Webinar Summary

Optimize your approach to mAb development

Authors

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As demand for monoclonal antibodies (mAbs) and their variants, e.g., bispecific antibodies and antibody drug conjugates, continues to grow, drug developers are looking to ensure robust and efficient process development approaches. Some of the critical areas of mAb process development include stable CHO cell line creation, optimized media and feed selection, and rapid downstream process development using resin high-throughput screening.

En route to manufacturing success, it is important to implement optimization to create efficiencies wherever possible. Optimization includes selecting CHO host cells strategically; identifying the media and feed, which will offer maximum growth and productivity while maintaining desired protein quality; and leveraging innovative resin high-throughput screening techniques for streamlined purification.

Prioritize stable CHO cell line development

In cell line development (CLD), drug developers are seeking high titers, short timelines, a host cell line that is regulatorily compliant, and low licensing costs. At Thermo Fisher Scientific, we offer Gibco™ Freedom™ Cell Line Development Kits, which achieve immunoglobulin G (IgG) titers of 3-6 g/L. With these kits, customers can expect to go from transfection to identifying top-producing clonal cell lines within four to five months. Each of the CLD kits comes complete with a cGMP-banked cell line in an animal origin-free, chemically defined medium, which eases the downstream burden for customers as they approach clinical trials and commercial production.

Figure 1 provides a comparison of the three different Freedom CLD kits available to Thermo Fisher Scientific customers.

The primary difference between CHO-DG44 and CHO-S is that DG44 cells undergo genetic amplification of the gene of interest following treatment with methotrexate (MTX) because of their deficiency in the dihydrofolate reductase (DHFR) gene. DHFR is provided exogenously with the pOpticVEC vector. Due to this amplification step, the timeline for generating stable clones with the CHO-DG44 kit is 36 weeks; the timeline for generating stable clones with the CHO-S kits is approximately 24 weeks. Regardless of the kit selected, you will gain access to a complete, step-by-step manual, technical support, and commercial licensing.

Optimize your CLD workflow

It is vital for your team to understand and optimize the steps of a traditional CLD process; Figure 2 demonstrates the workflow leveraged for the Freedom ExpiCHO-S kit.

The first step of CLD is vector construction; customers can leverage our proprietary GeneArt technology platforms for optimization of gene synthesis and vector construction. Vector construction is followed by transfection and then the first phase of selection. Once cells have recovered from phase one selection, phase two selection is applied and performed at higher concentrations. This approach can help selecting cells with higher protein expression for phase two stable pools. Following cell recovery from the second selection phase, single cell cloning is performed using the customer's method of choice.

Feature	Gibco™ Freedom™ CHO-S Kit	Gibco™ Freedom™ DG44 Kit	Gibco™ Freedom™ ExpiCHO-S Kit
Parental cell line	cGMP CHO-S™ cells	cGMP CHO-DG44 cells	cGMP ExpiCHO-S™ cells
Titer			
Medium	CD FortiCHO™	CD DG44 Medium and CD OptiCHO™ Medium	ExpiCHO™ Expression Medium and ExpiCHO™ Stable Production Medium
Growth rate	<24 hours	>30 hours	<24 hours
Vector	Freedom™ pCHO 1.0	pcDNA 3.3-TOPO™ and pOptiVEC™-TOPO™	Freedom™ pCHOm 3.1 and Freedom™ pCHOp 3.2
Primary application	mAb (IgG)	mAb and recombinant protein	mAb and recombinant protein
Amplification (with Methotrexate)	No	Yes	No
Timeline – transfection to stable clone	~24 weeks	~36 weeks	~24 to ~36 weeks
Complete manual	Yes	Yes	Yes
Support	Yes	Yes	Yes
Commercial licensing	Yes	Yes	Yes

Figure 1. Comparison of Freedom CLD kits

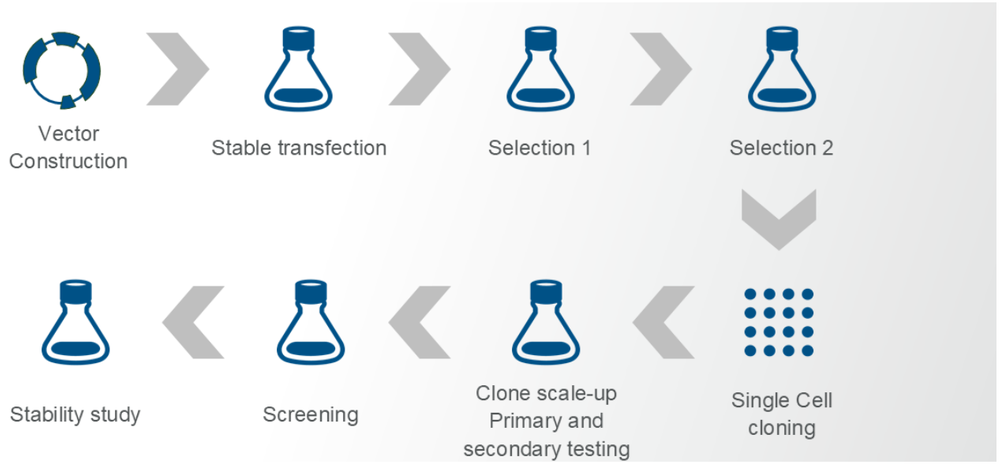


Figure 2. CLD workflow for ExpiCHO-S

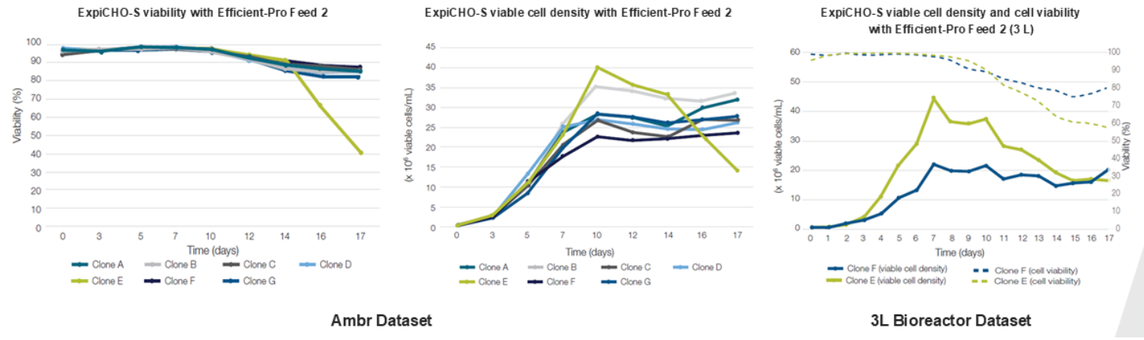


Figure 3. ExpiCHO-S clones provide viability and viable cell density

After clonal outgrowth occurs in 96-well plates, the primary screen is performed to identify the top producing clones for subsequent scale-up and secondary screening. After the top clones are scaled up, they are subjected to a final screening using the customer's method of choice. This is performed prior to the stability assessment. This CLD workflow takes approximately 24 weeks from transfection through cloning.

Figure 3 shows a data set depicting cell viability and viable cell density (VCD) for ExpiCHO-S clones assayed in both the Ambr®15 Cell Culture Bioreactor System (Sartorius) and 3L bioreactors; the data shown in the left and middle graphs were generated from a 17-day growth performance assay (GPA) using Ambr®15. Six out of seven clones maintained viabilities greater than 80% through day 17, and all clones remained above 85% viability through day 14. As shown in the middle graph, most clones sustained high VCDs throughout the duration of the GPA. Peak volumetric titers ranged from 4.5-5.5 g/L.

Following the Ambr®15 screens, clones E and F were scaled up to a 3L bioreactor, where they demonstrated similar viability in VCD profiles compared to the Ambr®15 experiment. Clones E and F reached peak volumetric titers of 3.6 g/L and 4.5 g/L respectively. This data demonstrates that clonal cell lines generated using the Freedom ExpiCHO-S kit are scalable between different bioreactors.

Recognize the importance of formulation diversity

CHO media and feed panel libraries offer a diverse array of formulations to screen across a variety of CHO clones or cell lines. The panel libraries are a great option for customers that have tested catalog media and feed but are looking for further improvement in cell growth, titer, and/or product quality. Customers can quickly screen with the panels, and if optimizations are identified, the formulations are ready to ship, available in different formats, and scalable to cGMP. Additionally, we provide customers with a sample study protocol to follow, field application scientist (FAS) consultation, evaluation support from technical teams, and next step recommendations.

Formulation diversity is critical for identifying a final medium that enables better production and higher titers. By evaluating a panel, you test a diverse set of formulations that offers a wide design space to capture the unique metabolic requirements of your clone(s). This enables you to identify an optimal formulation, which can ultimately reduce your timeline and cost of goods.

Figure 4 demonstrates heat maps of both the CHO media panel and the CHO feed panel. For both panels, you can see how rich or lean a particular formulation is relative to the other formulations within that panel to align with your clone's nutritional requirements.

To further demonstrate the importance of formulation diversity, consider data from an evaluation of the Gibco CHO media panel (Figure 5). Our team used a CHO-K1 cell line and a CHO-S cell line, each expressing a different IgG1 antibody to evaluate the media panel. Each cell line was thawed and recovered in its respective control growth medium for a minimum of three passages prior to adaptation into panel media 1-6, 9A, and 13-14. The cells then underwent another minimum of three passages in the panel media prior to inoculation of the Ambr®15. Adaptation was considered successful when the doubling time was consistent over three passages. For each cell line, a simple fed-batch (SFB) mode, 14-day Ambr®15 GPA was conducted in duplicate conditions to assess cell performance with the nine CHO media panel formulations and one control medium condition. The Ambr®15 was seeded at 0.3 x 106 viable cells/mL, and glucose was fed up to 6 g/L when culture levels fell below 3 g/L.

Gibco™ CHO Media Panel

Components	Medium #1	Medium #2	Medium #3	Medium #4	Medium #5	Medium #6	Medium #9A	Medium #13	Medium #14
AMINO ACIDS									
VITAMINS									
LIPIDS									
TRACE METALS									
AMINES									
NUCLEOSIDES									
ORGANIC ACIDS									

high
low

Gibco™ CHO Feed Panel

Components	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Amino acids					
Vitamins					
Lipids					
Trace metals					
Amines					
Nucleosides					
Organic acids					

high
low

Figure 4. Gibco Media and Feed Panel diversity

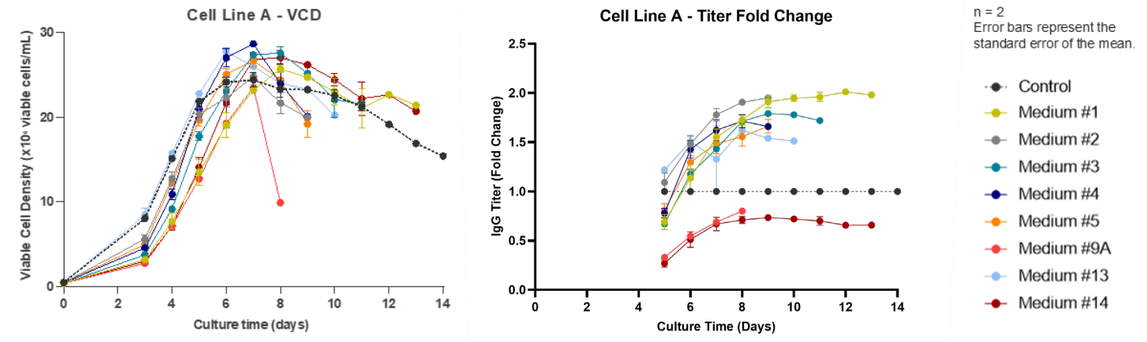


Figure 5. Gibco CHO Media Panel Evaluation: Cell Line A

Cell Line A demonstrated a wide spread with both VCD and titer fold change among the different conditions tested. In the left graph of Figure 5, peak VCD reached approximately 26 x 106 viable cells/mL at around day 7 for most conditions. The titer was normalized to the control medium condition, and Medium #1 was the lead performer, with a 2x increase in titer over the control.

Figure 6 demonstrates the distinct differences reflected in the Cell Line B data set.

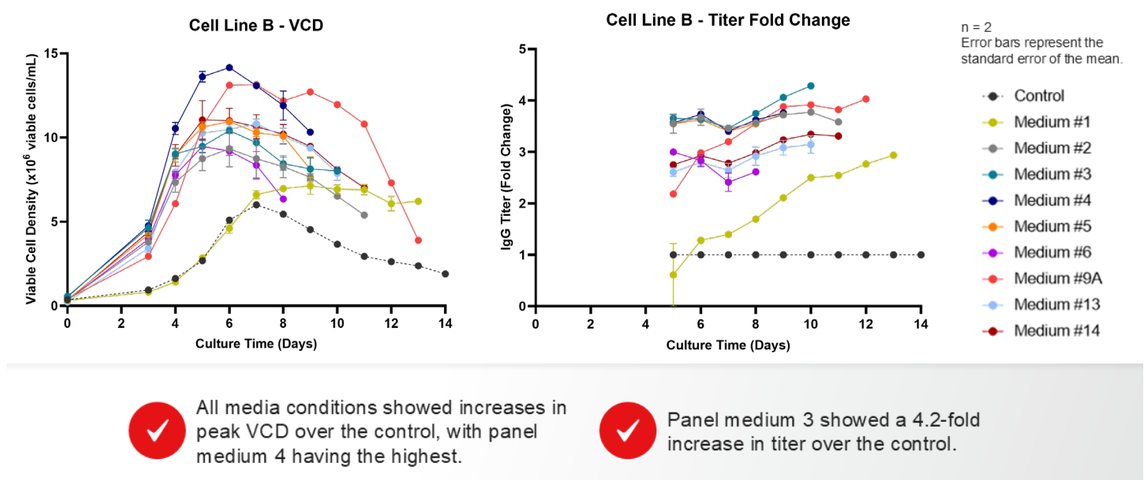


Figure 6. Gibco CHO Media Panel Evaluation: Cell Line B

In terms of VCD for Cell Line B, most conditions reached > 10 x 106 viable cells/mL at around day 6. For titer, Medium #3 showed the highest increase in titer over the control with a 4.2x increase. Overall, with a diverse set of formulations tested, titer performance varied based on the formulation used.

The titer and specific productivity (qp) heat maps in Figure 7 reflect the importance of panel diversity across both cell lines A and B.

On the left of Figure 7, normalized volumetric titers for both cell lines are shown; normalized specific productivity for the two cell lines are on the right. Overall, there was a wide range of productivity. As indicated by the arrows, a medium formulation that is optimal for one cell line may not be optimal for another.

Following a media panel evaluation, a customer might proceed with testing the feed panel to determine the optimal medium and feed combination. Once an optimal combination is chosen, the formulation can be scaled up to Gibco™ Rapid Prototyping (GRP) or to our cGMP manufacturing facility. If further improvements are desired, spent media analysis can be performed to determine an appropriate feed strategy or to modify the panel formulation through the GRP facility.

Titer at harvest day (fold increase)		
Panel medium	**Cell line A**	**Cell line B**
Control	1.0	
Medium #1	2.0	2.8
Medium #2	1.9	3.6
Medium #3	1.8	4.2
Medium #4	1.7	3.8
Medium #5	1.7	3.7
Medium #6	NA*	2.4
Medium #9A	0.8	4.0
Medium #13	1.6	3.1
Medium #14	0.7	3.3

Diversity observed

q^P (fold increase) day 6 through harvest day		
Medium	**Cell line A**	**Cell line B**
Control	1.0	
Medium #1	5.7	3.4
Medium #2	6.1	2.5
Medium #3	5.0	3.0
Medium #4	5.8	1.9
Medium #5	5.1	2.4
Medium #6	NA*	1.6
Medium #9A	2.9	2.8
Medium #13	3.0	2.0
Medium #14	1.9	2.1

Diversity observed

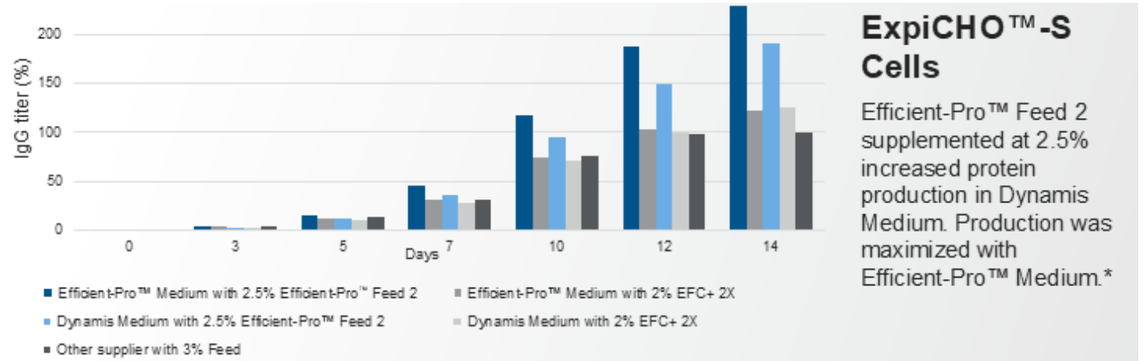
High level

Low level

* For cell line A, panel medium 6 was excluded due to limited conditions available in the ambr15 bioreactor.

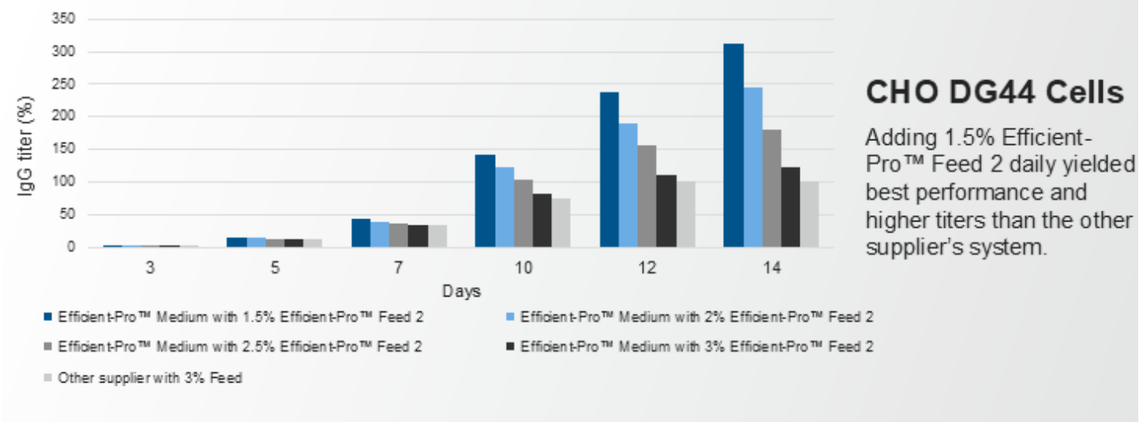
As indicated by the arrows, a medium formulation that is optimal for one cell line may not be optimal for another.

Figure 7. Heat maps for Cell Line A and B



ExpiCHO™-S Cells

Efficient-Pro™ Feed 2 supplemented at 2.5% increased protein production in Dynamis Medium. Production was maximized with Efficient-Pro™ Medium.*



CHO DG44 Cells

Adding 1.5% Efficient-Pro™ Feed 2 daily yielded best performance and higher titers than the other supplier's system.

Figure 8. Performance of ExpiCHO-S and CHO DG44 cells with Efficient-Pro medium and feeds

Leverage the benefits of the Gibco™ Efficient-Pro™ Medium and Feeds system

The Efficient-Pro system is a specific catalog medium and feed combination designed with versatility, performance, and scalability in mind. To enable process optimization and scalability, these formulations are nutrient rich to support higher cell density and titer. To demonstrate the benefits of the Efficient-Pro system, we evaluated its performance with ExpiCHO-S and CHO DG44 cells (Figure 8).

In the first experiment, protein production was evaluated in different media and feed combinations over 14 days. The combinations were Efficient-Pro Medium with 2.5% Efficient-Pro Feed 2, Efficient-Pro Medium with 2% EFC+ 2X, Gibco™ Dynamis™ Medium with 2.5% Efficient-Pro Feed 2, Dynamis Medium with 2% EFC+ 2X, and another supplier with 3% feed. Efficient-Pro Feed 2 at 2.5% demonstrated increased protein production in combination with Dynamis Medium and maximized production with the complementary Efficient-Pro Medium.

The bottom graph of Figure 8 shows the data from a feed titration with DG44 cells in an Ambr® 15. Bioreactor vessels were set up in triplicates to test Efficient-Pro Medium and Feed 2 for IgG production over 14 days. Efficient-Pro Feed 2 was tested at 1.5% to 3% compared to a commercially available medium and feed process. Overall, when Efficient-Pro Feed 2 was supplemented at 1.5% daily, it achieved 3x the titer compared to the other supplier's medium and feed.

In a scale-up study conducted at the Center for Biochemical Engineering and Cell Cultivation Technique at the Zurich University of Applied Sciences, Efficient-Pro Medium and Feed 1 were evaluated for scalability in 16-day fed-batch cultures with a CHO-K1 cell line. The Ambr® 250, Thermo Fisher Scientific 3L HyPerforma™ Bioreactor, and the Thermo Fisher Scientific DynaDrive™ Bioreactor were used for scale-up. We evaluated VCD, percent viability, and titer profiles and found very comparable results among the different bioreactor scales tested (Figure 9). Efficient-Pro Medium and Feed were shown to support consistent CHO-K1 cell growth and productivity.

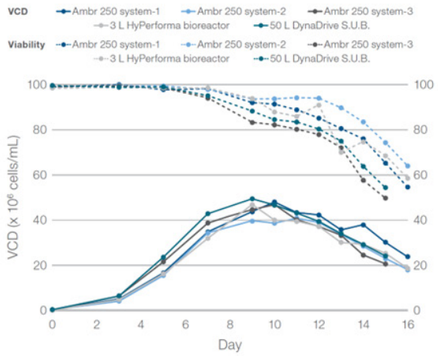


Figure 1. Cell growth and viability. VCD = viable cell density.

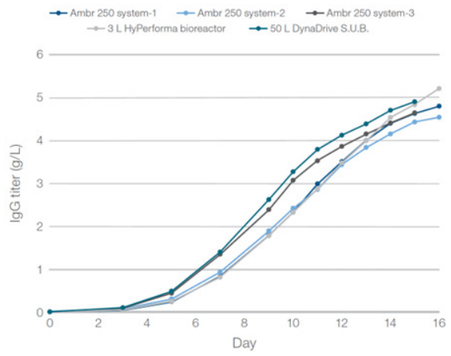


Figure 2. IgG titers.

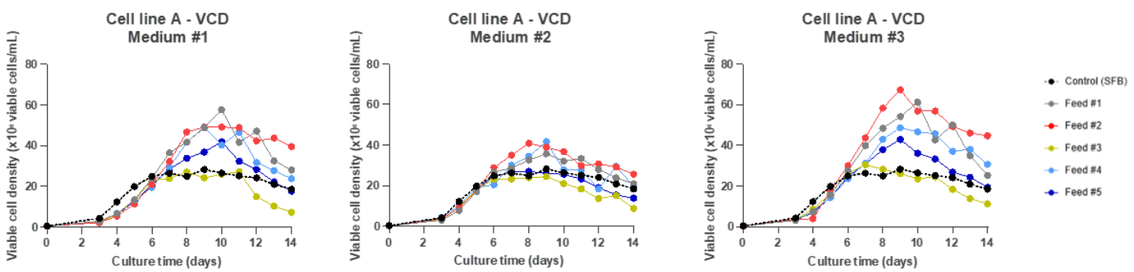
Efficient-Pro Medium and Feed 1 supported CHO-K1 scale-up from the Ambr 250 to the 50-liter DynaDrive S.U.B with strong growth profiles and peak VCDs ranging from 41 to 49 x 10^6 cells/mL by day 9 to 11 (Figure 1)

During scale-up, protein titers were maintained around 5g/L. (Figure 2)

Figure 9. Scale-up study results for CHO-K1 growth in Efficient-Pro medium and feeds

Consider the media and feed panel evaluation

The next study leveraged the same CHO-S and CHO-K1 cell lines to evaluate the nine different CHO media panel formulations in combination with five CHO feed panel formulations. Each cell line was thawed and recovered in its corresponding control growth medium and then adapted to the nine panel media for a minimum of three passages prior to the inoculation of the Ambr®15. For each cell line, a 14-day Ambr®15 GPA was conducted in fed-batch mode in duplicate to assess cell performance with the nine CHO media panel formulations and five CHO feed panel formulations along with one SFB mode control condition. The Ambr®15 was seeded at 0.3 x 106 vc/mL, each feed was fed at 3% daily on day 3 through day 13, and glucose was fed up to 6 g/L when culture levels fell below 3 g/L.



Medium 3 fed with feed 2 showed the highest peak VCD compared to other conditions.

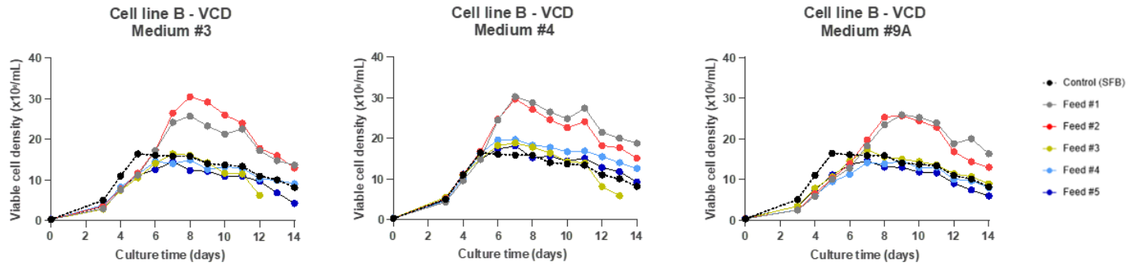
Figure 10. Gibco CHO Media and Feed Panel evaluation: VCD for Cell Line A

Starting with Cell Line A, the three lead media panel formulations were chosen to be Medium #1, #2, and #3 and are presented in Figure 10 in combination with all five feeds.

With a diverse set of formulations tested, cell growth performance varied based on the formulation used. Medium #3 conditions displayed higher peak VCDs than Medium #1 and #2. For Cell Line B, the three lead media panel formulations were Medium #3, #4, and #9A, which are shown in Figure 11 in combination with all five feeds. Panel feed #1 and #2, shown in gray and red, had overall higher peak VCDs compared to feed panel #3, #4, and #5 conditions.

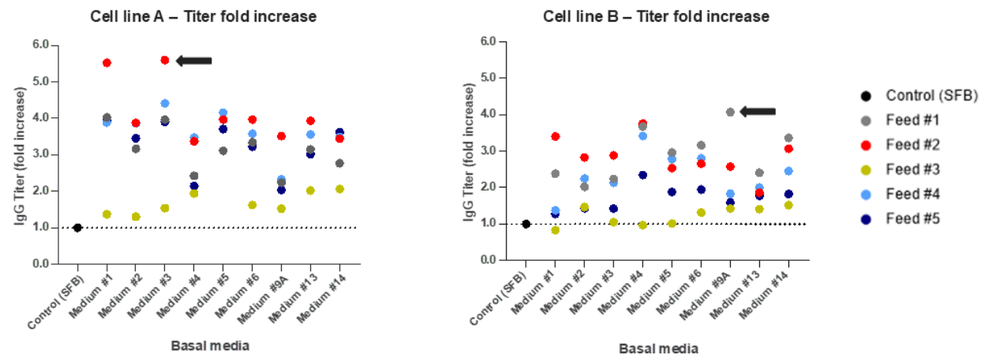
Figure 12 demonstrates the complete data set for normalized harvest titer for cell line A and cell line B with the nine media and five feeds, reflecting a wide range of productivity for both cell lines.

For cell line A, the highest productivity improvement, which was about 5.6x, was observed with Medium #3 fed with Feed #2. For cell line B, the highest fold improvement was seen with Medium #9A paired with Feed #1. These results demonstrate the importance of formulation diversity when screening a panel to identify the optimal medium and feed combination for a cell line.



Panel feeds 1 and 2 had overall higher peak VCD than feeds 3–5.

Figure 11. Gibco CHO Media and Feed Panel evaluation: VCD for Cell Line B



As indicated by the arrows, the greatest improvements in productivity for cell lines A and B were observed with medium 3 with feed 2 and medium 9A with feed 1, respectively.

Figure 12. Titer fold increase for Cell Lines A and B

Strategic downstream process development

Purification process development can be accelerated via high-throughput screening techniques and Thermo Fisher Scientific products. A typical downstream purification process for mAbs starts with cell culture clarification followed by capture affinity chromatography, viral inactivation, polishing chromatography, virus filtration, and formulation. Our CaptureSelect™ and POROS™ resin technologies aim to address challenges in the purification workflow and include the following:

- **CaptureSelect and POROS affinity resins:** high-performance affinity resins to support mAb therapeutics, viral vectors, mRNA, proteins, and vaccines. mAb-related options include MabCaptureC, CaptureSelect CH1-XL, CaptureSelect KappaXP, CaptureSelect LambdaXP, and CaptureSelect FcXP.
- **POROS ion exchange resins:** these options offer a performance independent of flow rate, high capacity, and salt tolerance. POROS offers four different ion exchange resins: two weak exchangers (D50 and PI50) and two strong exchangers (HQ50 and XQ).
- **POROS hydrophobic interaction resins:** these resins offer selectivity through a wide range of hydrophobic conditions. There are three hydrophobic resins available: POROS Ethyl, which works in bind/elute mode of moderately hydrophobic molecules; POROS Benzyl, which works in bind/elute mode or flow-through mode depending on the hydrophobicity of the molecule; and POROS Benzyl Ultra, which works in flow-through mode in lower salt concentration.
- **POROS Caprylate Mixed Mode Cation Exchange Chromatography Resin:** this resin offers high aggregate selectivity, increased productivity, and high scalability through flow-through application.

Implement high-throughput screening

High-throughput screening methods allow scientists to generate massive amounts of data through miniaturization and parallelization of experiments, enabling better process understanding, reduced time waste, and risk mitigation. Typical chromatography process development starts with high-throughput screening with miniaturized systems that allow for experimentation with multiple variables at the same time. At the next stage, you move to small bench-scale columns where you can fine-tune and optimize parameters. Finally, validation and scale-up evaluation are conducted on larger scale columns.

Resin slurry plates and miniature columns are two common approaches to high-throughput screening. Thermo Fisher Scientific offers the GoPure™ 96-well screening plates as well as RoboColumns™ for screening in miniature columns. Resin slurry plates allow for more conditions to be run in parallel with lower capital investment and a broader window of operation. They can be easier to operate and require less material. Plates are also more flexible in their setup; they can either be self-made or purchased prefilled with the resin of choice. A typical workflow for plate resin screening can be found in Figure 13. All process parameters relevant for DSP can be screened at the plates, and some examples are summarized in Figure 14.

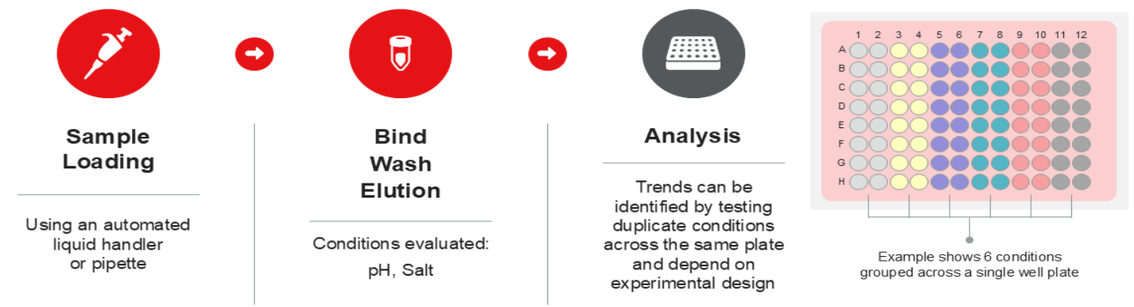


Figure 13. GoPure 96-well screening plates experimental setup

Experimental objective	Parameters to investigate
Determination of static binding capacity	• Sample concentration • Buffer composition (conductivity, pH, additives, etc.) • Incubation time
Screening of intermediate wash step(s)	• Sample concentration • Buffer composition (conductivity, pH, additives, etc.)
Screening of elution conditions	• Buffer composition (conductivity, pH, additives, etc.)
Screening of cleaning regime	• Buffer composition (alkaline/acid solutions, chaotropes, etc.)

Figure 14. Process parameters that can be evaluated in screening plates

Table 3. Summary of XQ purification process performance.

Antibody	Equilibration buffer	Process loading (mg/ml)	Yield (%)	HMW (%)	HCP (ng/mg)	DNA (pg/mg)	ProA (ng/mg)
mAb-A	20 mM Tris, pH 8.5 3.21 mS/cm	150	93	2.14	20	BQ	0.2
mAb-FL	50 mM Tris, pH 7.5 9.18 mS/cm	314	98	0.66	11	BQ	0.4
mAb-FY	50 mM Tris, pH7.5 10.10 mS/cm	114	91	1.31	26	BQ	BQ
mAb-R	20 mM NaPi, pH 6.8 7.50 mS/cm	100	89	1.36	7	ND	ND
mAb-T	20 mM NaPi, pH 7.2 6.00 mS/cm	100	92	0.58	15	BQ	ND
mAb-T (Pilot)	20 mM NaPi, pH 6.8 7.10 mS/cm	100	93	0.80	7	BQ	0.1
mAb-P	50 mM Tris, pH7.5 9.02 mS/cm	300	97	1.13	26	BQ	ND
mAb-C	50 mM Tris, pH7.5 10.10 mS/cm	100	90	0.54	4	BQ	BQ

BQ: Below quantification limit; HCP: Host cell proteins; HMW: High molecular weight species; ND: Not determined.

Up to 8-Fold HMW Clearance

Up to 9-Fold HCP Clearance

Effective impurity clearance while maintaining high product yield in flow-through mode

Figure 15. Results of POROS XQ case study

One example of screening performed in plates, which was then scaled up is demonstrated in research performed by Eli Lilly.[1] The researchers tested POROS XQ anion exchange immediately after Protein A for purification of seven acidic mAbs in flow-through (Figure 15). The goal of the research was to maximize impurity clearance in host cell protein, DNA, molecular weight, and leached Protein A and to maximize yield. They tested seven different mAbs using high-throughput screening in 96-well plates with 40 µl volume, then moved to small-scale columns before scaling up to 2.9 L of resin.

Per Figure 15, high-fold clearance was obtained for most of the impurities tested. The study demonstrated up to 8x high molecular weight clearance and up to 9x host cell protein clearance. POROS XQ reduced Protein A leachate levels to below quantification or within acceptable specification limits. All of the conditions used either moderate or high conductivity buffers, reflecting the salt capabilities of POROS XQ and its ability to eliminate dilution and diafiltration steps prior to loading.

Another example of screening conditions on 96-well plates is the POROS Benzyl Ultra resin for the removal of aggregates in flow-through mode. In this example, mobile phase conditions were evaluated to assess the efficacy of aggregate removal (Figure 16).

As Figure 16 shows, greater than 90% aggregate removal was achieved for all conditions; the results achieved under low-salt conditions are especially relevant.

Conclusions

Conditions: 0 - 150 mM Salt, pH 5.5 - 7.5

- **HIC-Benzyl ultra resin** demonstrates strong selectivity for mAb A aggregate removal in flow-through mode (data not shown) and is selected for scale-down model.
- **>90%** aggregate mass removal in static binding HTS format
- pH 6.8, 1.8 mS/cm selected for column optimization **(Same condition as mAb-A AEX-FT!)**

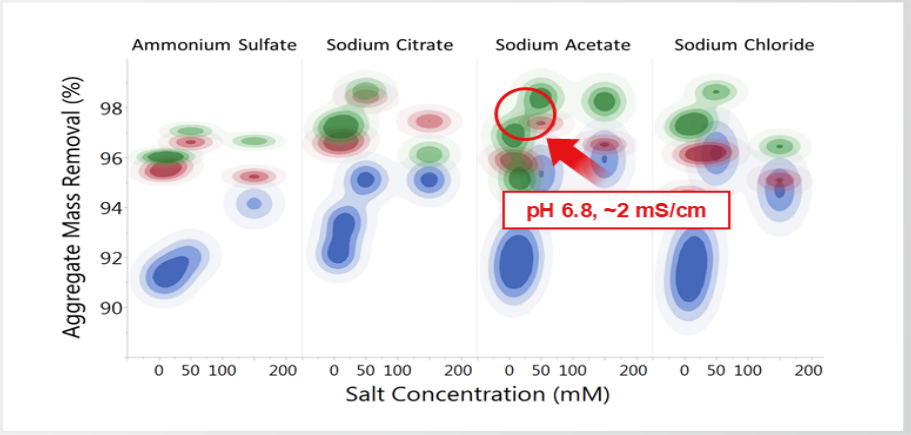


Figure 16. Results of POROS Benzyl Ultra case study

Think strategically

If you are looking to optimize your mAb process development, there are a wide range of options available. Consider how and where you can leverage strategic implementations to maximize cell growth and productivity while streamlining your processes. From there, identify suppliers that can deliver the starting materials and technologies to help your workflow thrive.

References

1. Kang et al Development of an acidic neutral antibody flow-through polishing step using salt tolerant anion exchange chromatography

Interested in leveraging Thermo Fisher Scientific's product lines for mAb development? Learn more at: **thermofisher.com/mabs**