

When producing biologics, cell yield, viability, and effective clarification are critical. Thomson's patented Optimum Growth™ Flask facilitates scalable mixing and high gas exchange rates to produce high density yields of viable cells. Now, to extend the Optimum Growth™ Flask product line

into downstream processing, Thomson has developed a revolutionary new technology to perform high speed clarification of cellular material. The Rapid Clear Cap® 3000 eliminates the multiple transfer steps involved in clarification and will filter between 2L-4L of high density

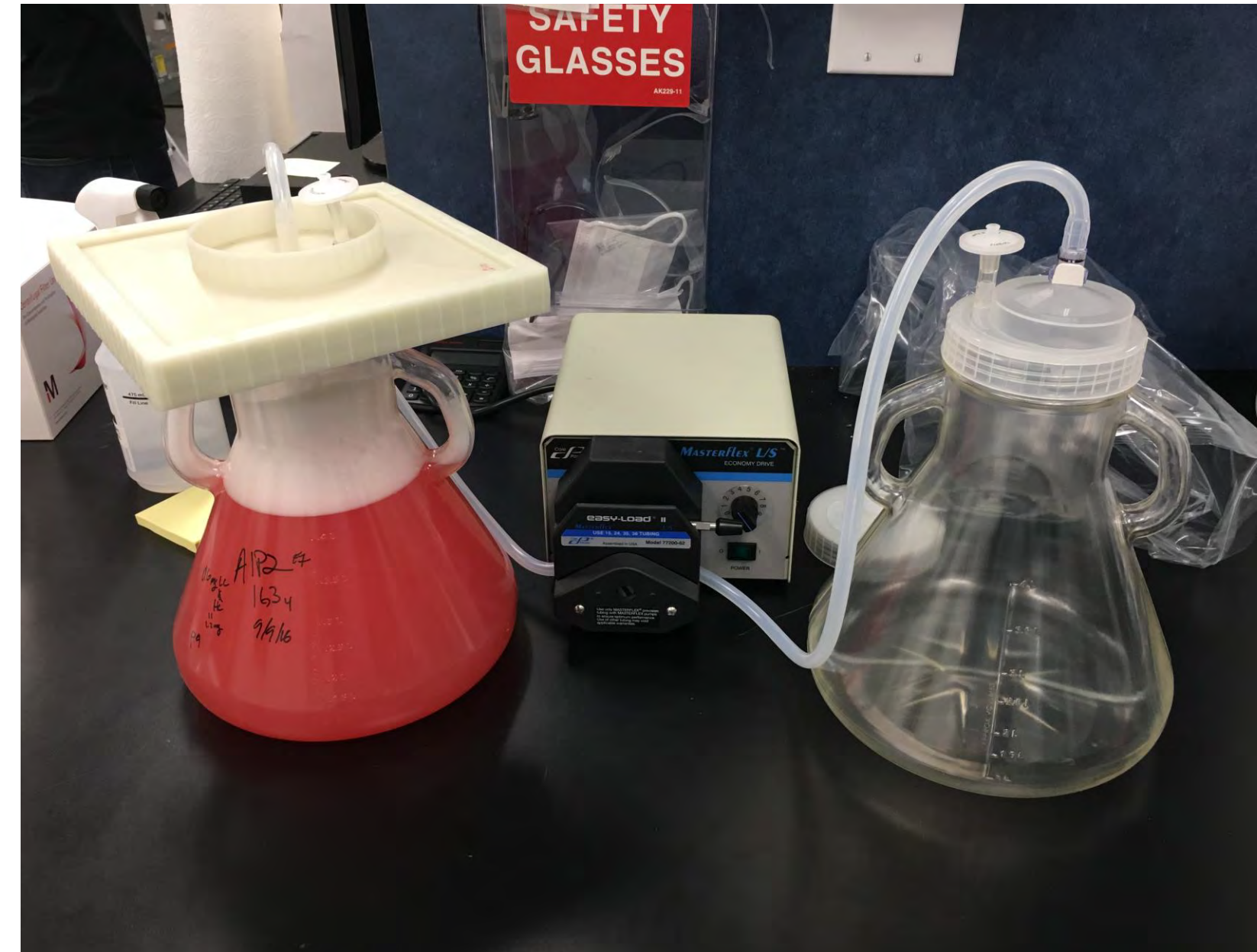
culture in under 35 minutes. This technique transforms the time consuming and laborious process of harvesting cells to a rapid, eco-friendly walk away procedure.

Method

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

Equipment

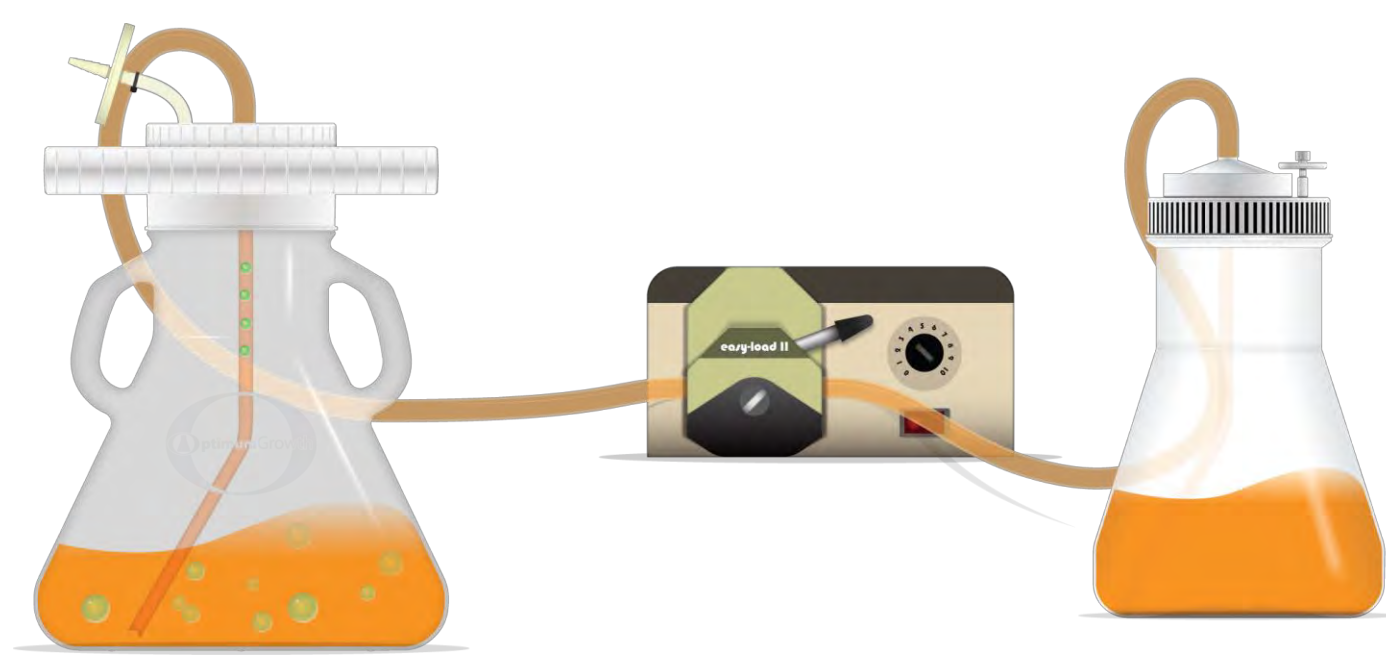
- Peristaltic Pump
- 5L Optimum Growth™ Flasks, 2x p/n 931116
- 2.8L Optimum Growth™ Flasks, 2x p/n 931114
- Rapid Clear® Cap 3000, 2x p/n 788116
- 800mL of Sterile PBS
- Centrifuge
- ULTA Prime GF 5µm 10" Capsule Filter
- ULTA Prime CG 0.2 µm 5" Capsule Filter, p/n 12410247



Rapid Clear® Cap Filtrations

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

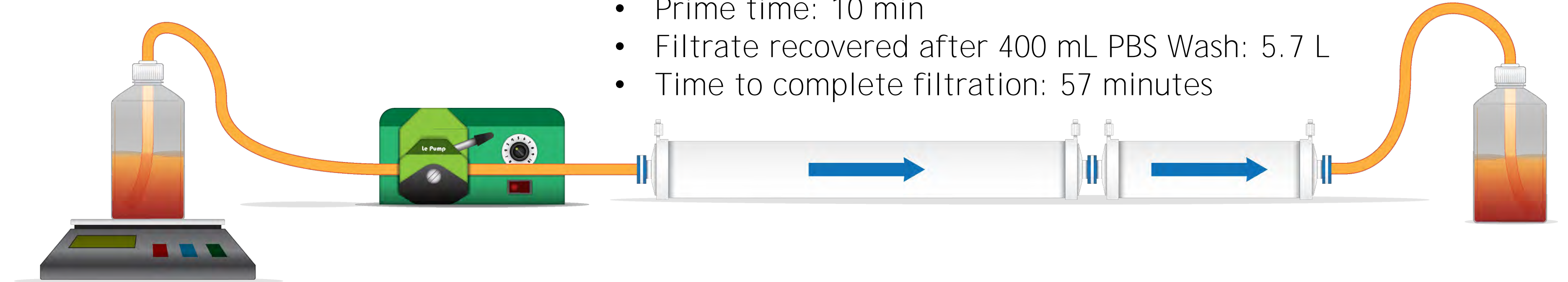
- Prime time: 50sec
- Max flow rate at beginning: 225 mL/min
- Filtrate recovered after 400mL PBS Wash: 4.2L
- Total Time to complete filtration: 25-30 min



ULTA Prime GF 5 µm 10" Capsule Filter & ULTA Prime CG 0.2µm 5" Capsule Filter

Two GE capsule filter columns were used in tandem. 10L of culture was 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 were utilized.

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



Purification

Clarified solutions from both the Rapid Clear® Cap 3000 and GE column method were purified using a 25mL MabSelect SuRe protein A capture column on an AKTA system. Two washes with buffer were performed prior to elution with pH 3.5 buffer to neutralize.

Results

Total recovery for both clarification systems was equivalent at 115mg/mL for the Rapid Clear® Cap 3000 and 100mg/L for the column purified 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

The purified solutions of from the Rapid Clear® Cap and GE were analyzed by both SEC and SDS Page for purity and total protein. The Rapid Clear® System yielded a slightly higher quantity of intact IgG, see fig 1 & 2, than the GE even though there was a lower volume of clarified culture, see table 1.

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.

- 1µg 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

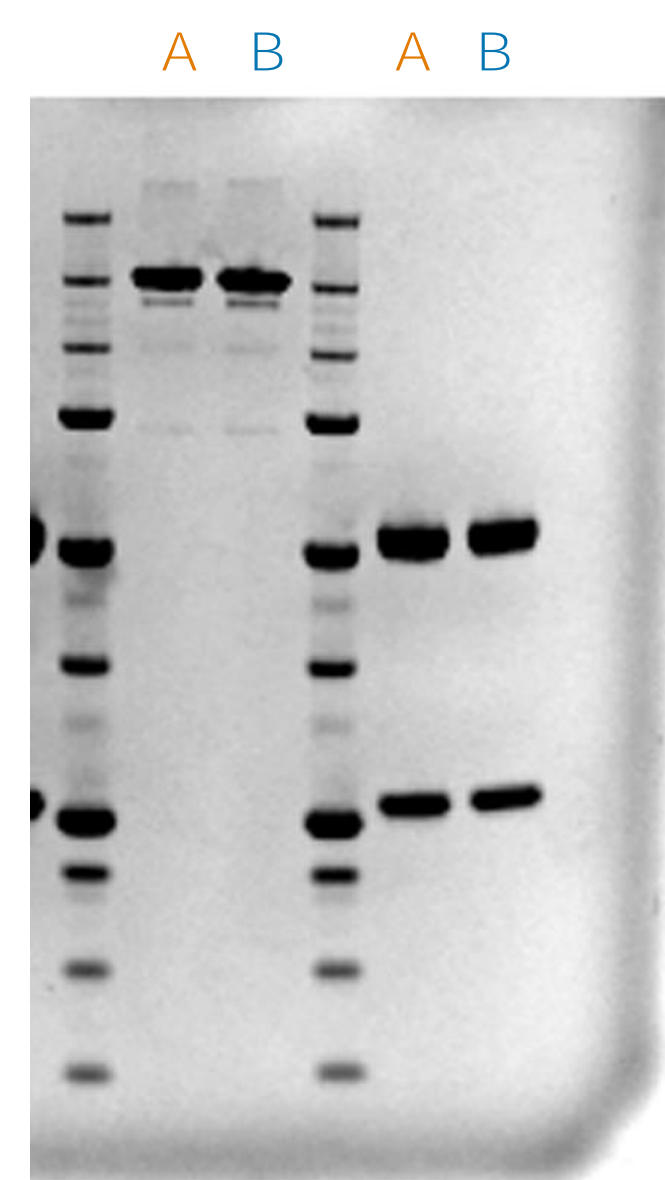


Fig 1. HPLC comparison of the purified IgG. The Rapid Clear® Cap 3000 is the red line and GE the blue line

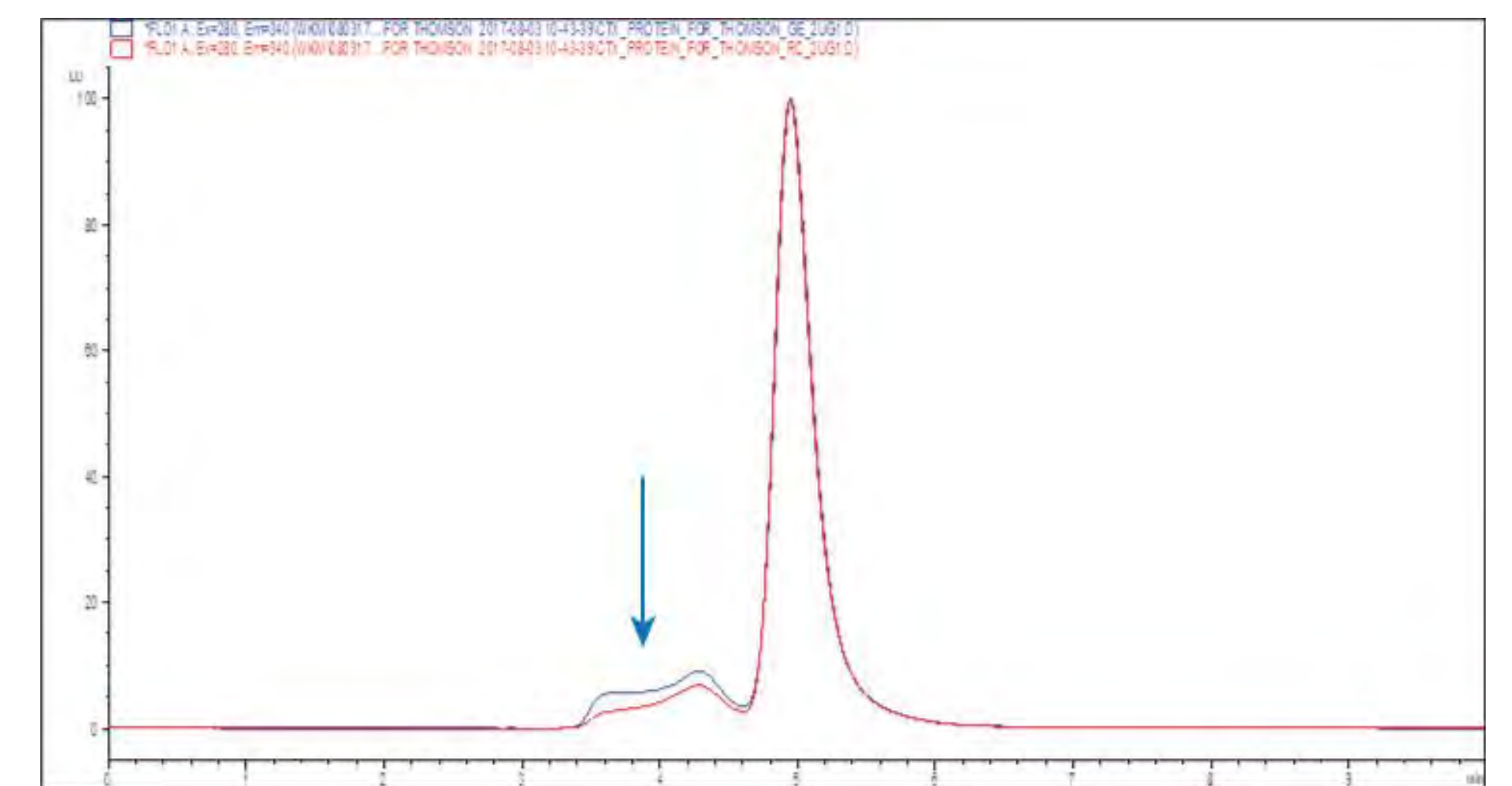


Table 1. Comparison of the GE vs Rapid Clear® Cap for the clarification of IgG

	GE	Thomson
Volume Recovered	5.7L	4.2L
proA yield	575mg	485mg
Purity of proA material	0.835	0.886
Relative Yield per HPLC purity	474mg	429mg
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

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.

Protein Yield Post Filtration & Final

