

Rapid Optimization of High Yield Processes Using Select™ CD1000 in a Platform Media Strategy

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ABSTRACT

Every cell line has unique nutritional requirements which must be properly balanced to achieve peak performance. However, production demands and tight timelines can make a cell specific base medium optimization challenging to perform. One solution is the use of a universal platform medium which can serve as the optimization starting point for all new clones. Utilizing a platform strategy can greatly reduce the time and costs associated with developing a high yield process.

One critical element to process optimization is the use of a nutritionally rich base medium that supports robust cell proliferation and protein production. Using Design of Experiments (DOEs), a chemically defined base medium was developed that supported the growth and antibody production of CHO cells. Through further investigation, it was determined that this formulation, designated Select™ CD1000, also supported the proliferation of and production from both hybridoma and NS0 cell lines. Additional studies with an expanded panel of cell lines confirmed that Select™ CD1000 is an ideal platform medium for process optimizations using CHO, hybridoma, or NS0 cell lines.

Hydrolysate screens were performed for all three cell types and significant production increases were observed after only one round of supplement optimization. Similar protein yield increases were achieved using chemically defined supplements, demonstrating the flexibility of the platform strategy to accommodate various process requirements. Through further process optimization, even higher yields were achieved using both chemically defined and hydrolysate containing feed strategies. By combining a robust platform medium with the proper supplementation and feed strategies, the average protein yields were increased over 200% in fewer than two months.

MATERIALS

CELL LINES

Three GS-CHO lines producing hu IgG (GS CHO-1, GS CHO-2, GS CHO-3),
One Neomycin resistant CHO line producing hu IgG (Neo CHO),
One Hybridoma line producing mu IgG

CULTURE MEDIA

BD Select™ CD1000,
Commercially available CD media,
BD Cell Animal Free,
BD Select™ CHO

HYDROLYSATES

TC Yeastolate UF,
Yeast Extract UF,
Phytone UF,
Developmental Wheat UF

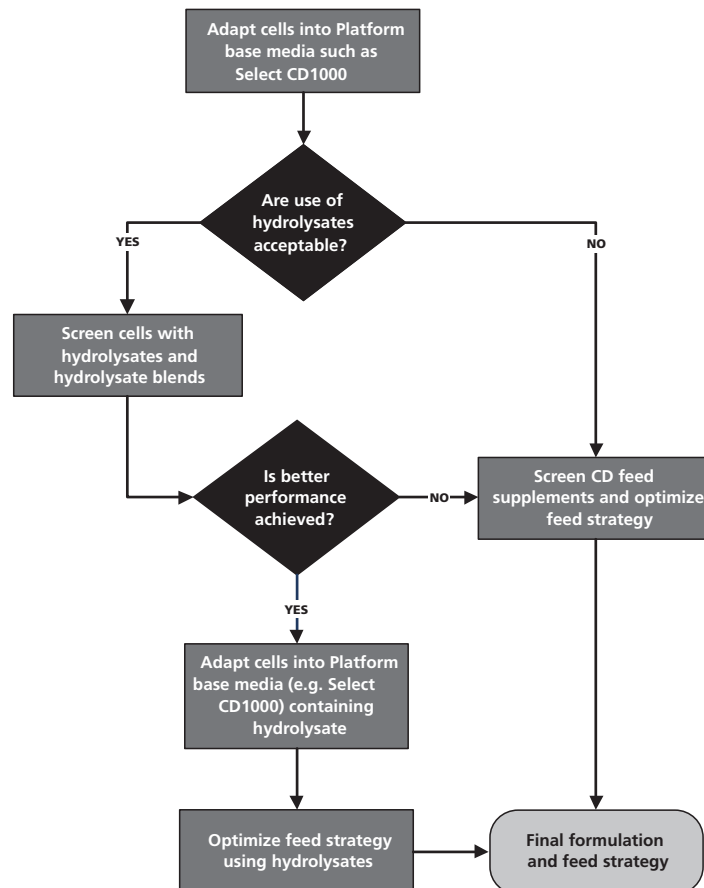
EQUIPMENT AND REAGENTS

125 mL Shaker flasks (Corning),
Octet (Forte Biosciences)

METHODS

- **Cell adaptation** into selected media formulations was performed in 125 mL shaker cultures. The cell cultures were passaged a minimum of three passages in the new formulation prior to initiating comparison studies. Shaker studies were carried for 7 to 14 days, as appropriate for the cell line. Production and Proliferation was monitored through out the culture life cycle. Only end point data, volumetric production, is displayed in this presentation.
- **Hydrolysate optimization** was carried out through the use of a proprietary design of experiment (DoE). The initial screen was conducted in 96 well culture dishes. The DoE for the hydrolysate screen was analyzed, and the predicted optimal hydrolysate blend(s) was confirmed in 125 mL shaker cultures. The cells were then adapted into the best performing condition for a minimum of three passages prior to evaluating the production levels in the hydrolysate containing medium versus the original chemically defined medium.
- **Feed strategy optimization** was performed in 125 mL shaker flasks. Feed supplement concentration, timing of initial addition of feed, and frequency of feed was evaluated and optimized in one experiment per BD proprietary study design.

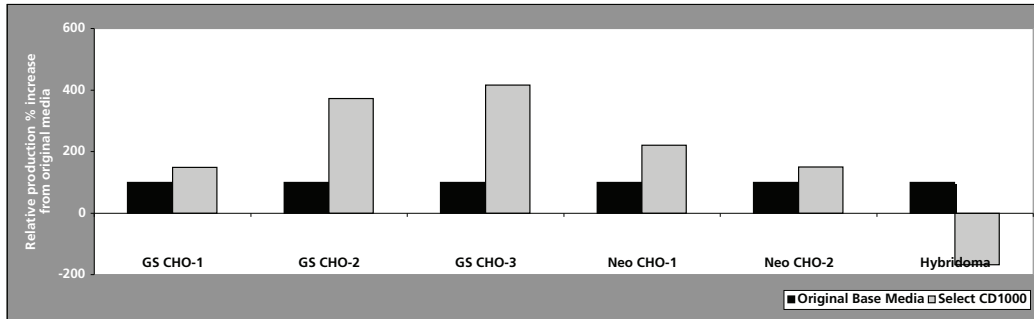
Figure1: Media Optimization Decision Tree for Platform Media Approach



RESULTS

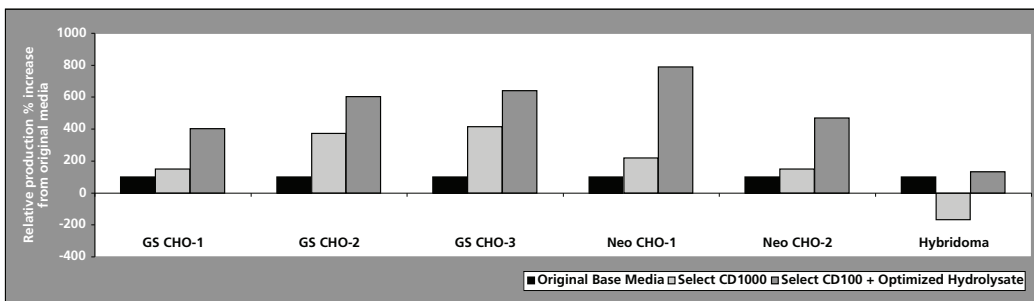
A variety of cell lines was adapted into Select™ CD1000 and passaged a minimum of three times. A shaker study was then conducted to evaluate the volumetric production of each cell line in their respective original media formulation versus Select™ CD1000 (Fig. 2). The CHO cell lines produced at higher levels in Select™ CD1000 than in their respective control chemically defined media. The hybridoma line showed a decline in production when adapted from the original hydrolysate containing media into the chemically defined Select™ CD1000.

Figure 2: Production Levels of Multiple Cell Lines in Select™ CD1000



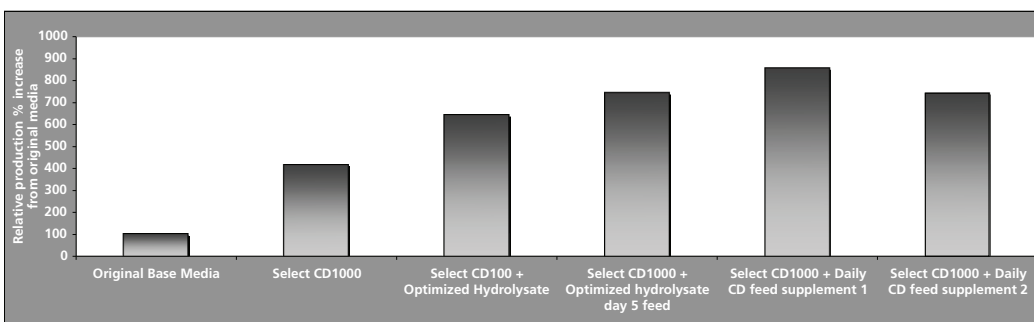
The cells now growing in Select™ CD1000 were then evaluated for impact of an optimized hydrolysate supplementation (Fig.3). In all cases, a hydrolysate supplementation improved volumetric production of each cell line. It should be noted that the addition of a hydrolysate to Select™ CD1000 with the hybridoma line returned the production levels to the equivalency of the original hydrolysate containing medium.

Figure 3: Improvement in Production Levels by Addition of Hydrolysates to Select™ CD1000



The GS CHO-3 cell line was further optimized through developing a hydrolysate feed strategy to yield an approximate 7-fold increase in protein production over original base media (Fig.4). In addition, equivalent or better production levels were obtained through the use of chemically defined feed supplements and feed strategy. This demonstrates the versatility of a robust platform media, such as Select™ CD1000, to allow media optimization within the parameters of the desired final formulation.

Figure 4: Select™ CD1000 Can Be Further Optimized with Feed Strategies Utilizing Hydrolysates or CD Feed Supplements



CONCLUSIONS

- Select™ CD1000 allowed for improved volumetric production for CHO cell lines tested.
- Further enhancement of protein production was obtained by the use of hydrolysate or CD Supplements and feed strategies.
- The average protein yields were increased over two fold in fewer than 2 months per cell line, by combining a robust platform medium, Select™ CD1000, with the proper supplementation and feed strategies.

Cell Line	Maximum Fold Increase from Original Culture Media
GS CHO-1	4.0 fold increase
GS CHO-2	6.0 fold increase
GS CHO-3	8.5 fold increase
Neo CHO-1	7.9 fold increase
Neo CHO-2	4.7 fold increase
Hybridoma	1.3 fold increase