OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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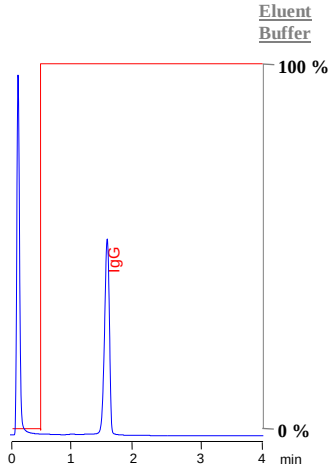
APPLICATION NOTE

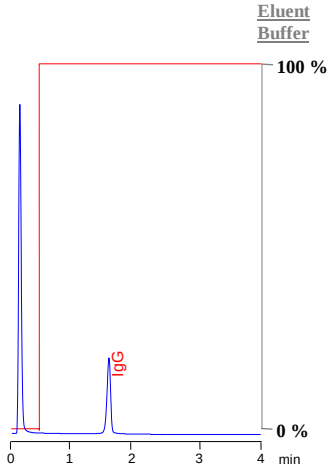
Rapid isolation of IgG From Human Serum on STYROS™ rA (Immobilized Recombinant Protein A on Simulated Monolith Polymeric Stationary Phase):

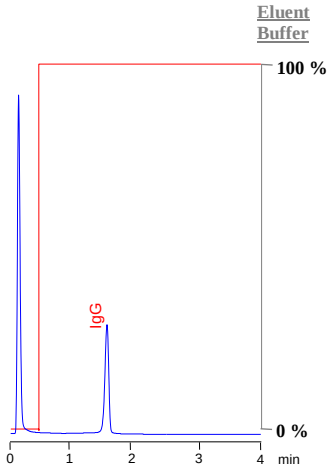
Commercially available immobilized Protein A stationary phases are known to have a number of disadvantages that include the cost, lifespan and most importantly leaching.

STYROS™ rA is a polymer based matrix developed by **OraChrom**. Unlike other polymer based Protein A columns, **STYROS™ rA** coating and immobilization processes are fully covalent. This eliminates any leaching of the stationary phase, makes the manufacture of the media more reproducible and therefore results in a far more stable and lower cost product.

STYROS™ Simulated Monolith columns can be run at high linear velocities. The following chromatograms show three serum samples run on a narrow bore column of 2.1mm ID with 33mm length. It is possible to fully separate the IgG from the serum in less than 2 minutes at 3,600 cm/hr of linear velocity.







Capture of IgG from three human serum samples, run at 2 ml/min (3,600 cm/hr) on a 2.1 x 33 mm **STYROS™** rA column.

Operating parameters for the chromatograms.

HPLC System	Agilent 1100, Standard Cell
Columns	2.1 x 33 mm.
Binding buffer	50 mM Phosphate, 150 mM NaCl, pH 7
Eluent buffer:	22 mM HCl, 150 mM NaCl, pH 1.9
Detection:	280 nm
Flow rate:	2 ml/min (3,600 cm/hr)
Temperature	30 °C
Injection volume	5 µ l
Sample:	Heat treated, non diluted human serum.

Human serum can be used without any dilution. The eluted IgG can be deposited onto a **STYROS™ R** reversed phase polymeric column or collected in a neutralizing buffer in order to preserve its activity.

The polymeric nature of the stationary phase, and the stable, covalent coating of the media, makes it possible to clean the column with organic solvents, as well as acidic and basic solutions.

Inc.