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ProductInformation

CHO Medium Component Optimization Kit 2

Product Code **C4364**
Storage Temperature 2-8 °C

Synonym: CHO Kit 2

TECHNICAL BULLETIN

Product Description

Chinese Hamster Ovary (CHO) cells are of great interest for bioprocess and pharmaceutical research and development. These cells are robust in culture and are able to produce a variety of recombinant glycoproteins at high levels on a large scale. However, different CHO cell clones often possess diverse nutritional requirements that are unique to each clone. As a result, medium optimization for CHO cells can be very challenging, often requiring the development of a custom medium for each particular clone.

The traditional approach to media development involves titrating each medium component individually to determine the optimal level of supplementation. This process involves extensive testing conditions and is very lengthy. CHO Kit 2 utilizes a statistical approach, Design of Experiment (DOE), to perform media optimization. This novel approach greatly reduces the time and effort needed to optimize a medium for a particular CHO clone.

CHO Kit 2 is a medium component optimization kit consisting of a concentrated basal medium, five concentrated basal supplements, and six concentrated optimization supplements. All optimization supplements have been determined to have significant effects on CHO cell growth and/or recombinant protein production.

The design of this kit allows for the testing of several supplements simultaneously at high and low levels, with all possible combinations based on factorial matrix design. This allows the researcher to recognize interactions between components in fewer conditions than the traditional approach without losing important information.

It is recommended that testing the six supplements should be done in two parts. The first part breaks up the six supplements into two factorial matrix experiments in order to determine the optimal levels of individual supplements. The second part brings these optimal levels together to look for interactions among all the supplements. This approach allows the user to quickly reach an optimal medium by designing and performing two independent 2^3 factorial matrix assays and one 2^2 factorial matrix assay.

Intended Use

For R&D use only. Not for drug, household or other uses.

Kit Components

This kit contains sufficient concentrated solutions to prepare at least 20L of optimized CHO AF PF medium. The Base Medium concentrate (Product Code C4739) includes inorganic salts, HEPES, sodium bicarbonate, essential and non-essential amino acids, vitamins, trace elements, phenol red, Pluronic® F-68, and other organic compounds. It does not contain plant hydrolysates, L-glutamine, antibiotics, antimycotics, or transferrin. It also does not contain hypoxanthine or thymidine to allow its use with dihydrofolate reductase (DHFR) gene amplification systems.

Basal Supplements are included to make a complete medium but not for optimization testing. The supplements are Iron Mix 2, (Product Code I3658), Glucose Solution, (Product Code G1669), Insulin Solution (Product Code I9278), Salts Mix (Product Code C4239), and Sodium Chloride (Product Code S3694). See Appendix 1.

The concentrated Optimization Supplements included for testing are Metals Mix (Product Code S4193), Amino Acid Mix (Product Code C7489), Vitamin Mix (Product Code C7614), Iron Mix 1 (Product Code C2115), Fatty Acid Supplement (Product Code F7050), and Hydrolysates Mix (Product Code H9162). See Appendix 2 for initial levels to test.

Preparation Instructions
The Base Medium is supplied as a sterile 10X liquid. All supplements are supplied as sterile, concentrated stock solutions and should be handled aseptically.

1. Follow Appendix 3 to set up Part 1A and Appendix 4 to set up Part 1B. For Part 2, the user will need to perform his own calculations to determine the amount of the supplements to add to the desired volume of the Base Medium in each condition. For an example, see Appendix 5.
2. Measure the amount of Base Medium and transfer to a beaker.
3. Add desired amount of L-glutamine. The recommended concentrations are 4-8 mM (20-40ml/L of 200mM, Product Code G7513).
4. Add the proper amount of the Basal Supplements (Glucose, Insulin, Iron Mix 2, and Salts Mix) to the beaker. **Note:** Hydrolysates (2.0 ml/L) should be added to restore their minimal level in the Base Medium for Part 1A conditions that are not testing Hydrolysates. If a chemically defined system is desired, do not add Hydrolysates.
5. Add the test supplements. For Part 1, add only three of the supplements for each matrix assay. For Part 2, add all six supplements.
6. Bring up to final volume using deionized water.
7. Adjust solution to desired pH. It is suggested to adjust pH to 7.4 using 1.0 N HCl (Product Code H9892) or 1.0 N NaOH (Product Code S2770).
8. Adjust the osmolality to 310 ± 10 mOsmo/kg using the 5M NaCl provided in the kit. Every 0.3 ml of NaCl added will increase the osmolality by 10 mOsmo/kg. **Note:** The Base Medium is designed to have low osmolality and basic pH levels due to the lack of supplements. Therefore, it is critical to adjust pH and osmolality after adding these supplements. See Appendix 6 for data chart.
9. Filter the medium using a 0.22 µm sterile filter and store at 4 °C prior to use.

Storage/Stability
The Base Medium and all the supplements are stable when stored at 2-8 °C and protected from light until the indicated expiration date on the label.

Procedures
Assay Format
This kit was developed for use in spinner flasks with a recommended working volume of 100 ml, but can be used with other systems.

Experimental Matrix Design
CHO Kit 2 enables the user to utilize factorial matrix design and analysis to quickly optimize media. For an organizational flow chart, see Appendix 7. Prior to adding the optimization supplements, the basal supplements (Glucose, Insulin, Iron Mix 2, and Salt Mix) should be added to all the formulations in the matrices. See Appendix 1 for the volumes required. For the test supplement optimization, we recommend a two-part strategy:

Part 1 consists of two initial experiments with each experiment testing three factors (one group) at two levels for each factor (a 2^3 matrix). This is to identify the supplements that have significant effects on growth and/or productivity and to determine their optimal concentrations with a manageable number of cultures. The two matrix experiments can be performed either sequentially or in parallel.

> **Part 1A** is designed to test Group 1 (Amino Acid Mix, Metals, and Iron Mix 1). The other three test supplements (Group 2) are NOT added to the Base Medium since their concentrations are kept at the base medium levels.
>
> **Part 1B** is designed to test Group 2 (Vitamin Mix, Fatty Acids and Hydrolysates). For this 2^3 matrix assay, Group 1 (Amino Acid Mix, Metals, and Iron Mix 1) are kept at the base medium levels and NOT added to the test formulations. Analyze the data using appropriate statistical software (see Procedures: Data Analysis).

Part 2 is designed to test all six supplements in one factorial matrix assay, in order to finalize the optimal concentrations and to test any interactions between the six test supplements. A 2^2 factorial matrix assay is recommended as a starting point, in which Group 1 and Group 2 are tested as the TWO factors at high and low levels. The levels of the test supplements that demonstrate significant positive effects in Part 1 are adjusted to new concentration intervals with the previous high point as the new center point. The levels of the test supplements that demonstrated significant negative effects in Part 1 are adjusted to a new interval with the previous low point as the new

center point in Part 2. The levels of the test supplements that demonstrated no significant effect in Part 1 should remain at the original center point level.

Analyze the results from Part 2 and determine the optimized formulation of all test supplements. The user may also perform further optimization by expanding the concentrations tested to higher or lower values than those initially suggested.

Data Analysis

The data can be collected and analyzed based on different optimization endpoints, such as maximum cell density, integrated cell area (cell days), or recombinant protein production. An example of the statistical analysis software, Design Expert®, may be obtained for a free 30-day trial at www.statease.com

In brief, the statistical program calculates the effect of any supplement or group of supplements based on the data collected using mathematical modeling and prediction. The Normal Probability Plot graphs these calculated effects and shows positive or negative effects of supplements against a calculated "line of chance". Any data point that falls off this line represents an effect on the criteria selected. Data points (as labeled on the plot) to the left of the line suggest a negative effect from the corresponding test supplements (i.e., more beneficial at low levels). Data points to the right of the line suggest a positive effect (i.e., more beneficial at high levels). Data points on the line suggest no significant effect. F values are calculated by Design Expert to compare model variance with error variance. If they were equal then the F value would equal 1. The larger the value, the more likely that error contributed to the model and none of the factors have a significant effect on the response. The F values between 0.05 and 0.1 from the Analysis of Variance or annotated ANOVA test also point out which supplements are significant. Cube graphs can confirm the effects seen in the Normal Probability Plot and can depict interactions between components. The three axes represent three supplements. The numbers on the axis corners indicate the effect of the corresponding supplements. A large difference in numbers represents a significant effect, while no change in number on a certain axis indicates no effect.

Design Expert also allows the user to assign importance rank values to the optimization endpoints based on his needs. For instance, if recombinant protein productivity is the critical endpoint, it can be assigned a value of 5 (the highest value), and if integrated cell area is less important in the optimization it is assigned a lesser value of 3. From these specifications, Design Expert analysis can predict an infinite number of combinations of the supplements to design solutions specific to your needs.

It is important to analyze and draw conclusions from a matrix experiment containing ALL designed conditions. Any scenarios that lead to missing more than one condition from the matrix makes the design unbalanced and non-orthogonal, which introduces undesirable properties to the statistical analysis. Performing analyses with such missing condition(s) may cause some important effects to be lost due to aliasing or points on the graph that do not fit with the analysis. We suggest that the user run all experiments exactly as planned or terminate the experiment early and repeat the designed setup in case of lost conditions in order to ensure the most accurate analysis.

Alternatively, the user can send his data back to Sigma-Aldrich Cell Culture R & D via email (cellculture@sial.com) for data analysis.

Product Profile

Sigma's CHO Kit 2 was used on several different recombinant CHO cell lines to determine the feasibility of using factorial matrix design for medium optimization. Data from such experiments using a CHO-K1 cell line are shown in Appendix 8. The CHO-K1 cells were adapted to serum-free conditions prior to the experiments. Cells were inoculated at 5×10^4 cells/ml. A new optimized medium was formulated according to the best-predicted values. Cultures grown in this formulation demonstrated a 1.5-fold increase in cell growth over the original Base Medium and two competitors' media. CHO Kit 2 provides a novel and important tool for rapid cell culture media optimization using factorial matrix design approach.

References

1. Bettger, W. J. and Ham, G. (1982), Advances in nutritional research. *The Nutrient Requirements of Cultured Mammalian Cells, Chapter 9*, (H. Draper, ed.), Plenum Publishing Corporation, pp. 249-281.
2. Moen, R., Nolan, T., and Provost, L., Quality Improvement through Planned Experimentation, Second Edition. McGraw Hill, Inc., New York (1999).

Precautions and Disclaimer

MSDS is available upon request or at **sigma-aldrich.com**. Pluronic is a registered trademark of

BASF Corporation. Design-Expert is a registered trademark of Stat-Ease, Inc.

Additional Materials Not Supplied in Kit

Product	Description	Unit
G7513	L-Glutamine 200mM	100ml

APPENDIX 1

Basal Supplement Levels

The basal supplements need to be added to each condition. They are left out of the base medium for stability reasons and are not for optimization. Hydrolysates are also not included in the base medium to give the user the option of having a chemically defined system. If a chemically defined medium is not desired, then hydrolysates should be added to at least a 20% level.

Product Codes	Supplements	Initial Level (10X Base)	Target Level	Volume (ml) to add to 0.5L to make a complete base medium
I3658	Iron Mix 2	0%	100%	0.25
G1669	Glucose	0%	100%	4.25
I9278	Insulin	0%	100%	0.15
C4239	Salts Mix	0%	100%	1.0
H9162	Hydrolysates	0%	20%	1.0
S3694	NaCl (5M)	0%	For adjusting osmolality only	

APPENDIX 2

Optimization Supplements Levels

The test supplements are the six supplements that will have the largest impact on medium optimization.

Product Codes	Supplement	Initial % in 10X Base	Volume to add to make 100% (ml/0.5L)	Low level to test	High level to test	Center point
S4193	Metals	12.5%	0.10	25%	175%	100%
C7489	Amino Acid Mix	25%	20.0	50%	150%	100%
C7614	Vitamin Mix	25%	25.0	50%	150%	100%
C2115	Iron Mix 1	0%	0.60	70%	130%	100%
F7050	Fatty Acids	20%	0.215	50%	150%	100%
H9162	Hydrolysates	0%	5.0	50%	150%	100%

APPENDIX 3

Part 1A Set-up

Part 1A tests Metals, Amino Acid Mix, and Iron Mix 1. The Vitamin Mix and Fatty Acids are not tested and therefore are not added. Hydrolysates are also not tested, but needs to be added back at 20% if a chemically defined medium is not required. A 2^3 matrix is illustrated as well as the amount of each supplement to add per 500ml condition.

2^3 Matrix			
Condition	Metals	Amino Acids	Iron Mix 1
1,2	Low	Low	Low
3,4	Low	High	Low
5,6	High	Low	Low
7,8	High	High	Low
9,10	Low	Low	High
11,12	Low	High	High
13,14	High	Low	High
15,16	High	High	High
17,18	Center point	Center point	Center point
19,20	Base Medium	Base Medium	Base Medium

Basal Supplements						
Condition #	Iron Mix 2 (ml/0.5L)	Hydrolysates (ml/0.5L)	Glucose (ml/0.5L)	Insulin (ml/0.5L)	Salts Mix (ml/0.5L)	Total ml of 10X Base Medium
1 - 20	100%=0.25	20% = 1.0	100% =4.25	100% =0.15	100% =1.0	50

Optimization Supplements					
Condition #	Metals (ml/0.5L)	Amino Acid Mix (ml/0.5L)	Iron Mix 1 (ml/0.5L)	Vitamin Mix (ml/0.5L)	Fatty Acids (ml/0.5L)
1,2	25%=0.013	50%=50	70%=0.42	0%	0%
3,4	25%=0.013	150%=25.0	70%=0.42	0%	0%
5,6	175%=0.163	50%=5.0	70%=0.42	0%	0%
7,8	175%=0.163	150%=25.0	70%=0.42	0%	0%
9,10	25%=0.013	50%=5.0	130%=0.78	0%	0%
11,12	25%=0.013	150%=25.0	130%=0.78	0%	0%
13,14	175%=0.163	50%=5.0	130%=0.78	0%	0%
15,16	175%=0.163	150%=25.0	130%=0.78	0%	0%
17,18 (center point)	100%=0.088	100%=15.0	100%=0.60	0%	0%
19,20(Base Medium)	0%	0%	0%	0%	0%

APPENDIX 4

Part 1B Set-up

Part 1B tests the Vitamin Mix, Hydrolysates, and Fatty Acids. For this test, the Hydrolysates are included with the test supplements instead of the basal supplements. Since Metals, Amino Acid Mix, and Iron Mix 1 are not tested, they do not need to be added. A 2^3 matrix is illustrated as well as the amount of each supplement to add per 500ml condition.

2^3 Matrix			
Condition	Vitamin Mix	Hydrolysates	Fatty Acids
1,2	Low	Low	Low
3,4	Low	High	Low
5,6	High	Low	Low
7,8	High	High	Low
9,10	Low	Low	High
11,12	Low	High	High
13,14	High	Low	High
15,16	High	High	High
17,18	Center point	Center point	Center point
19,20	Base Medium	Base Medium	Base Medium

Basal Supplements					
Condition #	Iron Mix 2 (ml/0.5L)	Glucose (ml/0.5L)	Insulin (ml/0.5L)	Salts Mix (ml/0.5L)	Total ml of 10X Base Medium
1 - 20	100%=0.25	100%=4.25	100%=0.15	100%=1.0	50

Optimization Supplements						
Condition #	Metals (ml/0.5L)	Amino Acid (ml/0.5L)	Iron Mix 1 (ml/0.5L)	Vitamin Mix (ml/0.5L)	Hydrolysates (ml/0.5L)	Fatty Acids (ml/0.5L)
1,2	0%	0%	0%	50%=6.25	50%=2.5	50%=0.065
3,4	0%	0%	0%	50%=6.25	150%=7.5	50%=0.065
5,6	0%	0%	0%	150%=31.25	50%=2.5	50%=0.065
7,8	0%	0%	0%	150%=31.25	150%=7.5	50%=0.065
9,10	0%	0%	0%	50%=6.25	50%=2.5	150%=0.280
11,12	0%	0%	0%	50%=6.25	150%=7.5	150%=0.280
13,14	0%	0%	0%	150%=31.25	50%=2.5	150%=0.280
15,16	0%	0%	0%	150%=31.25	150%=7.5	150%=0.280
17,18(center point)	0%	0%	0%	100%=18.75	100%=5	100%=0.172
19,20(BaseMedium)	0%	0%	0%	0%	20.0%=1.0	0%

APPENDIX 5

Part 2 Matrix Set-up

Part 2 combines the information learned in Part 1A with that in Part 1B. The low level and high levels for each component are determined based on the results from Part 1.The Basal Supplements will still have to be added at the original amounts. The 2^2 matrix of Part 2 will vary depending on the results from Part 1A and 1B and will be unique for every clone.

	Center Point	Low Level	High Level	
Metals	cp	Low	High	
Amino Acid Mix	cp	Low	High	**A**
Iron Mix 1	cp	Low	High	
Vitamin Mix	cp	Low	High	
Hydrolysates	cp	Low	High	**B**
Fatty Acids	cp	Low	High	

Condition #	Optimization Supplements
1,2	A-low, B-low
3,4	A-high, B-low
5,6	A-low, B-high
7,8	A-high, B-high
9,10	Center point
11,12	Base Medium

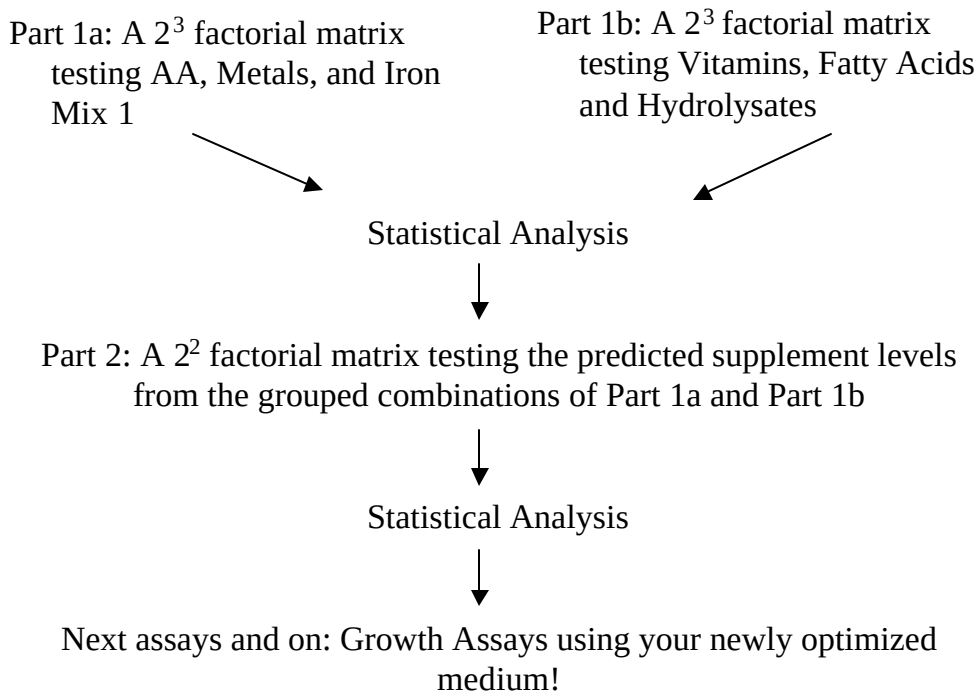
APPENDIX 6

pH and Osmolality Adjustment Sheet

Condition #	Date formulated
Initial pH =	Total supplements (ml) =
Final pH =	Total Base (ml) =
Initial osmolality =	Total H_2O (ml) =
NaCl added (ml) =	
Final osmolality =	

APPENDIX 7

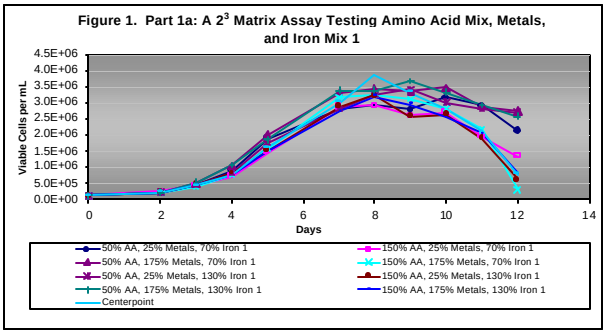
Organizational Flow Chart



Appendix 8

Part 1A

Medium optimization was performed for a CHO-K1 clone. The media was prepared as described in the Preparation Instructions and followed Appendix 3. Figure 1 depicts the cell growth curves generated in all media formulations and Base Medium.



The cell growth data were analyzed using Design Expert Software and a Normal Plot was generated as shown in Figure 2.

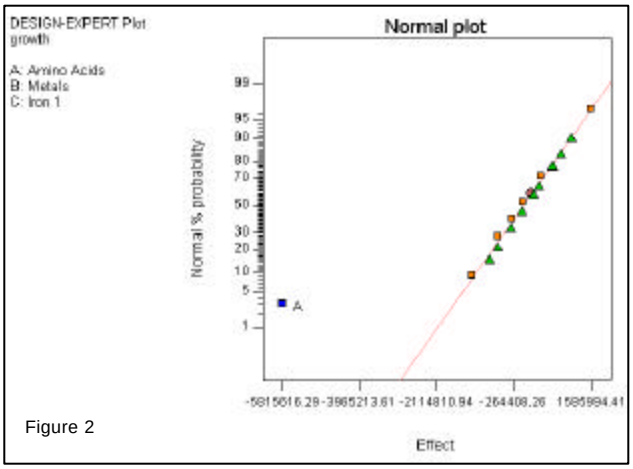
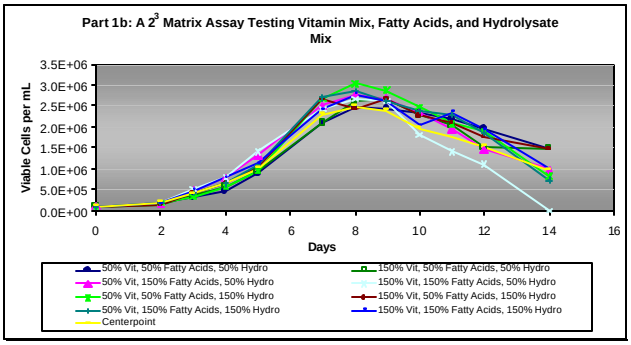


Figure 2

The Normal Plot indicates that the only significant factor is A: Amino Acid Mix. Since the factor is on the left of the "line of chance", Amino Acid Mix has a negative effect and is more beneficial at its lower levels. ANOVA indicates the effect is significant ($p<0.05$). Based on these analysis results the new center point for Part 2 was set as 50% (the low level tested in Part 1A) and tested additional levels at 25% (the new low level) and 75% (the new high level). On the other hand, Metals and Iron Mix 1 do not have any significant effect on cell growth and were tested at the original center point level from Part 1A (100%).

Part 1B

The media for Part 1B were prepared by following Appendix 4. The cell growth results are shown in Figure 3.



Design Expert analyzed the cell growth data and a Normal Plot was generated as shown in Figure 4.

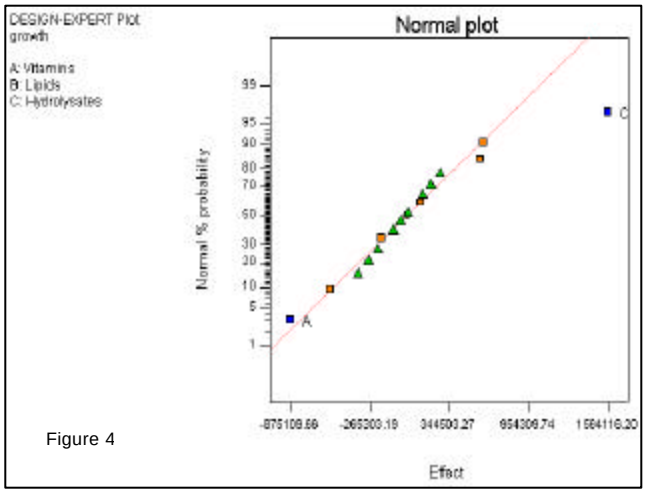


Figure 4

This normal plot indicates that C: Hydrolysates have a strong positive effect because it is very far to the right of the line. Factor A: Vitamin Mix, has a slight negative effect. For Part 2, the center point for Hydrolysates was set at 150% and the new low and high level was set at 100% and 200%, respectively. Since Vitamin Mix 1 is slightly negative the new center point was set at 50% and the low and high levels at 25% and 75%, respectively. Fatty Acids had no effect, so they were left at the same levels as in Matrix 1B.

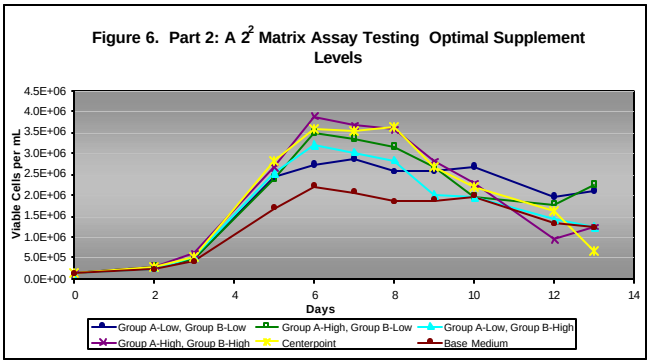
Part 2

Based on the results from Part 1, Figure 5 was generated illustrating a 2^2 factorial matrix.

Figure 5

	Center-point	Low level	High Level	
Metals	100%	25%	175%	
Amino Acid Mix	50%	25%	75%	**A**
Iron Mix 1	100%	70%	130%	
Vitamin Mix	50%	25%	75%	
Hydrolysates	150%	100%	200%	**B**
Fatty Acids	100%	50%	150%	

Using the above table and Appendix 5 as a guide, the necessary media were prepared and cell growth curves were shown in Figure 6.



The cell growth curves generated in Part 2 (Figure 6) suggest that the center point is the optimal medium. Design Expert analysis indicated no significant factors, indicating this is the optimal medium formulation (Figure 7).

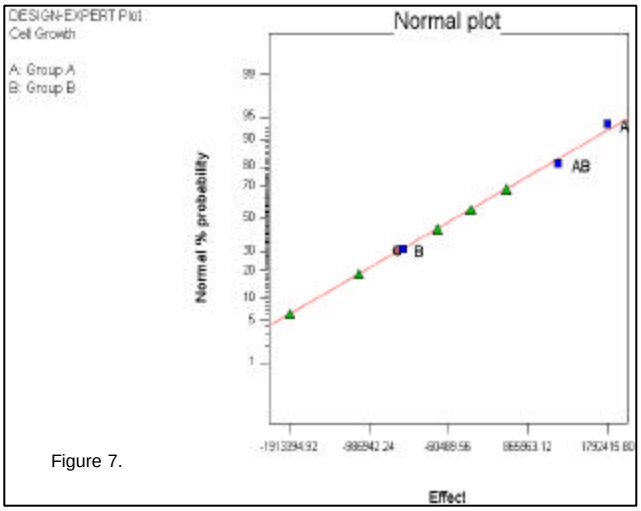
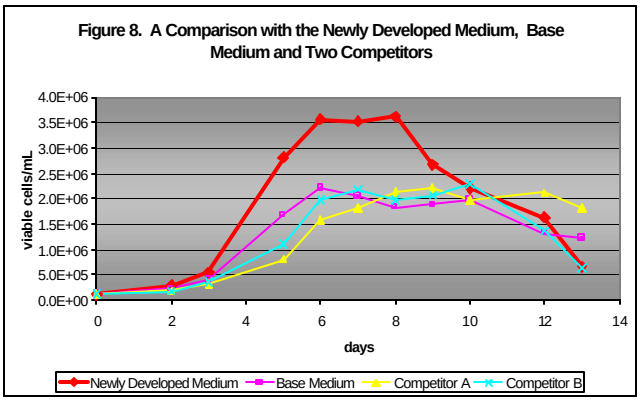


Figure 7.

We performed a final growth study using this optimized formulation, two competitors media and the Base Medium in CHO Kit 2. As demonstrated in Figure 8, over 1.5-fold increase in peak cell density and accumulated integrated cell area was achieved in the optimized formulation compared with the other three media.



As shown, by setting up the recommended factorial matrix assays, the user can develop an optimized medium for their particular clone.