

CHO Media Library – An Efficient Platform for Rapid Development and Optimization of Cell Culture Media Supporting High Production of Pharmaceutical Proteins in Chinese Hamster Ovary Cells

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Abstract

Chinese Hamster Ovary (CHO) cells are used extensively to produce recombinant proteins that require post-translation modification to express full biological function. Recombinant CHO cells are challenging to culture because each clone has diverse nutritional requirements. To save critical development time, we have developed very efficient medium optimization processes.

When optimizing medium for a specific CHO clone, pharmaceutical companies frequently follow a traditional approach of testing one medium component at a time to determine its optimal level. This strategy is labor intensive, costly and time consuming. To reduce costs and decrease development time, we adopted a newer approach based on statistical design. Design of Experiment (DOE) software can be customized to a user's needs and desired development criteria. We can analyze multiple criteria simultaneously to determine synergistic responses between them. We can then identify one or more media mixtures that provide the desired user outcomes.

SAFC Biosciences (SAFCB)' CHO cell culture platform, that includes high-throughput screening technology, a diverse selection of CHO media (both animal-component free and chemically-defined formulae), state-of-the-art cell engineering, and DOE design software, allows us to make CHO medium optimization an extremely efficient procedure. We will present the new strategic approach by making "CHO Media Library" (Figure 1) to speed medium development. An example depicts use of the "CHO Media Library" combining DOE analysis to efficiently develop several optimized formulations for a humanized IgG-producing CHO clone.

Methods and Materials

Cell lines and media:

Table 1 shows the CHO cell lines tested with CHO Media Library. The stock cultures of these cells were cultured in animal-component free media (SAFCB C8862 or proprietary formulations). The stock cultures were pre-adapted to the test formulations or directly seeded into the test formulations without adaptation. All SAFCB formulations and two competitor media were supplemented with 6 mM L-Glutamine unless otherwise specified. The data for this presentation was from 125-mL shake flasks culture.

Cell Line	Cell Line Lineage	Recombinant Protein Produced
CHO-K1	CHO-K1 parental	None
CHO-S	CHO-S parental	None
CHO-AP1	CHO-K1 derived	Alkaline Phosphatase
CHO-AP2	CHO-K1 derived	Alkaline Phosphatase
CHO-hGH1	CHO-K1 derived	Human Growth Hormone
CHO-hGH2	CHO-K1 derived	Human Growth Hormone
Recombinant CHO line 1	CHO-K1 derived	Human IgG
Recombinant CHO line 2	CHO-S derived	Human IgG

Table 1. CHO Cell Line Models for CHO Media Library Study.

Analytical methods and productivity assays:

Alkaline phosphatase activity: alkaline phosphatase activity was measured with Alkaline Phosphatase Fluorescence Detection Kit according to the manufacturer's protocol (Sigma-Aldrich, AP-F);

Human growth hormone: hGH productivity was quantified with hGH ELISA Detection Kit (Roche). The procedures were conducted at the manufacturer's protocol with minor modifications;

Human IgG: IgG concentration was measured with Protein G affinity chromatography.

Statistical analysis:

In the Design of Experiment (DOE) mixing experiment, User-Defined Design category was used to generate the mixing combinations to make a "Pyramid" model (Figure 2). The DOE screening data was analyzed by Design-Expert® Version 7.0.1 (Stat-Ease). The model with *p*-value less than 0.0001 by Analysis of Variance (ANOVA) indicates the statistical significance. In addition, the model F-value (50.42 for ICA, 33.57 for productivity) also implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise.

CHO Media Library

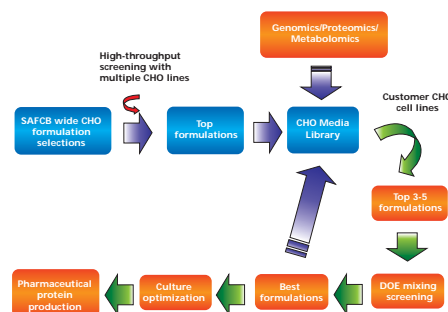


Figure 1. CHO Media Library Platform.

To develop CHO Media Library, multiple CHO cell lines have been tested with a wide selection of CHO formulations from SAFC Biosciences and screened for top formulations based on the cell growth and protein productivity. Customer CHO cell lines will be screened with the CHO Media Library and the top 3-5 formulations will be selected for Design of Experiment (DOE) mixing screen to identify the best formulation mixtures supporting the customer cell lines. After optimization of cell culture conditions (e.g. temperature shift and cell feeding), the selected formulations will significantly improve the pharmaceutical protein production in an efficient manner. Meanwhile, the selected top formulations and SAFCB's ongoing state-of-the-art systems biology studies (Genomics/Proteomics/Metabolomics) will continue enriching the contents of CHO Media Library.

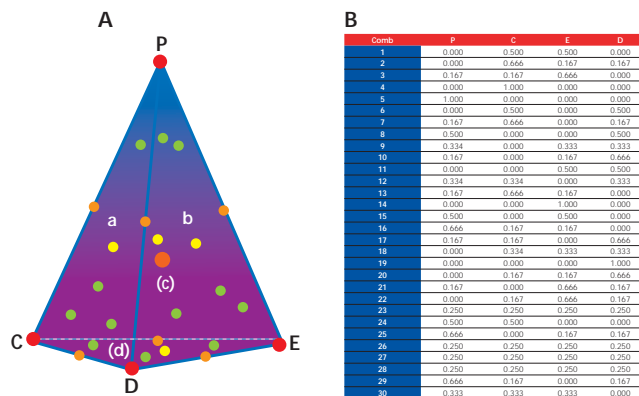


Figure 2. DOE "Pyramid" Design.

A) Schematic representation of DOE design. Four SAFCB formulations (C, D, E, and P) have been chosen for DOE mixing analysis. To simplify the experimental design, only the combinations on the surfaces (A-D) of pyramid have been evaluated, including vertices points (red spots), centers of edges (orange spots), surface centroids (yellow spots), axial check blends (green spots), and overall centroid (big red spot).

B) DOE combination table (including 26 combinations and 4 replicates of overall centroids).

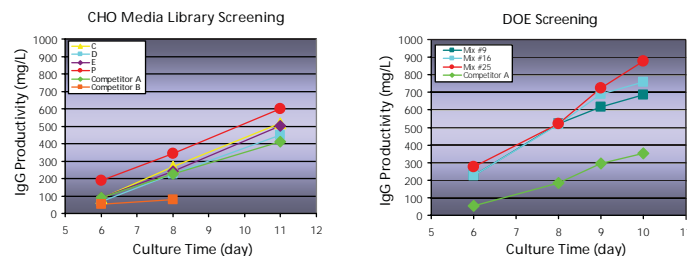


Figure 3. IgG Productivity Comparison.

A) IgG production in selected formulations from CHO Media Library along with 2 competitor media.

B) IgG production in selected mixing formulations after DOE screening along with competitor medium A. DOE mixing dramatically improved the IgG productivity ~ 2-fold higher than that in competitor medium A.

Conclusions

- SAFC Biosciences' cell culture platform (high-throughput screening, diverse selections of CHO media, cell line engineering, DOE design, and systems biology methodologies) facilitates the new strategic approach (CHO Media Library) to speed medium development. Comparing to the traditional medium development approach, this platform would dramatically reduce the costs and development time (usually less than 5 months).
- In this study, we first present the four-component DOE analysis (pyramid model) which turns out to be more informative and efficient than the conventional three-component DOE analysis (triangle model).
- With an IgG-producing CHO clone, CHO Media Library platform has developed several well-performing formulations supporting IgG production and one of them (Mix 25) nearly triples the IgG productivity compared to the control (competitor A) after culture modification (temperature shift), which further proves the efficacy of this platform.

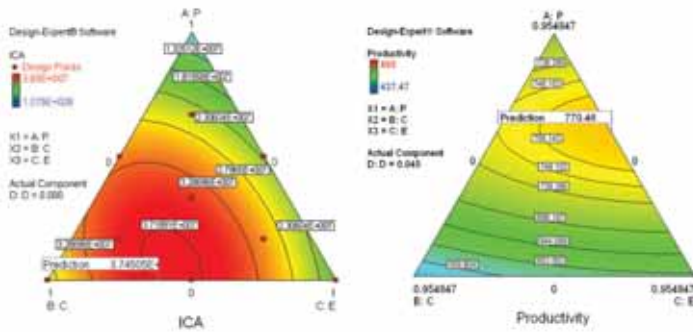


Figure 4. DOE Statistical Analysis and Numerical Optimization.

A) DOE optimization based on the cell growth (ICA: specific cell growth). It is obvious that more of formulation C would be beneficial to improve cell growth.

B) DOE optimization based on IgG productivity. More of formulation P would be beneficial to improve IgG production. Therefore, based on the features of the specific cell lines, the different mixing strategies will be applied to fit the needs.

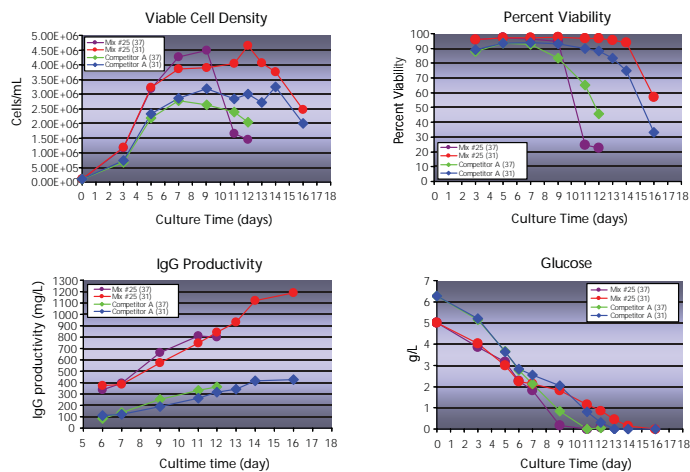


Figure 5. Temperature Shift Study on Mix 25 (Culture Condition Optimization).

A, B) Cell growth comparison. A) Viable cell density; B) Cell viability. Lower culture temperature (cultures were switched from 37°C to 31°C on day 6) has significantly extended the cell growth in Mix 25 and competitor A.

C) IgG productivity in Mix 25 and competitor A. Temperature shift has greatly improved IgG production in Mix 25 and has nearly tripled the IgG productivity than that in competitor A.

D) L-Glucose consumption rate has been significantly slowed down in 31°C cultures that could be the mechanism for the extended cell growth, which provides useful information for cell feeding strategy.