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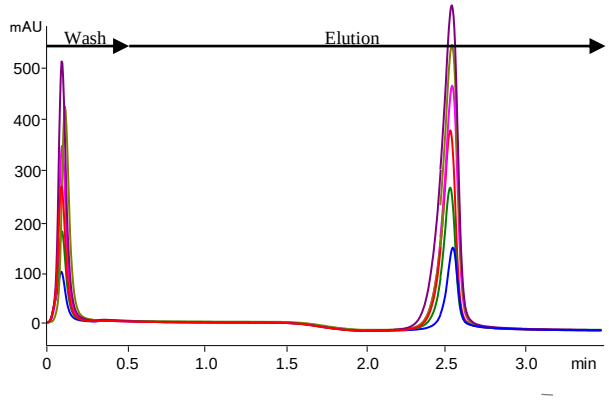
APPLICATION NOTE

Quantitation of Monoclonal Antibodies with Immobilized Protein rA on Simulated Monolith Polymeric STYROS™.

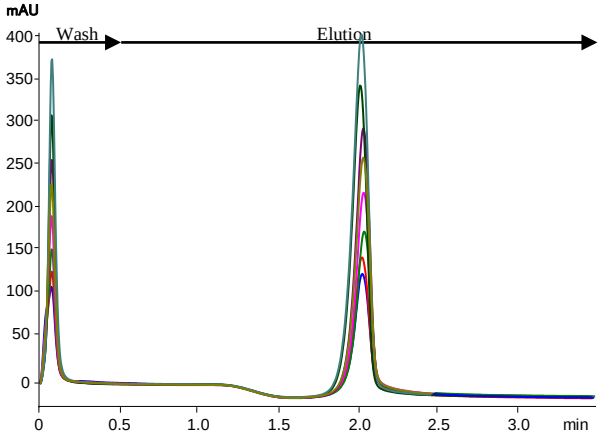
STYROS™ rA columns are made of Simulated Monolith polymeric tethered with recombinant Protein A. They can enable rapid and accurate assay of monoclonal antibodies in sample solutions such as harvested cell culture fluid as well as downstream products.
Any Fc containing biomolecules can also be retained by the column and assayed.
STYROS™ rA comes in many formats to consider the minimal use of the samples, the time of the assay and the generation of as little waste as possible.
In the present Application Note we have used a 1 mm ID column with a length of 3 cm or 5 cm to run samples as little as 0.1 µl to 1.2 µl with complete reproducibility.



Reproducibility of Injections at 1 ml/min (7,600 cm/hr) on a 1 x 30 mm **STYROS™** rA/MB column.

HPLC System	Agilent 1100, Standard Cell with column heater.
Columns	**STYROS™** rA/MB 1 x 30 mm (0.024 ml)
Binding buffer	50 mM Phosphate, 150 mM NaCl, pH 7
Eluent buffer:	22 mM HCl , pH 1.9
Detection:	280 nm
Flow rate:	1 ml/min (7,600 cm/hr)
Temperature	30 °C
Injection volume	0.1 to 1.2 µ l
Sample:	Harvested cell culture with monoclonal antibody content (mAB).

It is recommended that samples be filtered prior to the injection into the column. The column size (0.024 ml) allows small amounts of sample to be detected. The samples could also be injected into a larger column of 5 cm length at a higher flow rate of 1.5 ml/min that is the equivalent of 11,500 cm/hr of linear velocity.



Reproducibility of Injections at 1.5 ml/min (11,500 cm/hr) on a 1 x 50 mm **STYROS™** rA/MB column.

HPLC System	Agilent 1100, Standard Cell with column heater.
Columns	**STYROS™** rA/MB 1 x 50 mm (0.104 ml)
Binding buffer	50 mM Phosphate, 150 mM NaCl, pH 7
Eluent buffer:	22 mM HCl , pH 1.9
Detection:	280 nm
Flow rate:	1.5 ml/min (11,500 cm/hr)
Temperature	30 °C
Injection volume	0.6 to 2.0 µ l
Sample:	Harvested cell culture with monoclonal antibody content (mAB).

In some cases the addition of EDTA (10 mM) can help the chelation of ferric salts in the cell culture fluid that would otherwise result in nonspecific binding of impurities to the column.
Common excipients such a Tween, Triton x-100 at less than 1% or polysaccharides or glycols at less than 20% does not interfere with the assay.
The elution buffer can be hydrochloric acid as used in the present case or citrate, acetate, glycine, phosphate or other salt with buffering capabilities at low pH.
Due to the polymeric nature of **STYROS™** and its stable, covalent coating, it is possible to clean the column with organic solvents, as well as acidic and basic solutions.

Inc.