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Comparison of the Rapid Clear® Cap 3000 vs GE Capsule Filters for clarifying monospecific IgG

Introduction

Two methods to clarify 10L of post transfected IgG harvested from culture are compared. The culture was harvested on day 4 with 75% viability and a yield of 3.44e6/mL VCD.

Equipment

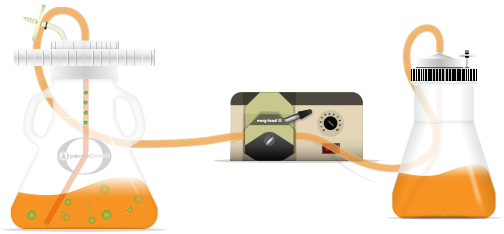
- Pump
- 5L Optimum Growth™ Flask
- Rapid Clear® Cap 3000
- Centrifuge
- ULTA Prime GF 5µm 10" Capsule Filter
- ULTA Prime CG 0.2 µm 5" Capsule Filter, p/n 12410247

Method

Rapid Clear® Cap Filtrations

10L culture was split in half and run through 2 Rapid Clear® Cap 3000's

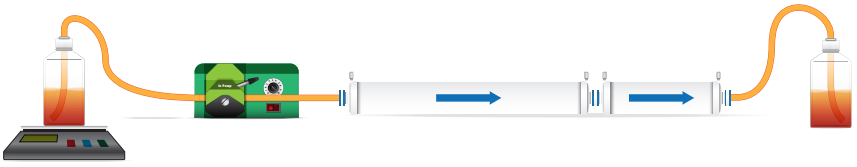
- Prime time: 50 S
- Max flow rate at beginning: 225 mL/min
- Filtrate recovered after 400mL PBS Wash: 4.2L
- Time to complete filtration: 25-30 min



GE Filtration ULTA Prime GF 5 µm 10" Capsule Filter and ULTA Prime CG 0.2µm 5" Capsule Filter

Two GE capsule filter columns are used in tandem. Culture is pumped through the columns and collected in a flask. ULTA Prime GF 5 µm 10" Capsule Filter and ULTA Prime CG 0.2 µm 5" Capsule Filter, p/n 12410247 was utilized.

- Prime time: 10 min
- Filtrate recovered after 400 mL PBS Wash: 5.7 L
- Time to complete filtration: 57 minutes



Purification & Analysis

All filtered material was analyzed by HPLC to determine quantity and purity. A 25mL Mab Select Sure proA capture column was used on an AKTA system. Two washes with buffer were performed prior to elution with pH 3.5 buffer to neutralize. Total recovery for both clarification systems was equivalent at 115mg/mL for the Rapid Clear® Cap and 100mg/L for the GE method for post column purification comparison:

- Inject 2µg of post proA material on TOSOH TSKgel SuperSW, 4.6 x 15 mm, 4µm
  - Take average of 3 runs for percentage POI
  - GE filter material: 83.56%*
  - Rapid Clear® filter material: 88.36%*

* Note: this IgG is known from prior work to have < 90% purity post proA

Results

Protein Yield Post Filtration & Final

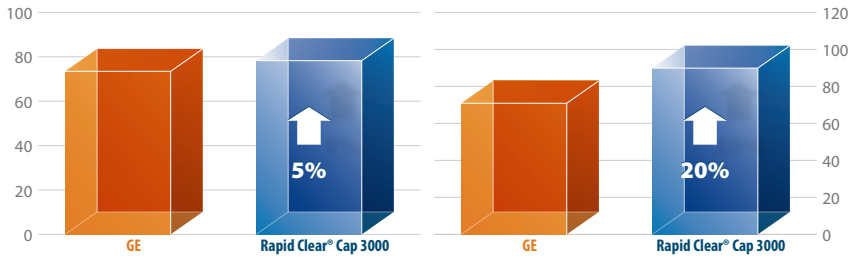


Fig 1. Protein Yield Post Filtration (left) Post Purification (right) - For data see table 1 below

HPLC comparison of post purified Rapid Clear® Cap and GE column was performed using a proA column purification was performed for both the Rapid Clear® System and the GE clarified solutions. The Rapid Clear® System yielded a slightly higher quantity of intact IgG, see fig 2, than the GE even though there was a lower volume of clarified culture, see table 1.

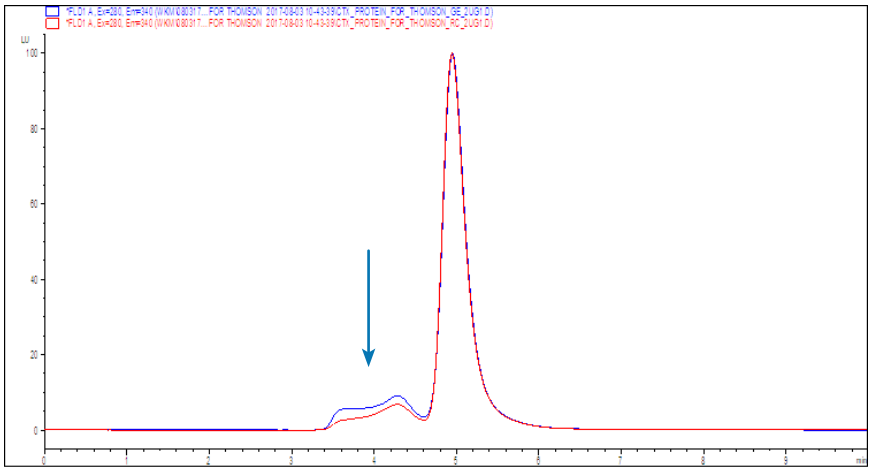
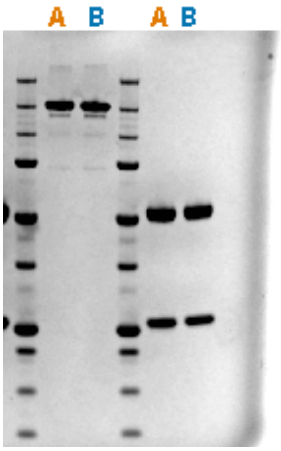


Fig. 2. HPLC comparison of the purified IgG. The Rapid Clear® Cap is the red line and GE the blue line.



- 1ug of post proA protein per lane
- Both Reduced and Non-reduced samples
- 4-12% Bis Tris Gel from Life Tech
- Run in MES buffer @ 200 V for 30 minutes
- Stained with Safe Stain
- Ladder is Precision Plus from Biorad

A: GE filtered material
B: Rapid Clear® Cap 3000 filtered material

Fig 2 Both GE and Rapid Clear® clarified IgG were run on an SDS Page gel for comparison: Lanes 1 & 4 are molecular weight standard ladder; Lanes 2&3 are non-reduced; lanes 5&6 are reduced.

Conclusion

Using the Thomson Rapid Clear® Cap 3000 was quick and easy. It needed less bench space and equipment increasing efficiency and minimizing waste. The Rapid Clear® Cap 3000 resulted in high quality Mab with higher yield per recovered volume over the GE, Table 1. The Rapid Clear Cap® 3000 had 20% higher recovery of intact protein than the GE capsule columns as calculated by % purity by HPLC divided by the total recovered volume.

Tabl 1. Comparison of the GE vs Rapid Clear® Cap for the clarification of IgG

	GE	Thomson
Volume Recovered	5.7 L	4.2 L
proA yield	575 mg	485 mg
Purity of proA material	0.835	0.886
Relative Yield per HPLC purity	474 mg	429 mg
Yield per recovered volume*	83 mg/L	102 mg/L
Time for clarification	75-80 min	25-30 min

* Yield on intact purified protein as determined by HPLC