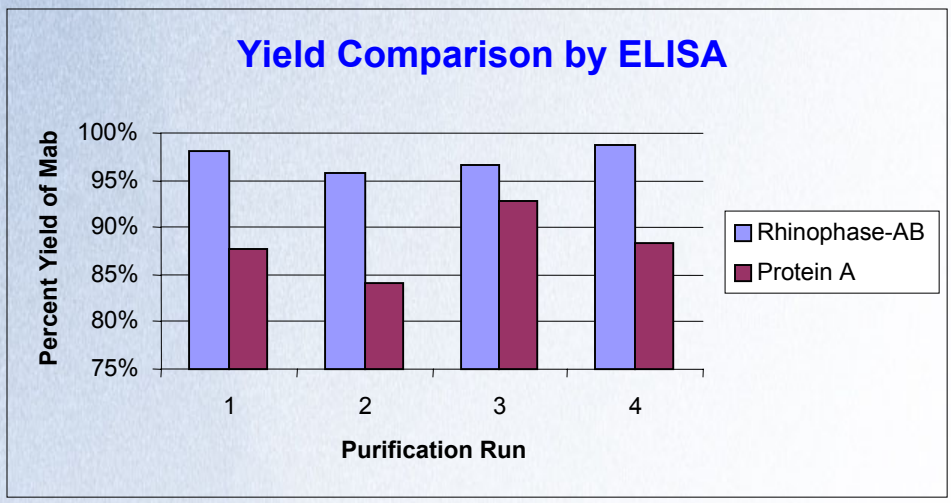
Lane 1 shows a molecular weight ladder. Lane 2 shows a 3 *u*g application of pure BSA. Lanes 3 and 7 show application of pure BSA and MAB at a total protein level of 2 *u*g, respectively. Lanes 4 and 8 show an application of cell culture supernatant at a total protein level of 4 *u*g. The cell culture supernatant has two distinct protein bands corresponding to BSA with a molecular weight of 56 KDa and MAB (IgG) with a molecular weight of 150 KDa with some additional minor bands. Lane 5 shows the unretained fraction from Run #1 at a total protein level of 3 *u*g. Lane 9 shows the unretained fraction from Run #2 at a total protein level of 3 *u*g. The fall through fraction gave a band around 56 kDa that is similar to the pure BSA in Lane 2. Lane 10 shows the elution fraction from Run #2 at a total protein level of 3 *u*g. The eluted fraction gave a band around 150 kDa that is similar to the pure MAB in Lane 3. The purity of the MAB in the eluate fraction (Lanes 6 and 10) is estimated to be greater than 96% by digital image processing.

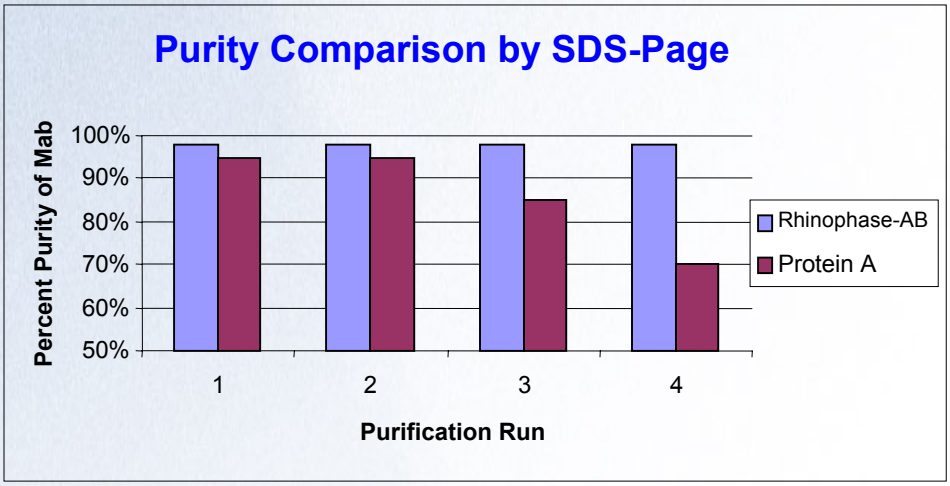
Head to Head Purity Comparison Using Protein G and 25 Micron Rhinophase®-AB

Purified Mab was equally pure using Protein G and Rhinophase®-AB .

Silver-stained, SDS-PAGE gel Comparing IgG_1 purified by Protein G (row 1 from right) and Rhinophase®-AB (row 3 from right). Electrophoresis was run under reducing conditions. Sample loading at 1 *u*g per lane. All other lanes are standards.

Purity and Yield Comparison in the Presence of Serum Proteins

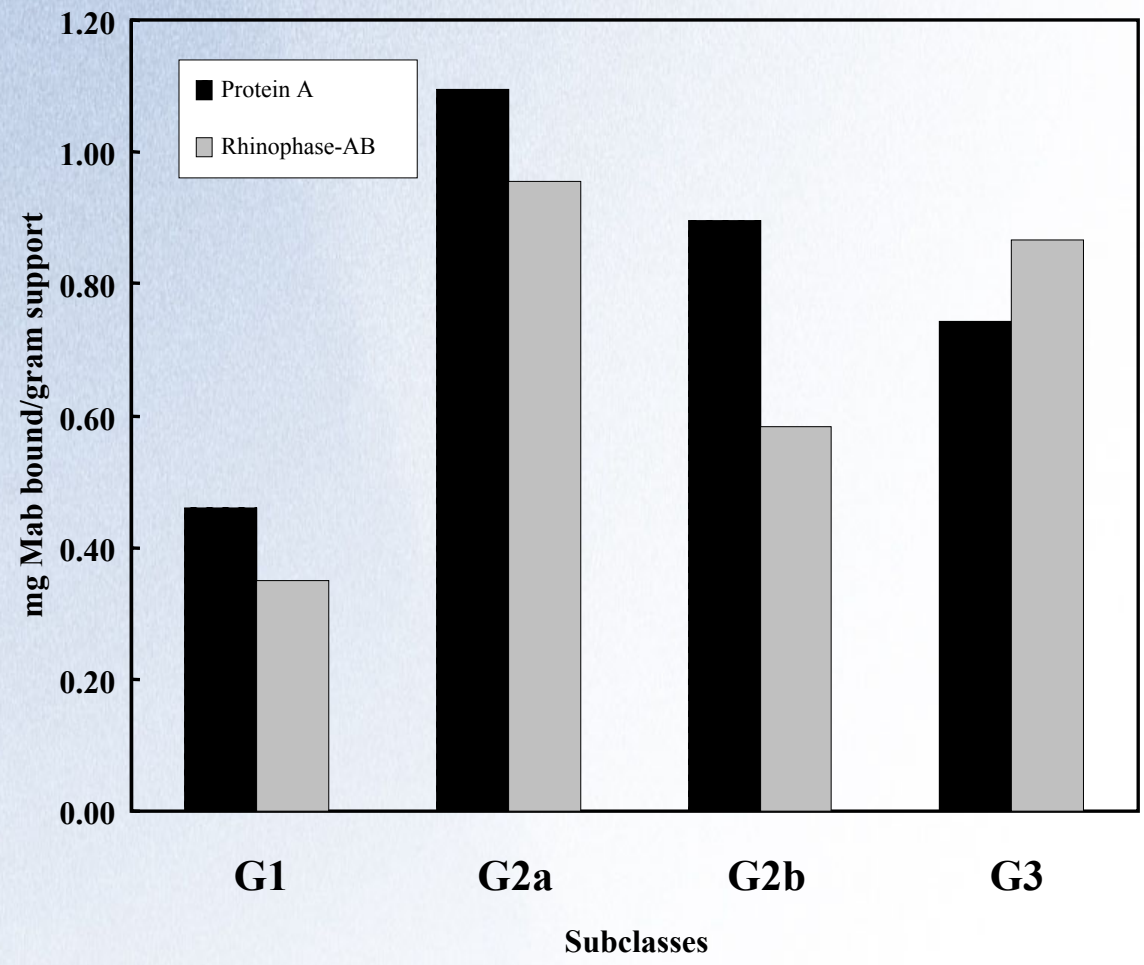




Discussion:

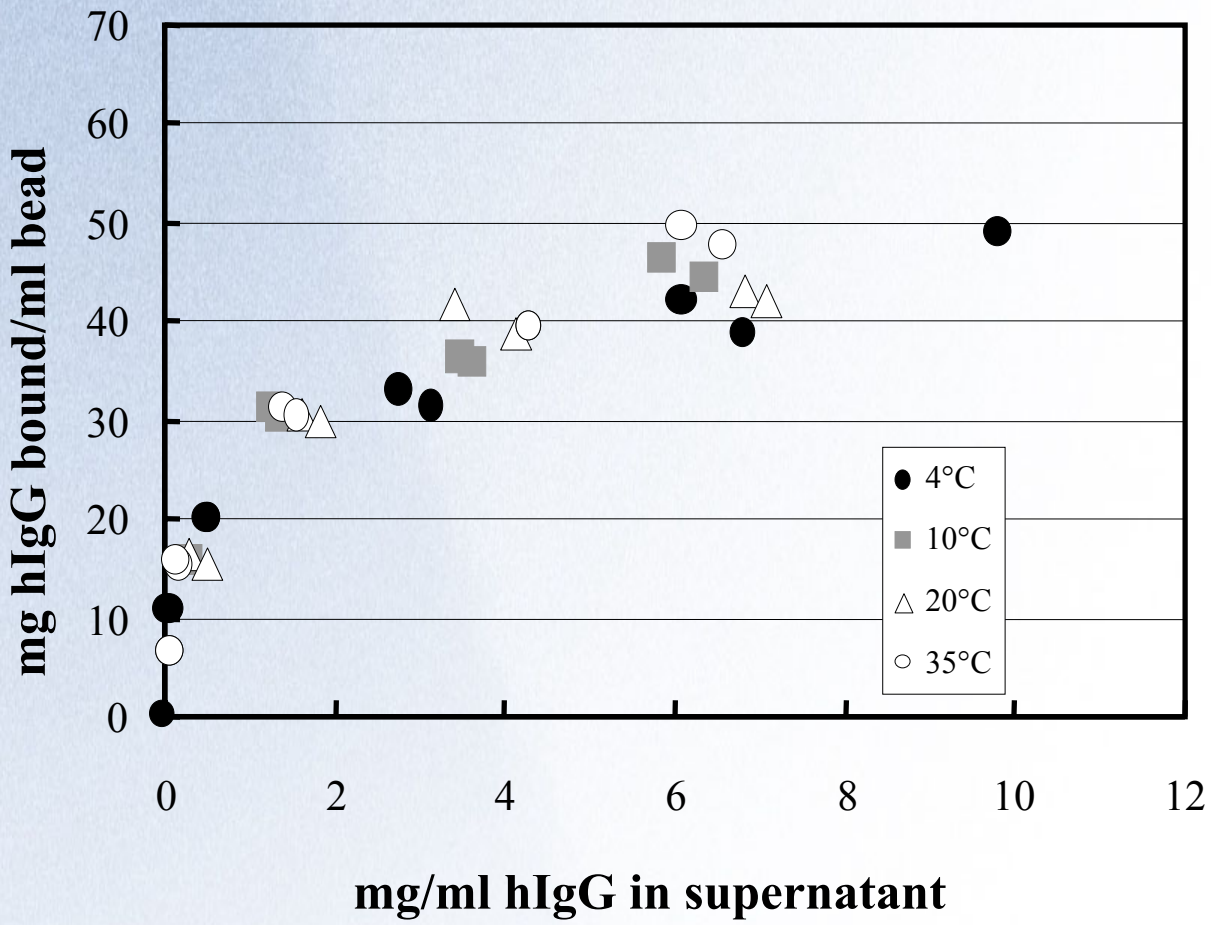
The sample used for this study had a high level of serum proteins (and Mab) in the cell culture supernatant, which quickly degraded the performance of the Protein A material. The Rhinophase®-AB material (25 micron) did not show any decrease in performance in the study as the serum proteins do not bind to the column and are flushed out during the loading step. The Protein A support was purchased from Pierce Chemical Company and is an agarose-based support, which is incapable of supporting high linear mobile phase flow velocities.

Rhinophase®-AB is Useful for Different Subclasses of IgG



Rhinophase®-AB had a high binding capacity for a variety of different IgG subclasses and is comparable to Protein A media.

Rhinophase®-AB's Binding Capacity is Unaffected by Temperature



Rhinophase®-AB is Useful for a Variety of Immunoglobulins

	Rhinophase®-AB
Sample	Capacity (mg antibody/ml particles)*
hIgG	28
hIgA	9
hIgM	2

*All values are reported as an average of 3-independent replicate experiments. The standard deviation is less than 5%. All capacities are reported as mg Ig bound per ml of beads.

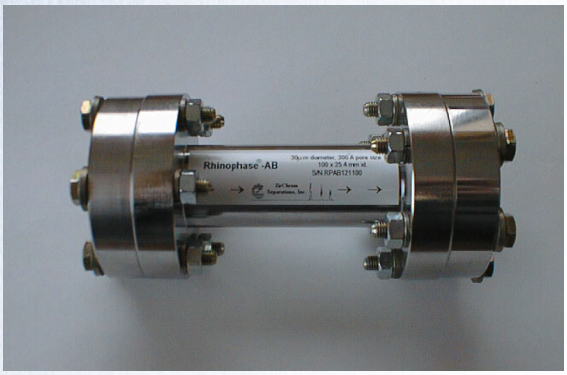
Rhinophase®-AB is Useful for Immunoglobulins from Different Animal Sources

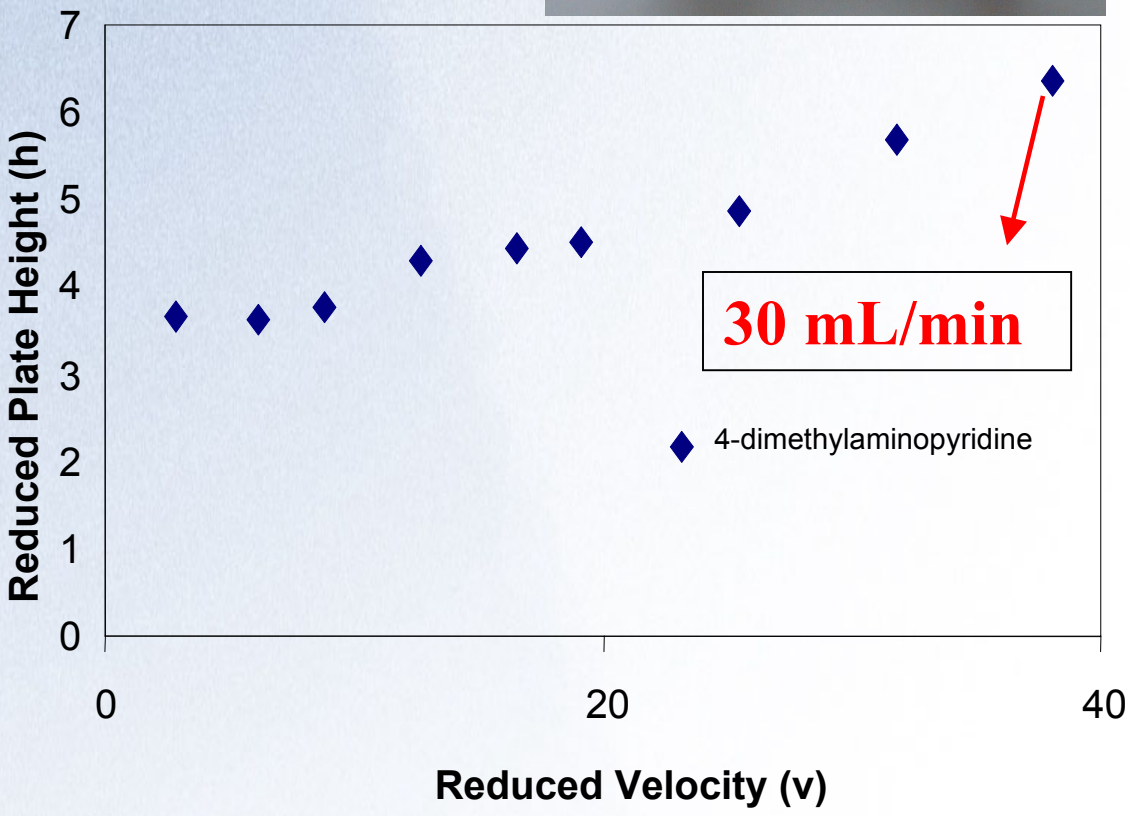
Rhinophase®-AB		
Sample	Offered	Bound
	mg/ml beads*	mg/ml beads*
Porcine IgG	37	28
	18	11
	9	8
	5	4
	1	1
Bovine IgG	22	19
	11	10
	6	5
	3	3
	1	1
Human IgG	22	19
	11	11
	6	5
	3	3
	1	1

*All values are reported as an average of 2-independent replicate experiments. The standard deviation is less than 5%. All capacities are reported as mg Ig bound per ml of beads.

Rigid Zirconia Particles Can Be Operated at High Flow Rates

Picture of a 100 x 25.5 mm Preparative Rhinophase®-AB Column





Rigid zirconia particles can withstand high pressure and high linear flow velocities.