A rapid murine IgG productivity screen based on prepacked Protein A columns.

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Abstract

The ability to produce milligram quantities of purified rIgG from transient mammalian expressions of multiple rIgG constructs is an important component of the selection process for antibody development. In our facility, screens of constructs containing variations in the amino acid sequence of the IgG variable regions typically contain 12-24 candidates and the production of 5-10 milligrams of purified protein from each construct supports the analytical processes required to select the candidates that will move forward in the development process. The performance of the 1 mL prepacked Protein A columns used in the purification of these antibodies is a critical factor in the process. In this study we compared the performance of three pre-packed Protein A columns with respect to binding capacity, elution profile, purity and throughput using a protocol using two flow rates (3 ml per min. and 5 mL per min. The columns SUPrA Cartridge, (BioRad), MabSelect Sure, (GE) and Protein A Capture Device , (Gore) were used to perform parallel purifications on aliquots of serum-free culture supernatant from 10 individual murine rIgG constructs and a single stable recombinant CHO cell line. Transient expression was performed using HEK-293 cells co-transfected with heavy and light chain plasmids complexed with PEI. The culture supernatants were collected 120 hours post-transfection, filter sterilized and purified in parallel. Comparisons of the performance of the three Protein A capture systems will be presented.

Materials and Methods

Cell Line: HEK-293
Plasmid: pcDNA3.1
Transfection Reagent: 1 mg/mL PEI Max, Polysciences, Inc.
Media Formulation: Expi293, Thermo Fisher
Additive: 10 mM Sodium Butyrate, Sigma
PEI:DNA Ratio: 4:1
HC to LC Ratio: 1:1
Total Plasmid Concentration: 1 mg/L
Culture Vessel: 3L Non-Baffled Low-Profile Fernbach Flask, CellTreat
Temperature: 37°C
Atmosphere: 95% Air: 5% CO_2
Agitation: 100-125 rpm
Purification System: Bio-Rad Biologic LP
Protein A Columns:

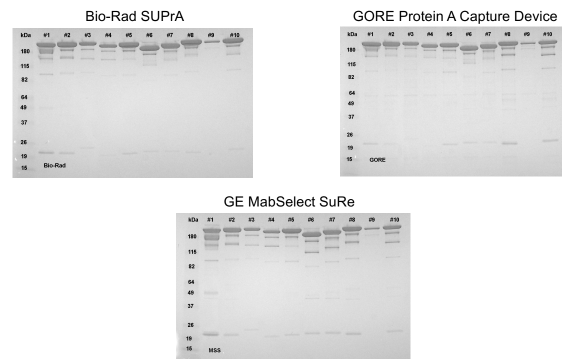
SUPrA , Bio-Rad 64200664
MabSelect SuRe, GE 29-0491-011
GORE Protein Capture Device with Protein A, Gore PROA101

Heavy and light chain plasmids for each of the ten murine rIgG constructs were amplified and purified using DH5-alpha cells cultivated in Plasmid Plus medium (Thomson Instrument Company) and Nucleobond endotoxin-free kits (Macherey-Nagel). The HEK-293 cells were split to 8x10e5 cells per mL 24 hours prior to transfection. The PEI/DNA complex was prepared by adding 1 mg of total plasmid (0.5 mg HC and 0.5 mg LC) diluted to 40 mL in DI water. Four milliliters of PEI Max solution (1 mg/mL) was added to the diluted plasmid and the solution was mixed by inversion for 20 seconds and allowed to incubate at room temperature for 5 minutes. The PEI/DNA complex was added to a 1 liter culture of cells and the culture was returned to the shaker-incubator. A concentrated stock of sodium butyrate in DI water was added 16-24 hours post-transfection to yield a final concentration of 10 mM. The cultures were harvested 120 hours post-transfection using centrifugation and the supernatant was filter sterilized and held at 4°C prior to purification. For the purification, 25 mL of culture supernatant was loaded onto the selected 1 mL Protein A column (equilibrated with PBS pH 7.2) at a flow rate of 3 mL per min. The columns were washed with 10 column volumes of PBS, eluted using 5 mL of 0.1 M Glycine pH 2.5 and neutralized with 0.2 mL 1M Tris pH 9. The flow rates for the wash and elution steps was 3 mL per min. For the comparison of binding capacity 100 mL of supernatant from a stable recombinant CHO cell line with a murine IgG titer of 495 mg/L was loaded onto the three columns at two flow rates (3 and 5 mL per min.) giving a total of six purificartion runs. The columns were washed with 20 column volumes of PBS, eluted using 10 mL of 0.1 M Glycine pH 2.5 and neutralized with 0.5 mL 1M Tris pH 9. The flow rates for the wash and elution steps were the same as the flow rate for the load (3 and 5 mL/min. respectively). The concentration of IgG was determined in each sample using UV absorbance at A_{280} and an extinction coefficient of 1.4. SDS PAGE analysis was performed on each elution fraction using 4-20% Tris Glycine gels (Thermo Fisher) run under non-reduced conditions and stained with Coomassie-Blue.

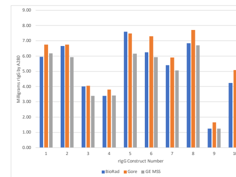
Results

rIgG Productivity Screen: 10 Constructs

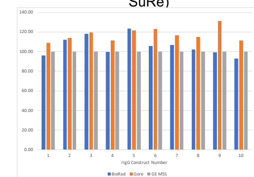
Coomassie-Stained SDS PAGE



Total IgG Recovered (mg)

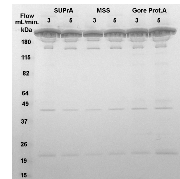


Total IgG Recovered % of Control (MabSelect SuRe)



Binding Capacity: Stable CHO Construct (495 mg/L)

Coomassie-Stained SDS PAGE



Total IgG Recovered and Predicted Purified Yield

	Total mg IgG Eluted	Predicted Purified Yield (mg/L)
SUPrA 3 mL/min.	19.70	197
SUPrA 5 mL/min.	15.48	155
MabSelect Sure 3 mL/min.	10.71	107
MabSelect Sure 5 mL/min.	15.29	153
Gore Protein A Device 3 mL/min.	17.60	176
Gore Protein A Device 5 mL/min.	20.30	203
100L Purification (Actual Yield)		250

Conclusions

1. In the productivity screen all three columns performed in a similar manner under the conditions tested.
2. The GORE Protein A Capture Device and the Bio-Rad SUPrA column captured approximately 10-20% more IgG than MabSelect Sure under the conditions used.
3. The purity of the IgG in the eluate pools was higher in the material processed over the GORE Protein A Capture Device than the other two columns.
4. The total cycle time for the IgG purification screen was 20 minutes with a 25 mL load.
5. The binding capacity screen demonstrated that, under the conditions used, the Gore Protein A device loaded at 5 mL per min. and the SUPrA protein A column loaded at 3 mL per min. generated the most accurate prediction of the actual purified yield (81% and 79% of the actual yield respectively).
6. Total assay time at the 3 mL per min. flowrate was 45 minutes
7. Total assay time at the 5 mL per min. flowrate was 30 minutes
8. The ability to accurately predict the purified yield of IgG increases the accuracy of material estimates and limits overproduction or underproduction of the protein of interest and their associated effects on the cost of production.

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