

# cell culture

## Medium Optimization Kit for CHO Cells: Using Factorial Matrix Design of Experiment to Increase Cell Performance

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### Application Notes

- Statistical approach to medium development using Design of Experiment software
- Animal component-free, with chemically-defined formulation options
- Efficient medium optimization of any CHO clone

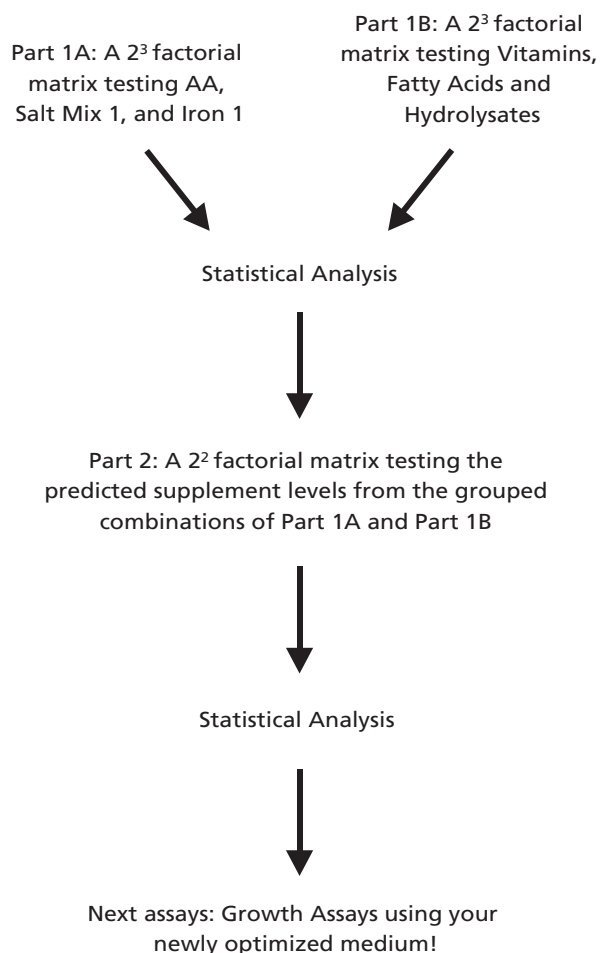
### Introduction

Chinese Hamster Ovary (CHO) cells are of great interest for bioprocessing 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. The most challenging aspect of culturing recombinant CHO cell clones is providing for the diverse nutritional requirements that are unique to every transfected cell line – often requiring the development of a custom medium for each particular clone. The traditional approach to media development involves titrating each component individually to determine the optimal level of supplementation. This process involves extensive testing conditions and is very lengthy. Therefore, using factorial matrix statistical assays to accelerate the optimization of cell culture medium has received great attention in many pharmaceutical companies.

### Design of Experiment (DOE) provides efficient experimental procedures and easy analysis

In order to assist pharmaceutical companies in improving their medium optimization process, Sigma-Aldrich has developed CHO Kit 2 (Product Code [C 4364](#)) – a medium component optimization kit that utilizes DOE. The

design of this kit allows the researcher to test 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. This novel approach greatly reduces the time and effort needed to optimize a medium for a particular CHO clone. Figure 1 outlines the procedure recommended for the testing of the kit supplements.



*Figure 1. Organizational flow chart for CHO Kit 2 experimental procedures. The first part divides 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. Analyze the results and determine the optimized formulation of all test supplements.*

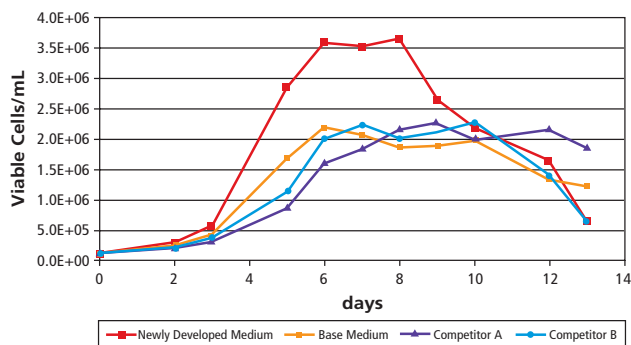
The statistical analysis software, Design Expert®, is used with CHO Kit 2 to analyze the factorial matrices. In brief, the statistical program calculates the effect of any supplement or group of supplements against variance in the assay based on the data collected using mathematical

modeling and prediction. 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. Design Expert analysis can predict an infinite number of combinations of the supplements to design solutions specific to your needs.

### Optimized media components increase cell growth and protein production

The kit consists of one concentrated, chemically defined basal medium. It is deficient in several nutrients so that any component may be added back for optimization. Several basal supplements are included to make a complete medium, but not included in optimization testing. These supplements are glucose, insulin, salts, iron chelator, and sodium chloride. Six concentrated grouped media supplements included for optimization testing are amino acids, vitamins, iron chelator, fatty acids, hydrolysates and metals.

The CHO-K1 cells were adapted to serum-free conditions prior to the experiments. Cells were inoculated at  $5 \times 10^4$  cells/ml. All assays were run in duplicate in 125-ml spinner flasks and stored at 37 °C, 5% CO<sub>2</sub>, with 80 rpm stir speed. Samples were counted on a CASY®-1 cell counter (Scharfe Systems, Reutlingen, Germany) and by the trypan blue exclusion method. Data was then input into Design-Expert for analysis. From the first two factorial matrix assays using CHO-K1 cells, we obtained two predicted optimal growth formulations. Combining data from the two assays, a new medium was prepared: 50% amino acids, 100% metals, 100% iron, 50% vitamins, 100% lipids, and 150% hydrolysates. The cell growth performance of this new medium was tested on CHO-K1 cells (Figure 2). The result clearly showed that CHO-K1 cells grew to a 1.5-fold higher cell density in this new medium than in the original Base Medium or in the two competitors' media tested.



**Figure 2.** A comparison of the newly developed medium, the original base medium, and two competitors. A new optimized medium was formulated according to the best-predicted values from Design Expert analysis. Cultures grown in this formulation demonstrated a 1.5-fold increase in cell growth for CHO-K1 cells over the original Base Medium and the two competitors' media.

### Kit provides medium optimization for several CHO clones

We have tested this kit using several CHO cell lines to statistically predict the most optimal levels of each component for unique medium supplementation. Our results demonstrate that using this medium optimization kit and Design Expert software on diverse CHO cell lines can generate different optimized media formulations (Table 1). Overall, this kit provides a novel approach to medium development, allowing the customer to easily perform factorial-matrix experiments while reducing the time and effort needed to optimize a medium for a particular CHO clone.

**Table 1.** Comparison of the most optimal medium supplementation values for four CHO clones.

		CHO-K1	IgG Clone 1	IgG Clone 2	Alk Phos
Group A	Amino Acids	50%	150%	100%	50%
	Metals	100%	25%	175%	175%
	Iron 1	100%	70%	100%	100%
Group B	Vitamins	50%	50%	100%	100%
	Fatty Acids	100%	150%	150%	100%
	Hydrolysates	150%	150%	150%	150%

*Some of the components showed similar results between the cell lines. For example, hydrolysates were seen to be beneficial in all clones at 150%. Most of the components showed a difference overall in each cell line, demonstrating that CHO Kit 2 provides diverse nutritional requirements for several cell lines.*

### Ordering Information

Product	Description	Unit
<a href="#">C 4364</a>	CHO Kit 2	1 kit