Cellufine™ SPA-HC

An affinity chromatography resin designed for the isolation of immunoglobulins, including monoclonal antibodies (mAb) from complex matrices, such as cell culture supernatant and serum/plasma.

Cellufine SPA-HC resin is based on a spherical highly cross-linked cellulose bead functionalized with an alkali stable rProtein A affinity ligand. The resin shows excellent flow properties, low ligand leachate levels, high dynamic binding capacity and good retention of binding after multiple cycles of base cleaning in place (CIP) and re-use. The highly cross-linked cellulose base beads were developed to accommodate the higher flow rates required to meet the demands of future high productivity purification workflows. This new high-performance affinity resin accelerates development of efficient purification processes for downstream purification of therapeutic monoclonal antibodies. Resin properties are summarized in Table 1 below.

Table 1, Performance characteristics of Cellufine SPA-HC

Property	Characteristic
Ligand	Alkali-stable rProtein A affinity ligand produced in *E.coli*
Matrix	Highly cross-linked cellulose beads
Particle size	Average 70 µm diameter
Ligand coupling method	Coupling via formyl groups on resin
Flow velocity	≧ 650 cm/h (0.3 MPa) with 10 cmID x 20 cmL Column
Dynamic binding capacity (DBC)	≧ 70 mg /ml (DBC C_{10} with Polyclonal IgG) [1]
Recommend CIP solution	0.1 M NaOH
Temperature stability	4 - 50 ºC No significant change in performance after 1-week storage.
Storage	2 - 8 ºC in 20 % (v/v) ethanol up to 2.5 years provisionally.
Chemical stability	No significant change in performance after 1-week storage in 30 % (v/v) isopropanol, 20 – 70 % (v/v) ethanol, 8 M Urea, 6 M Guanidine-HCl or 0.1 M Acetic acid.
pH working range	3 - 12

[1] DBC C_{10} measured at 10 % break through point at a residence time of 6 min using human polyclonal gamma globulin (Human IgG for infusion).

High dynamic binding capacity comparison with other commercial media

Human polyclonal pAb dynamic binding capacity (DBC) was compared to commercial rProtein A resins and showed that Cellufine SPA-HC has one of the highest adsorption capacities available to support IgG capture in bioproduction. Data comparing SPA-HC to an 85 µM crosslinked agarose bead over a wide range of residence times is summarized in Figure 1. Table 2. shows a wider resin comparison at 4 and 6 min residence times.

Fig.1, pAb DBC comparison to a commercial Protein A resin

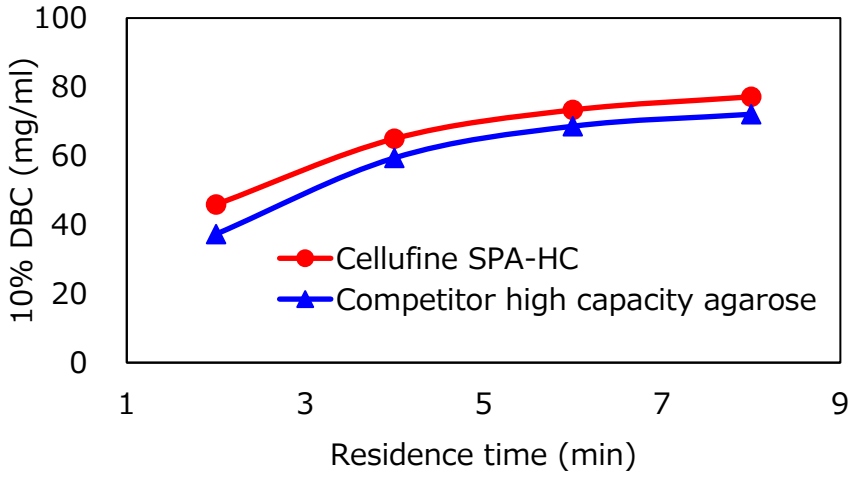


Table 1, DBC at 4 and 6 min residence times for a range of commercial resins

Resin Base polymer	Mechanical feature		pAb 10% DBC (mg/mL)	
	Bead size* (µM)	Flow rate** (cm/h at 0.2MPa)	4 min	6 min
Cellufine SPA-HC	70	200	67	72
Cross-linked agarose	85	220	57	68
	60	130	75	79
Polyvinyl ether polymer	50	-	52	55
Polymethacrylate	45	120	68	75
Methacrylic polymer	50	100	67	71
Cross-linked cellulose	75	260	60	69

*Bead size was taken from vendor information except for Cellufine SPA-HC.

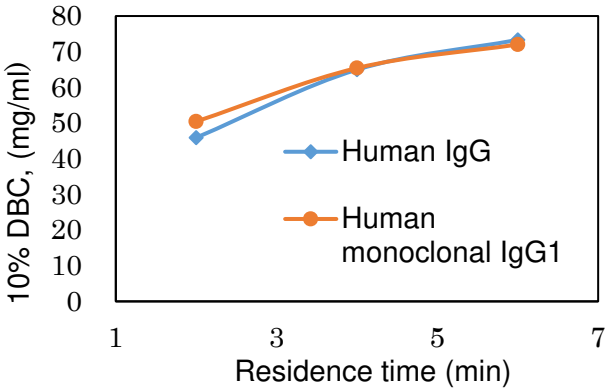
**Flow rate data was taken from a published report (see J. Chro.A 1554 (2018) 45-60). A 11 mmID column was used at a bed height of 10 cmL. The data for Cellufine SPA-HC was extrapolated from the above experimental data according to the column dimensions.

A pre-packed 1 mL column (6.7 mmID x 3 cmL) was equilibrated in 20 mM Na-Phosphate buffer + 0.15M NaCl at pH 7.5. Polyclonal human antibody (pAb) stock solution (50 mg/mL Human IgG for infusion) was adjusted to 5 mg/mL IgG concentration with equilibration buffer and filtered with a 0.22 µM pore size bottle top filter to remove particulates. DBC was measured at 10% breakthrough point.

Purification of mAb from CHO cell culture samples

Cellufine SPA-HC has also demonstrated high dynamic binding capacity with a human mAb sub-type 1 (see Figure.2) indicating that this new resin can be used for efficient mAb purification workflows.

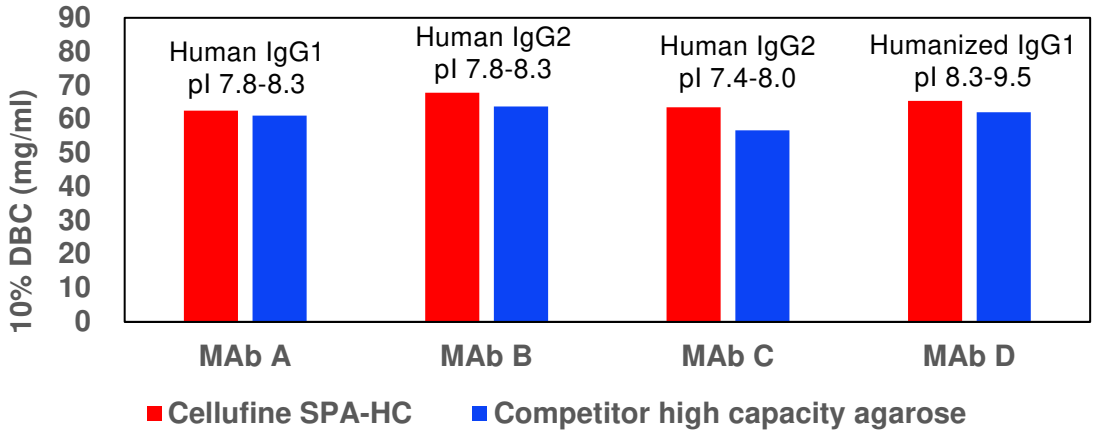
Figure 2, mAb and pAb DBC comparison



Human pAb (IgG for infusion) was purchased commercially. Human monoclonal antibody IgG1 was purified from CHO cell culture.

mAb's with a range of isoelectric points (pI) and sub-types were used to investigate whether the molecular charge state had any impact on the dynamic binding capacity of the resin. The results indicate that Cellufine SPA-HC can adsorb mAb's with a wide range of pI's and show higher DBC capacity compared to an 85 µM high capacity agarose competitive resin. A summary is shown in Figure 3, below.

Figure 3, DBC data on a range of pI and sub-type mAb's



A mAb expressed in CHO cell culture was captured from the clarified supernatant, adjusted to pH 7.5 and 15 mS/cm conductivity with 1 M Tris base and 1 M NaCL. Sample volume was also adjusted for the antibody titer (1.25 g/Lt.) to target a load of 80% of the 10% DBC measured for the 4 min. residence time. After loading, the 1 mL columns were washed down to baseline with 10 CV equilibration buffer. Retained mAb was eluted with 10 CV of 60 mM Sodium Acetate pH 3.0. The collected fractions were neutralized with 1 M Tris base and tested for; a) mAb recovery (by A280), b) ppm CHO-HCP by ELISA (P/N F550 Cygnus Technologies, Southport, NC) and c) % aggregates in the recovered mAb fractions by SEC analysis. A full summary of the recovery and contaminant levels are shown in Table 2, below.

Table 2, mAb purification summary

Sample	mAb Load (mg/mL)	Elution CV	% Yield	CHO-HCP (ppm)	Leached rProtein A (ppm)	% Aggregates by SEC
Load	-	-	-	8.68×10^5	-	-
SPA-HC	53	3.0	89	1800	4	1.4
Cross linked Agarose	51	3.9	89	1820	8	1.9

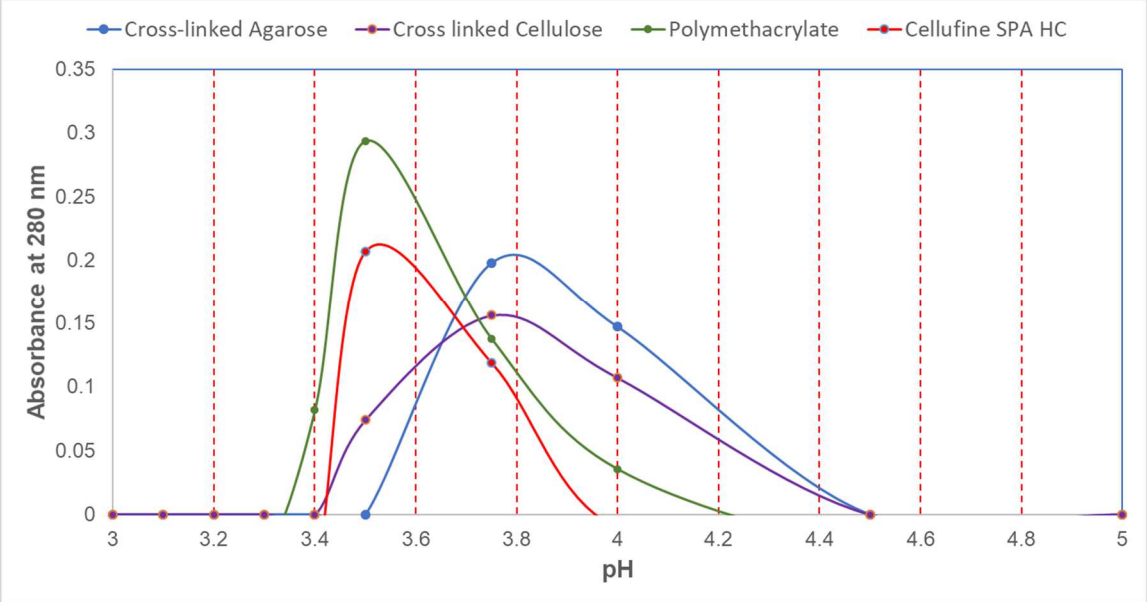
This study shows that a mAb derived from CHO culture supernatant was efficiently captured by Cellufine SPA-HC achieving a high level of impurity removal in this first capture step of a purification workflow.

Elution pH screening in high throughput format

Screening for elution pH was carried out in a high throughput format using a centrifugal spin device (Ultrafree MC with a 0.45 µM pore size filter, EMD Millipore) as follows; 1) pipet 0.02 mL of a 50% (v/v) slurry of the resin to test into the above device, 2) wash 2 x with 0.4 mL of loading buffer (20 mM Na Phosphate buffer + 0.15 M NaCl at pH 7.5), 3) Load with 0.4 ml of 5 mg/mL purified Gamma globulin IgG in loading buffer, 4) Wash 2 x with 0.4 mL of load buffer, 5) Serial elute with 0.1M Glycine pH 5.0, 4.5, 4.0, 3.75, 3.5, 3.4, 3.3, 3.2, 3.1 and final 3.0. 6) Collect the filtrate from each elution step in a multiwell plate (low UV acrylic, Corning) and measure the eluted protein at 280 nm in a UV plate reader (Molecular Dynamics).

Data is summarized in Figure 4 below for Cellufine SPA-HC, cross-linked agarose (85 µM bead size), polymethacrylate (45 µM bead size) and a cross-linked cellulose (75 µM bead size).

Figure 4, Elution pH screening in high throughput format



The above result shows that Cellufine SPA-HC and the polymethacrylate based resin both show peak elution at pH 3.5 and the other two resins at pH 3.8. This difference in elution pH presumably reflects the structure of the different rProtein A affinity ligands on these commercial resins. This methodology is a very quick way to screen elution pH and uses very little material.

Base Stability and CIP – Cycle to Cycle CHO-HCP "cross-over":

Cleaning in place (CIP) with base is routinely applied during cycles of re-use of rProtein affinity resins. Cellufine SPA-HC has been evaluated for 0.1 base CIP for up to 150 cycles of re-use. Results are summarized in Figure 4 below.

Figure 4, 0.1M NaOH CIP study with 15 min contact for 150 cycles of re-use.

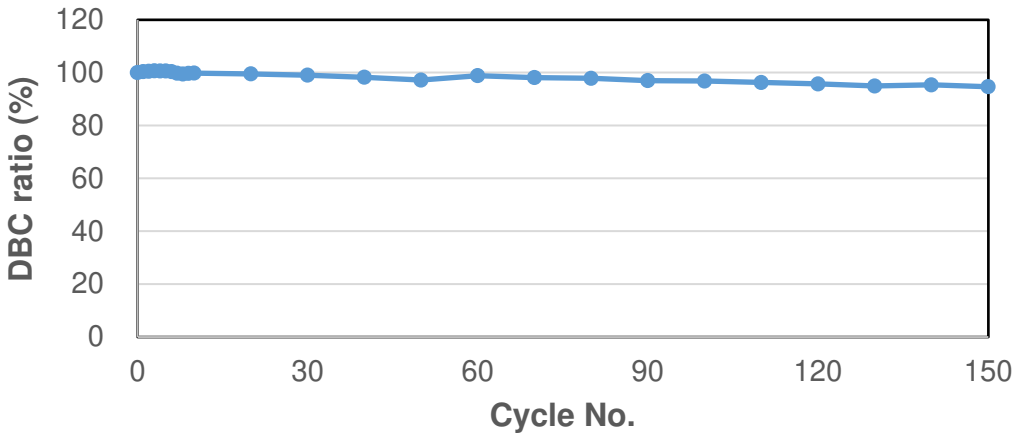


Figure 4 shows high retention (>95%) of DBC after 150 cycle 0.1M NaOH CIP. The capacity at cycle zero was 75 mg/mL and changed to 72 mg/mL at the end of the study.

CHO-HCP "carry-over" between cycles of re-use was also investigated by alternating mAb (from cell culture) and pAb capture and assessing the level of CHO-HCP in the pAb fraction by ELISA assay. Results are summarized in Table 3 below.

Table 3, Summary of CHO-HCP "carry over" between cycles of re-use

Sample	CHO-HCP (ppm)	% HCP Clearance by SPA-HC	% "Carry-over" to next cycle
Load	777,000		
mAb Elution Cycle 1	306	> 99	28.2
pAb Elution – NO CIP	86	-	
mAb Elution Cycle 2	238	> 99	5.5
pAb Elution – 0.1M CIP	13	-	
mAb Elution Cycle 3	260	> 99	3.2
pAb Elution – 0.5M CIP	8	-	

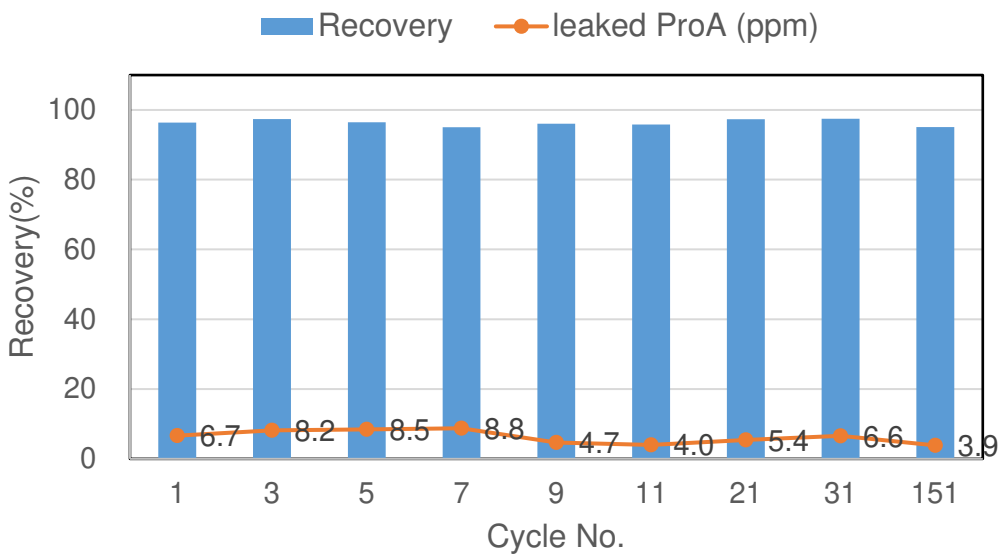
This study showed a high CHO HCP clearance over the three cycles of re-use. There was some "carry over" between cycles of re-use at very low levels which was further reduced by base CIP in-between each of cycle of re-use.

Base Stability and CIP – rProtein A Leachate after 150 cycle of 0.1 M NaOH CIP

A 1mL Cellufine SPA-HC mini-column was equilibrated in 0.02M Tris-HCl buffer, 0.15M NaCl pH 7.5 and loaded with 60 mg/mL polyclonal antibody (80% of 10% breakthrough DBC of 75 mg/mL at 6 min residence time) at a flow rate of 0.125 mL/min. The retained fraction was eluted with 60 mM Sodium Acetate pH 3.0. After a 100 mM wash with Acetic acid the column was subjected to cleaning in place with 0.1M NaOH contact for 15 min.

Recovery of DBC (> 95%) was also maintained over all 150 cycles CIP with 0.1 M NaOH and showed very low Protein A leachable levels (< 9 ppm). A summary of the results from this study are shown in Figure 5, below.

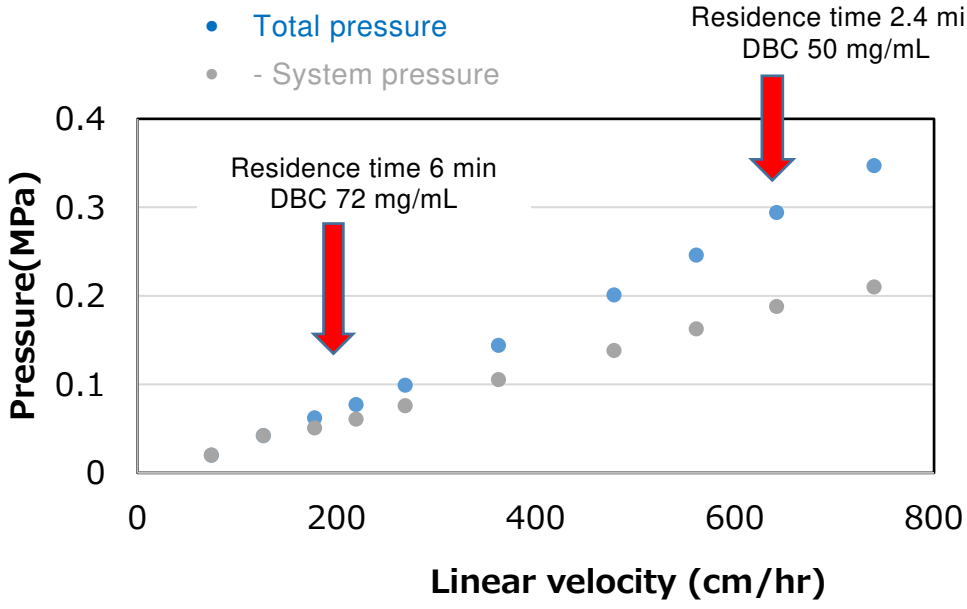
Fig.5, Summary of elution mAb recovery study and measurement of rProtein A leachate over 150 cycle of CIP and re-use.



Pressure/Flow properties in a pilot process column (1-2L)

As Cellufine SPA-HC is based on a highly cross-linked cellulose bead structure and is very adaptable for use in conventional manufacturing with large scale columns. Pressure-flow rate studies indicate that Cellufine SPA-HC developed back-pressure <0.3MPa at 650 cm/h in a 10cmID x 20 cmL bed height (1.57 L) column. A summary of the back-pressure/flow curves measured at a range of flow rates up to 750 cm/h is shown in Figure 6, below.

Figure 6, Pressure/Flow curve for SPA-HC packed pilot scale process column



Pressure/Flow test was carried out in a 10 cmID x 20 cmL column (Axichrom 10/300, 1.57L CV, GE healthcare) with a water mobile phase at 20-25 °C.

The above data suggests that Cellufine SPA HC resin packed in a pilot process column can be operated at a residence time of 2.4min at < 0.3MPa with a flow rate of 650 cm/h and achieve a mAb capture loading of 50 mg/mL from a clarified CHO cell sample.

Recommended Polishing Steps after rProtein A Capture

After Protein A capture the following contaminants need to be removed in the subsequent "polishing" steps; a) CHO host cell protein (HCP), b) leached Protein A ligand, c) residual dsDNA and d) mAb aggregates > 300 kDa molecular weight. In some cases, removal of mAb dimers is also required. Cellufine resins are available to remove these contaminants and are listed below.

Cellufine MAX IB, a new mixed mode primary amine based hydrophobic modified resin for flow through removal of CHO- HCP, leached Protein A and mAb aggregates.

- Adjust to pH 7.0 with Tris base,
- Tolerant of a wide conductivity range up to 0.2 M NaCl.

Cellufine MAX GS, a dextran coated cation exchange resin with high binding capacity for bind and elute removal of dimers and aggregated mAb's.

- Adjust to pH 4.5 with Tris base and lower conductivity < 5 mS/cm.

Cellufine MAX Q_h, a high capacity anion exchange resin that can be used to remove HCP, leached Protein A and residual dsDNA in a flow through format.

- Adjust to pH 8.5 with Tris base and conductivity ~12 mS/cm.

More details on these resins can be found on the web site at;
http://www.jnc-corp.co.jp/fine/en/cellufine/

Storage

Store unopened container at 2 – 8 °C. **Do not freeze**.
Adsorption capacity did not change with a month storage at an elevated temperature of 40 °C which corresponds to 6-month storage at room temperature (25oC) and 2.5-year storage at 10 °C.

Longer storage should be in 20 % (v/v) ethanol, at 2 – 8 °C. **Do not freeze.**

Ordering Information

Description	Quantity	Catalogue No.
Cellufine SPA-HC	1 x 1 mL (mini-column)	21900-11
	5 x 1 mL (mini-column)	21900-51
	1 x 5 mL (mini-column)	21900-15
	10 mL	21900
	50 mL	21901
	500 mL	21902
	5 L	21903
	10 L	21904

Purchase/Technical Support

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