An Efficient Approach to Cell Culture Medium Optimization for CHO Cells

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Application Notes

- Six unique animal component-free media for maximum recombinant protein production
- Convenient format and rapid screening of multiple media formulations
- Detailed instructions for media mixing, experimental design, and data analysis
- Simple yet powerful mixing experiments using DOE methodology

Introduction

One of the most challenging aspects of culturing recombinant CHO cell clones is providing for the diverse nutritional requirements that are unique to every transfected cell line. In order to minimize the amount of time required for medium development, we have recently developed a medium optimization kit, CHO Kit 1 (Product Code CH0001). This kit consists of six diverse animal component-free CHO media formulations to provide for a wide range of nutritional requirements. This format serves not only as a quick and easy screen for multiple media, but also as a platform for statistical medium optimization by using a three-point mixing design. By selecting the top three performing media from this initial screening, it is possible to further increase growth and productivity by following a statistical approach to media mixing provided by Design Expert® computer software. Taken together, our data strongly suggests that using CHO Kit 1 with a combination of media screening and a statistical approach to media mixing can facilitate the rapid development of an optimized medium for any recombinant CHO clone.

Flexible format, rapid screening and media mixing

The six diverse animal component-free media in this kit are designed to maximize cell growth and recombinant protein production in a wide variety of CHO cell clones. Two of these six media are chemically defined and all six media differ in concentrations of amino acids, vitamins, salts, trace elements, recombinant human insulin, and other organic compounds. The format of the kit allows the user to rapidly screen all six of the media for cell growth, recombinant protein production or whatever other criteria the user deems important. If this initial screen yields satisfactory results, the researcher may decide that no further optimization is required. However, if the initial screening does not satisfy the specified criteria, a series of media-mixing assays can be completed.

Media mixing is the most efficient way to meet the diverse nutritional requirements of a particular cell line. As a result, the media selected for these mixing assays are absolutely critical. In order to select the best candidates to be mixed, it is important to examine all of the data from the initial screen and determine which criteria (i.e. growth kinetics, productivity) are most important. The format of this kit will use the three best performing media selected from the initial screen to perform the blending assays. Figure 1 depicts the diagram and mixing table used for the blending of three media selected (media A, B, and C).

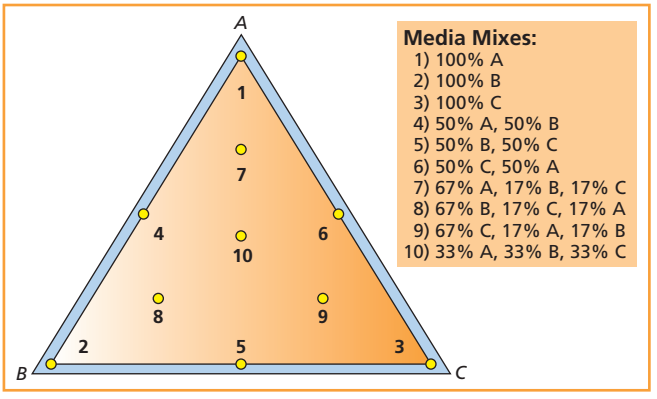
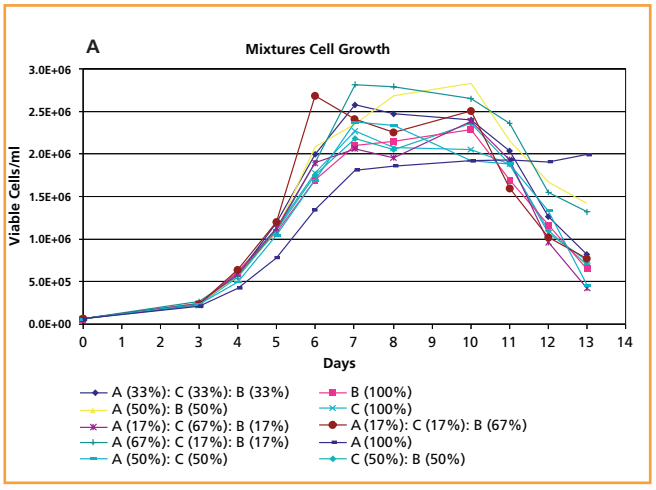


Figure 1. Representation of the three-point mixture triangle with the associate media mixes. *Each medium is set to 100% at vertices of the triangle (points 1, 2 and 3). Mixing begins at 50% between two of the media along the sides (points 4, 5 and 6). This is followed by a 67%, 17% and 17% mix of all three media within the interior of the triangle (points 7, 8 and 9). The final mixture will be 33% of all three media as seen at the axis of the triangle (point 10).*

Once the media-mixing experiments have been completed, there are two options for data analysis. The first option is to visually analyze the data for each criterion, as can be done by examining viable cell growth (Figure 2A). At the same time, maximum cell growth data can be normalized and plotted on the mixture triangle to see where the best mixes might be located (Figure 2B). The same method can be applied to analyze the productivity of recombinant protein and any other criteria.



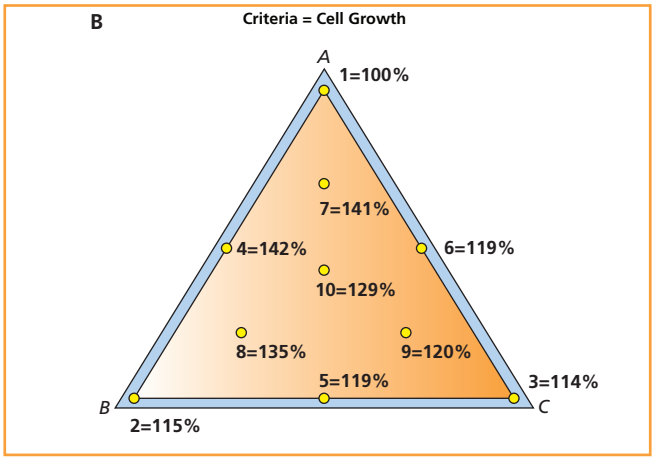


Figure 2. Representative comparison of cell growth for media mixes performed. *Media mixes above were formulated and tested based on results of the initial screen of all six media in the kit.* ***A).*** *Ten cell growth curves were obtained from the three-point medium-mixing assay as indicated in the three-point mixture triangle.* ***B).*** *The maximum cell densities from the different media mixes were normalized by using the number obtained from medium A as 100%. This data was plotted on the mixture triangle in order to estimate where the best mixes might be located.*

The second method of data analysis is more in-depth and involves the use of a design-of-experiment software package such as Design Expert®. The software analyzes the media-mixing data and allows the researcher to assign importance values to each criterion. Based on these inputs, mathematical models are used to predict the outcome of an infinite number of combinations of the three media, and their desirability based on which criteria are most important. The final outcome is one or several best-fit media designed specifically to meet the nutritional requirements of a particular cell line. This is illustrated in Figure 3 by a contour plot generated by the software to depict where on the mixture triangle the most desirable media mixes are located.

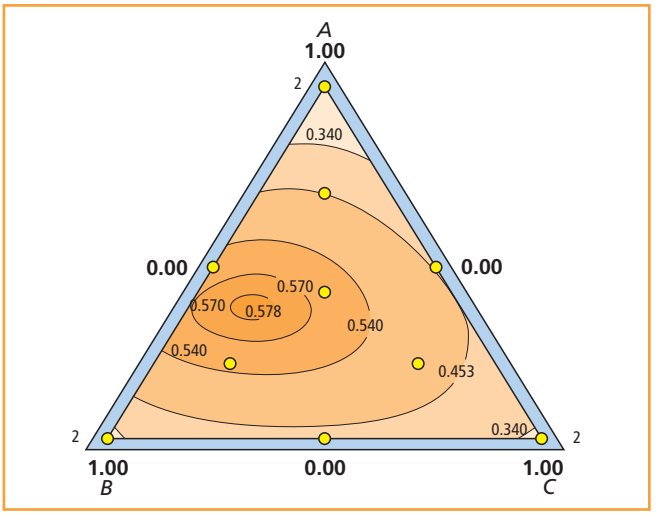


Figure 3. Contour plot indicating where the most desirable media mixes exist. *After inputting all the data collected from the mixture assay, importance values were assigned to each criterion. Design Expert then analyzed the data and generated a contour plot. Values indicated on the graph are based on 1.000 being the most desirable. Any point on the graph can be selected and identified by the exact ratio of the three media.*

As more and more recombinant CHO clones are developed, it becomes increasingly important to streamline the medium optimization process. To meet this need, CHO Kit 1 has been developed to provide researchers with a format for medium development that is efficient yet powerful. The convenient format allows for a rapid screening of multiple diverse CHO formulations designed for maximum recombinant protein production. In addition, the powerful mixture experiments coupled with the DOE software provide an invaluable tool for boosting cell growth and productivity. In either application, it is obvious that CHO Kit 1 will meet the needs of the majority of medium optimization projects.

Ordering Information

Product	Description	Unit
CH0001	CHO Kit 1, Animal Component-Free	1 kit

Kit Components

C 5467	CHO Medium, Animal Component-Free	1 L
C 8862	CHO DHFR⁻ Medium, Animal Component-Free	1 L
C 4726	CHO Medium, Chemically Defined, Animal Component-Free	1 L
C 9737	CHO Medium 4, Animal Component-Free	1 L
C 0363	CHO Medium 5, Animal Component-Free	1 L
C 0488	CHO Medium 6, Chemically Defined, Animal Component-Free	1 L