Mab Capacity Study

The static loading capacity was measured on the same 25 micron **Rhinophase®-AB Media** using the following conditions:

Sample:
Purified Ms x hPARG IgG1 clone (3.69mg/mL) was diluted in Loading Buffer (20mM MES, 50mM NaCl, 4mM EDTPA, pH=4.0) to make a 1mg/mL IgG1 solution by diluting 5.42mL of the purified IgG1 in 14.58mL of Loading Buffer.

Column:
A 1cm x 2.1mm i.d. guard was slurry packed with 0.067g of **Rhinophase®-AB**, lot#16-140. The amount of material in the column was determined by unpacking the column and drying down the material after the study was completed.

Pump and Detection:
HP1100 pump run at 0.20ml/min and UV-Vis detector set at 280nm with a standard flow cell were utilized for the study.

Experimental procedure:

1. The plumbing to the mixing valve was disconnected and the direct feed into the pump was used to pump the sample into the column. This was done to eliminate the mixing valve and all the tubing (i.e. volume) required from the solvent bottle through the degasser to the mixing valve.
2. Small 15mL vials were used as solvent reservoirs to hold the Loading Buffer, IgG1 sample, and Elution Buffer (20mM MES, 2.0M NaCl, 4mM EDTPA, pH=4.0). Tubing was then run to bypass the injector and go directly into the guard hardware.
3. A piece of tubing was run directly from the end of the guard holder into the detector.
4. Another piece of tubing was run from the detector into a graduated cylinder to collect the sample.
5. The column was pre-conditioned with Loading Buffer to equilibrate the column. Once a good baseline had been achieved a blank injection was done (zero volume injection of ACN/Water) to begin acquiring data for each run. As the instrument injected, the vial was switched from the Loading Buffer to the IgG1 sample at 1mg/mL. The tubing to the graduated cylinder was quickly moved to an empty 5mL cylinder at the start of the loading.
6. The IgG1 sample was loaded until it was apparent from the chromatogram that the column was overloaded (25minutes).
7. One fraction was collected upon loading.
8. The pump was then turned off and the Loading Buffer replaced with Elution Buffer.
9. The graduated cylinder was emptied into a centrifuge tube and labeled and then replaced to collect the elution fractions.

10. The pump was turned back on and another blank injection initialized for a 10min run. Two fractions were collect during the IgG1 elution.
11. Each fraction was measured and placed in a labeled centrifuge tube.
12. After the first run, the column was re-equilibrated with the Loading Buffer again and the whole procedure repeated to obtain two separate runs. The fractions were analyzed by ELISA to determine the amount of Mab present in each fraction.

Table 7. CAPACITY BASED ON ELISA RESULTS

Run	Fraction #	IgG Quant (ug/mL)	Fraction Volume (mL)	Mass of IgG (mg)
Test 1	Load	210	3.74	0.79
	Elution #1	790	0.69	0.55
	Elution #2	1070	0.68	0.73
Test 2	Load	215	3.49	0.75
	Elution #1	783	0.72	0.56
	Elution #2	798	0.69	0.55

Column: 1cm x 2.1mm I.d. containing 0.067g of media

RESULTS: Test #1 18.955 mg/mL

Average: 17.8 mg/mL

Test #2 16.567mg/mL

* Note: 1g = 1mL