# Development of a next generation cellulose based high capacity rProtein A capture resin for high through-put Mab purification in both batch and continuous purification formats

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Abstract: A new product development approach will be described for the affinity capture of Mab's from cell culture materials employing a novel base stable rProtein A ligand. Using a stable cellulose base bead with excellent flow properties coupled with a novel immobilization methodology, a next generation rProtein A capture resin has been developed with a high level of antibody binding capacity. The new Cellufine™ rProtein A resin shows $C_{20\%}$ dynamic binding capacity (DBC) of >50 mg/ mL with polyclonal antibodies at a residence time of 8 minutes. This resin has been shown to retain > 95% of its original binding capacity after 100 cycles of re-use with a 0.1M NaOH CIP step every 10th cycle. In continuous purification format, where more of the resin capacity is utilized >65 mg/mL DBC has been demonstrated with shorter 4-minute residence times. A high through-put screening (HTS) assay format has been developed to screen pH elution conditions. Polyclonal antibodies show efficient elution at pH 3.5 with a 0.1M Glycine HCL buffer.

## Experimental details and Results

### Chromatography resins and other materials:

Cellufine spherical bead cellulose (90 µM) was coupled with a novel base stable rProtein A ligand vis reductive amination to yield a high ligand density on the bead surface. The resin was flow packed in water in a 0.66cmID x 5.0 cmL glass column (Omnifit™ EZ, Diba Industries Inc, Danbury, CT) at up to 600 cm/h till a stable bed height was reached. The final column length was then adjusted to apply axial compression to a final ratio of 1.015 – 1.020.

### Dynamic Binding Capacity Measurements:

The above column was equilibrated in 20 mM Na Phosphate buffer + 0.15M NaCl at pH 7.5 at 150 cm/h flow rate on an AKTA Purifier 10 instrument for 10 column volumes (CV). For DBC measurements a 5 mg/mL polyclonal human Gamma globulin (Golden West Biologicals, Inc®, Temecula, CA) was prepared in the above loading buffer and filtered with a 0.22 µM pore size bottle top filter (Nalge NUNC, Thermo Scientific, Waltham, MA) to remove particulates. Note: based on the initial non-retained breakthrough peak the protein was estimated to be 95% pure material that was retained by Protein A. The overall protein concentration was measured at A280 nm and used an extinction of 1.38 for a 1 mg/mL IgG in a 1 cm path length cuvette. A series of breakthrough curves are overlaid in Figure 1 and the calculated DBC at 20 and 50% of saturation is summarized in Table 1. A summary of the recovered IgG after low pH elution (0.1M Glycine pH 3.0) is shown in Table 2 for both peak area and A280 estimation of protein concentration.

**Figure 1,** Dynamic Binding Capacity of Cellufine rProtein A resin over a range of flow rates

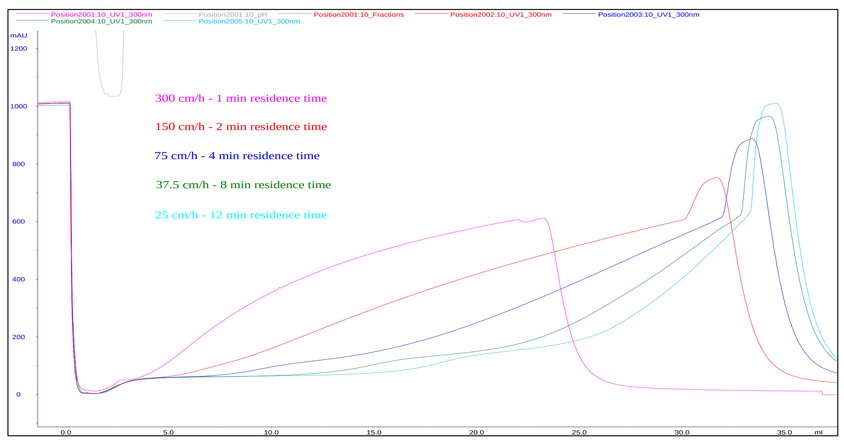



**Table 1**, Summary of DBC over a range of residence times

| Residence time (min) | DBC- $C_{10\%}$ (mg/mL) | DBC – $C_{50\%}$ (mg/mL) | C10/C50 |
|---|---|---|---|
| 1 | 10.9 | 32.1 | 0.195 |
| 2 | 23.1 | 53.2 | 0.230 |
| 4 | 41.7 | 65.9 | 0.311 |
| 8 | 52.9 | 71.7 | 0.432 |
| 12 | 58.9 | 75.1 | 0.516 |

**Table 2**, Protein recovery over a range of residence times

| Residence time (min) | Peak Area (mAU/mL) | Recovered Protein (mg) | Recovery /ml resin volume |
|---|---|---|---|
| 1 | 9685 | 88.1 | 51.5 |
| 2 | 12572 | 111.2 | 65.0 |
| 4 | 12894 | 116.7 | 68.3 |
| 8 | 13477 | 121.6 | 71.1 |
| 12 | 13531 | 130.9 | 76.5 |

## Cycles of Re-Use with 0.1M NaOH Base CIP:

Multiple cycles of re-use were carried out on the above packed column with a Gamma globulin load of 80% of the $C_{10\%}$ DBC (24.8 mg ≈ 6 mL volume) at a flow rate of 37.5 cm/h (8 min residence time). The protein retained was eluted with 5CV of 0.1M Glycine pH 3.0 and collected as described above. Recovery (mg) was calculated after measuring the volume and A280 (1/10 dilution in water). Every 10 cycles of re-use the column was cleaned with 0.1M NaOH in a static soak (no flow) for 30 min. A summary of the recovery of eluted IgG is shown in Figure 3 and a comparison of DBC measured on cycle 1 and 100 is summarized in Table 3.

**Figure 2,** Protein recovery over 100 cycles of re-use with 0.1M NaOH CIP

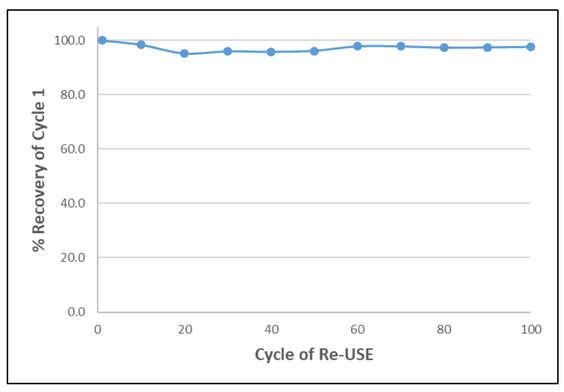


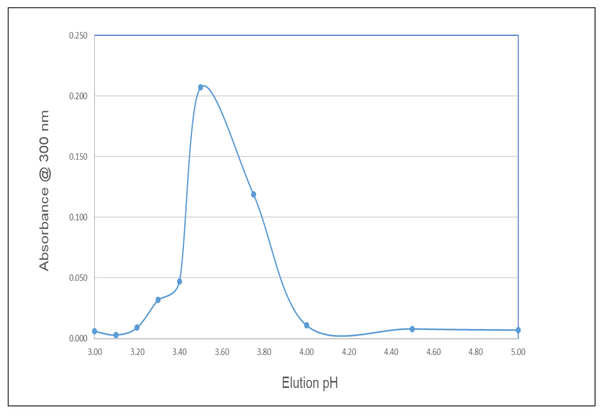

**Table 3**, Retention of DBC after 100 cycles of re-use

| Cycles of Re-Use | DBC- $C_{20\%}$ (mg/mL) | |
|---|---|---|
| | 4 min | 8 min |
| Cellufine rProtein A Cycle 1 | 41.7 | 52.9 |
| Cellufine rProtein A Cycle 100 | 41.4 | 51.8 |

## HTS Screening of Elution pH Conditions:

Elution conditions for polyclonal human IgG were screened in a high throughput screening format using a centrifugal membrane spin device (Ultrafree MC with a 0.22 µM pore size filter, EMD Millipore, Billerica, MA) with a series of 0.1M Glycine elution buffers at pH 5, 4.5, 4, 3.75, 3.5, 3.4, 3.3, 3.2, 3.1 and 3.0. Carry out a serial elution with the above series starting at pH 5 and place 0.1mL of the eluted fractions (in duplicate) into a UV clear 96 well plate (Corning, Billerica, MA) and read at 300 nM in a plate reader with a water reference. A summary of the pH elution profile of a retained antibody is shown in Figure 3 below.

**Figure 3,** Protein elution profile over a range of pH




## Conclusion

- Cellufine rProtein A showed $C_{20\%}$ dynamic binding capacity (DBC) of >50 mg/ mL with polyclonal antibodies at a residence time of 8 minutes (37.5 cm/h).
- The retained polyclonal IgG elutes at pH 3.5.
- Very high retention of IgG recovery after 100 cycles of re-use with a 0.1M NaOH CIP every 10th cycle.
- Little or no difference seen in DBC after 100 cycles of re-use + base CIP.
- At residence times > 4 min the recovery of IgG after $C_{50\%}$ saturation binding as expected in continuous purification in SMB, PCC or MCC modes is > 65 mg/mL.
- Samples of this resin are available in 1 and 5 mL pre-packed formats.