

APPLICATION NOTE

Assessing Monoclonal Antibodies (mAB) with Immobilized Protein rA on Simulated Monolith Polymeric STYROS™: Dynamic Capacity.

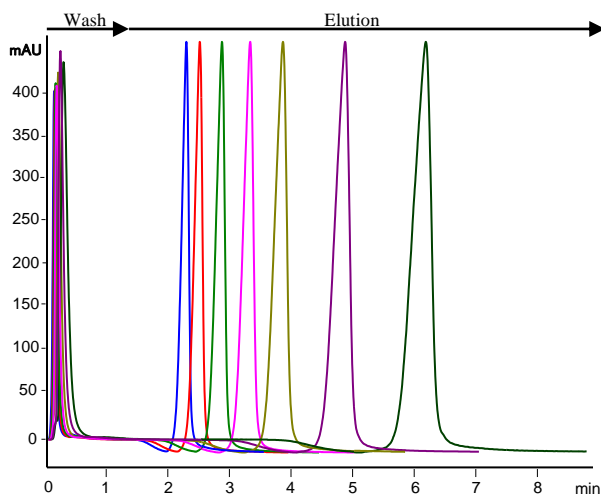
We have shown in the previous application note that **STYROS™ rA** columns can enable rapid and accurate assay of monoclonal antibodies in sample solutions such as harvested cell culture fluid as well as downstream products.

We also noted that **STYROS™ rA columns** can be acquired 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 and compared it with a narrow bore column of 2.1 mm ID of similar length.

Same amounts of sample were used in both cases with acceptable detection level.

The goal was to monitor the effects of linear velocities on the dynamic capacity of the media.



Injections from 1 ml/min (7,600 cm/hr) down to 0.4 ml/min (3,000 cm/hr).

Column: 1 x 30 mm **STYROS™ rA/MB**.

The changes in dynamic capacity are within experimental errors at present high linear velocities.

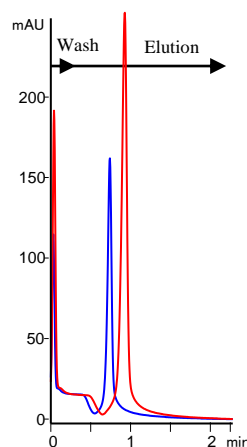
The percentage of retained mAB remains the same as compared with the non-retained eluent of the sample.

A minimal amount of sample is used for each injection (0.1 µl).

It is noteworthy to understand that no product is lost in the waste stream due to the drop in capacity at higher flow rates as it is the case with soft gel media or other hard gel

media with “shrink wrapped” coating that has the propensity to leach as well as release fines.

The same pattern is seen with a Narrow Bore column of 2.1x30mm dimension. The percentage of the retained product as compared with the non-retained part remains the same.



Injections at 3 ml/min (5,200 cm/hr) and 4 ml/min (6,900 cm/hr).

Column: 2.1 x 30 mm **STYROS™ rA/NB**.

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| HPLC System | Agilent 1100, Standard Cell with column heater. |
| Columns | STYROS™ rA/MB 1 x 30 mm (0.024 ml) STYROS™ rA/NB 2.1 x 30 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 ml/min (7,600 cm/hr) for 1x30 mm 4 ml/min (6,900 cm/hr) for 2.1x30 mm |
| Temperature | 30 °C |
| Injection volume | 0.1 µl |
| Sample: | Harvested cell culture with monoclonal antibody content (mAB). |

At 4 ml/min of volumetric flow (6,900 cm/hr of linear velocity) the back pressure of the column is only 41 bar that is well within the range of pressure tolerance of the column rated at 140 bar for routine operations.

