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ProductInformation

CHO Kit 1
Without L-Glutamine

Product Code CH0001
Storage Temperature 2-8 °C

Synonym: CHO PF-AF Medium Kit

Product Description
CHO Kit 1 is a diverse collection of six protein-free animal component-free medium formulations. Each of these media has been selected and optimized for enhanced recombinant protein expression in Chinese Hamster Ovary (CHO) cell systems.

The expression of recombinant proteins has increased in importance in both research and pharmaceutical manufacturing applications. CHO cells are one of the most frequently used systems for the expression of a variety of recombinant proteins. As such, individual CHO cell clones have highly specific nutritional needs in order to reach maximum cell growth or productivity. To determine which medium is optimal for an individual CHO cell clone, it may be necessary to screen multiple medium formulations. This kit provides a platform from which the user can screen several unique CHO modifications. Initial screening of the six media for growth and productivity may offer enough improvement over the current culture system to suffice. However, if further medium optimization is desired, the user can select the top three performing media based on cell growth or productivity. These three formulations can be combined in a mixture experiment and analyzed to determine an optimum medium based on growth and productivity.

Intended Use
For research use.

Components
The formulations include inorganic salts, HEPES, sodium bicarbonate, essential and non-essential amino acids, vitamins, recombinant human insulin, trace elements, and other organic compounds. In addition, four of the media formulations (Catalog no's C 5467, C 8862, C 9737 and C 0363) also contain plant-based protein hydrolysates. The remaining two formulations (Cat. no's C 4726 and C 0488) are without plant hydrolysates and are considered chemically defined. The addition of a surfactant to any of the media (such as Pluronic® F-68) is not required.

The formulations do not contain L-glutamine, phenol red, antibiotics, antimycotics, transferrin, and recombinant peptides. These media do not contain hypoxanthine or thymidine to allow their use with dihydrofolate reductase (dhfr) gene amplification systems.

Preparation Instructions
These media are supplied as a sterile 1X liquid. Aseptically add 4-8 mM L-glutamine (20-40 mL of 200mM L-glutamine, Sigma cat. no. G 7513) to each liter of medium prior to use.

Storage/Stability
These media are stable when stored at 2-8 °C and protected from light until the indicated expiration date on the label.

Procedure
Freezing and Thawing
CHO cells grown in CHO PF-AF Medium (Cat. no. C 5467) have been successfully frozen in liquid nitrogen and recovered. Cells must be in the mid-logarithmic phase of growth with greater than 90% viability.

1. Pellet cells by centrifugation for 5 minutes at 200 X g. Re-suspend at a concentration of 5×10^6 cells/mL in a 50:50 mixture of fresh CHO PF-AF Medium and conditioned CHO PF-AF Medium supplemented with DMSO at a final concentration of 7.5%.
2. Freeze cells in liquid nitrogen according to standard procedures (1 °C decrease per minute).

3. Recover cells by rapidly thawing the vial in a 37 °C water bath.
4. Dilute cells 1:10 in fresh CHO PF-AF Medium. Mix and centrifuge suspension at 200 x g for 5 minutes.
5. Re-suspend the pellet in 1 mL CHO PF-AF Medium. Add 9 mL additional fresh CHO PF-AF Medium.
6. Transfer suspension to a T-75 flask containing fresh CHO PF-AF Medium at a final volume of 30 mL. Suspension culture can be transferred to appropriate spinner culture after 2-3 days.

Adaptation to CHO PF-AF Medium

CHO cells adapted to serum free conditions are more readily able to provide good bases for medium development. It is recommended that CHO cells be adapted to serum-free conditions before using this kit. Minimal time is required to adapt CHO cells from serum-containing medium to CHO PF-AF Medium. For good adaptation, it is critical that cell viability is at least 90% and the cells are in the mid-logarithmic growth phase. Cells grown in serum-containing medium should be inoculated at a viable cell density of 2×10^5 cells/mL in a 1:1 mixture of serum-containing medium and CHO PF-AF Medium. Allow cells to reach a density of 1×10^6 cells/mL. Subculture at an initial density of 2×10^5 cells/mL into medium containing increasing proportions of CHO PF-AF Medium, first at 1:3 mix and then 1:7 mix (serum-containing medium: serum-free medium). Titration may be required at each subculture step to achieve a good single-cell suspension. Cells are considered adapted when the cell density reaches 1×10^6 cells/mL. This usually occurs within 7 days after inoculation. The time interval required for adaptation will vary by individual CHO clone. All cultures should be incubated at 37 °C in a humidified atmosphere at 5% CO_2.

Assay Format

This kit can be used in a variety of formats, including plate, T-flask, or spinner flask assays. The spinner format may have additional benefits for predicting upstream scale up issues. If spinners are the desired format, the recommended working volume is 100mL.

Inoculation

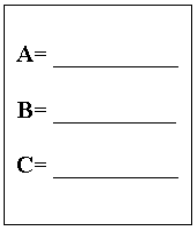
Prior to inoculation, CHO cells must be adapted to serum-free conditions and in the mid-logarithmic phase of growth with greater than 90% viability.

1. Harvest the appropriate volume of cells in conical centrifuge tubes. Pellet cells by centrifugation for 5 minutes at 200 x g.

2. Aspirate supernatant and re-suspend in HBSS at 5mL more volume than needed to inoculate at 1mL per flask.
3. Inoculation should be between 5×10^4 and 2×10^5 cells/mL.

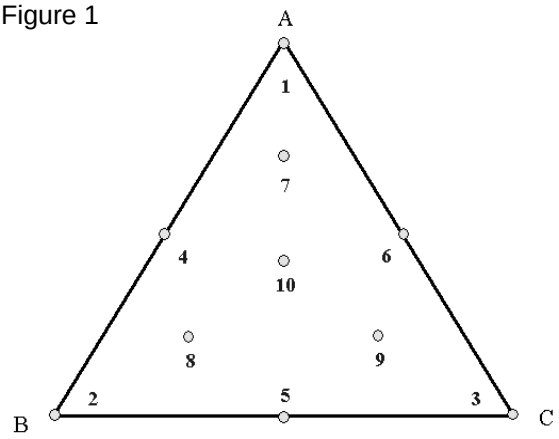
Media Screening and Media Mixing Procedure

CHO Kit1 allows the user to screen 6 of Sigma's CHO media at the same time. All media testing should be done in duplicate. After the initial screening of all six media, the user may decide that further optimization may be required in order to increase cell growth and productivity. Based on these criteria, the user can select the three best performing media (such as A, B and C) and perform a mixing assay.



As illustrated by the following diagram and mixing table (as shown in Figure 1), the three media will be set at 100% at one of the vertices of the triangle (Fig. 1, points 1,2 and 3). Mixing will begin at 50% between two of the media along the sides (Fig.1, points 4,5 and 6). This will be followed by a mixture of 67%, 17%, and 17% of all three media within the interior of the triangle (Fig.1, points 7,8 and 9). The final mixing will be 33% of all three media as seen at the axis of the triangle (Fig.1, point 10).

Figure 1



Media Mixes:

1.) 100% A
2.) 100% B
3.) 100% C
4.) 50% A, 50% B
5.) 50% B, 50% C
6.) 50% C, 50% A
7.) 67% A, 17% B, 17% C
8.) 67% B, 17% C, 17% A
9.) 67% C, 17% A, 17% B
10.) 33% A, 33%B, 33%C

Data Analysis

Data analysis can be done by two methods. The first method is for the user to normalize the data and make a visual comparison of the mixtures based on criteria such as cell growth or productivity. An example of this method is presented in Figures 4 and 5 of the product application section.

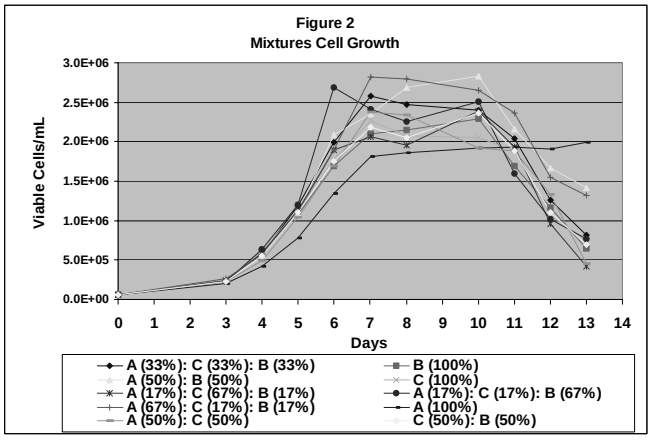
If the user desires a more in depth analysis, a design-of-experiment (DOE) software package such as Design-Expert (Stat-Ease, Inc. A free 30 day trial can be obtained at www.statease.com) can be used. By using DOE software such as Design-Expert, it is possible to find one or several best-fit media for a particular CHO clone. The software accomplishes this by using mathematical modeling to predict the outcome of an infinite number of combinations of the three media selected. One unique feature of this program is that specified criteria can be assigned importance values based on the user's need and desired outcome. Design-Expert can then recommend one or several combinations of the media that were mixed, in order to provide the user with the best medium possible.

Alternatively, the user can normalize the data and send it back to Sigma-Aldrich's Cell Culture R&D group via e-mail (cellculture@sial.com) for data analysis.

Product Application

Sigma's CHO Kit 1 was tested by using several different recombinant CHO clones to determine the value of mixing media after an initial screen of all six media. Representative data obtained from using one of these recombinant clones is presented in Figures 2-5. CHO cells were adapted to serum-free conditions prior to the start of the experiments. Cells were inoculated at a density of 5 x 10^4 cells/mL. After the first quick screening of the six CHO media, three media were selected for additional media optimization (media A, B and C).

Figures 2 and 3 show that by mixing the top three performing media from the initial screen, it was possible to find several combinations that increased performance. In this case, performance was evaluated based on cell growth and productivity separately.



A quick comparison of the normalized data is shown in the following figures. The maximum growth and productivity data was normalized and ranked, as shown in Figure 4. The growth data was then plotted on the data application sheet, as shown in Figure 5. In this example, it is clear that the best media mixtures for cell growth are located around points 4, 7, 8 and 10.

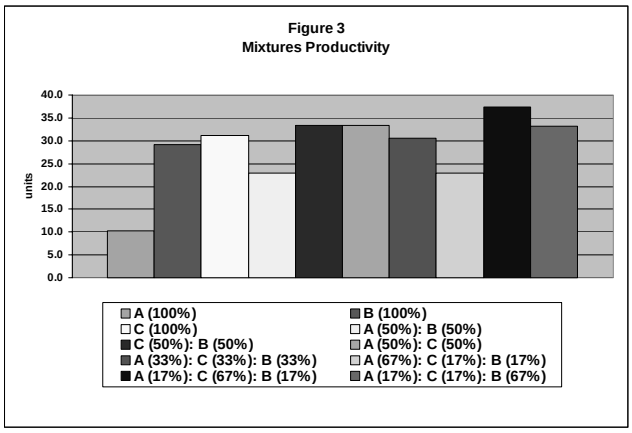
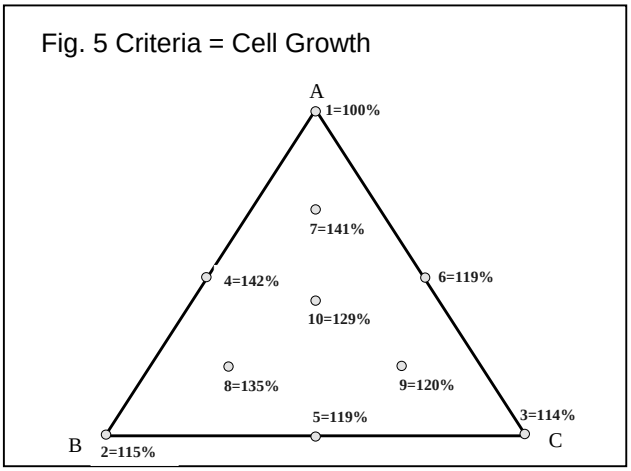


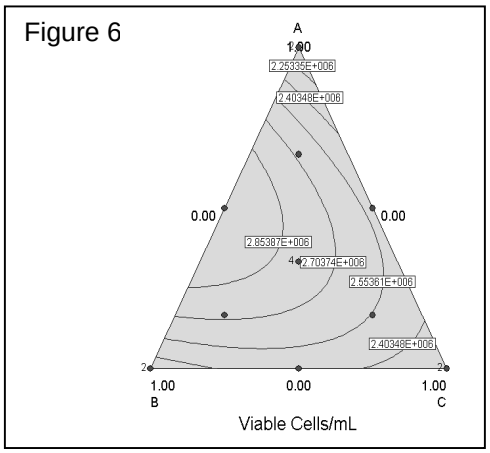
Figure 4

Normalized Mixture Data

Growth		**Productivity**	
50% A, 50% B	= 142%	67% C, 17 % A, 17% B	= 367%
67% A, 17% B, 17% C	= 141%	50% A, 50 % C	= 327%
67% B, 17% C, 17% A	= 135%	50% C, 50% B	= 327%
33% A, 33% B, 33% C	= 129%	67% B, 17% C, 17% A	= 325%
67% C, 17% A, 17% B	= 120%	100% C	= 306%
50% A, 50% C	= 119%	33% A, 33% B, 33% C	= 300%
50% C, 50% B	= 119%	100% B	= 286%
100% B	= 115%	50% A, 50% B	= 225%
100% C	= 114%	67% A, 17% B, 17% C	= 225%
100% A	= 100 %	100% A	= 100%



For a more precise analysis and optimization, Design-Expert Software was used. It is important to note that this program can be used to analyze each criteria separately, or multiple criteria together. We start by entering the data collected for each criteria. Figures 6 shows the contour plot for our first criteria, cell growth. Figure 7 is a similar plot for our second criteria, production. Once all data has been entered, the software will then analyze our data using mathematical models. After the analysis comes optimization in which we can select each criteria we want to optimize and assign importance values based on the desired outcome. Figure 8 is a graphical representation of the desirability of all mixtures that can be formulated from the optimization step. The desirability ranges from 0 to 1, and indicates how the predicted mixtures might perform. In our experiment we assigned equally high importance values for both criteria. The result is a predicted medium mixture of 51% of A, 46% of B and 3% of C that will produce the maximum cell growth and productivity for our particular clone.



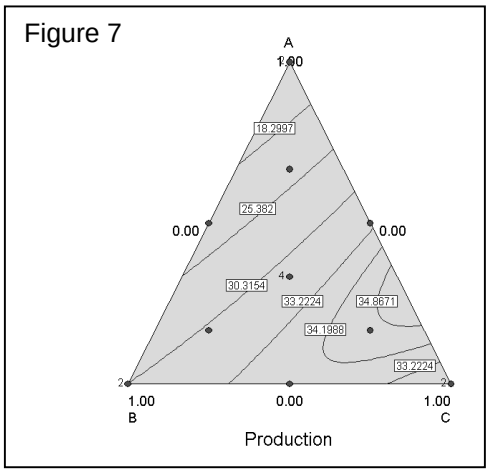
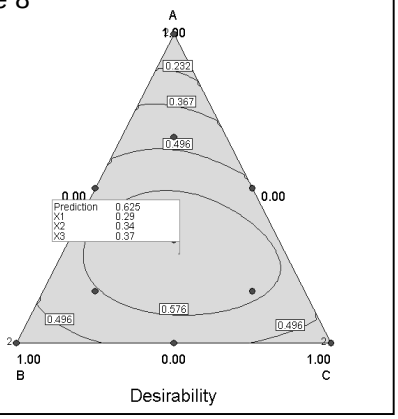


Figure 8



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Precautions and Disclamer

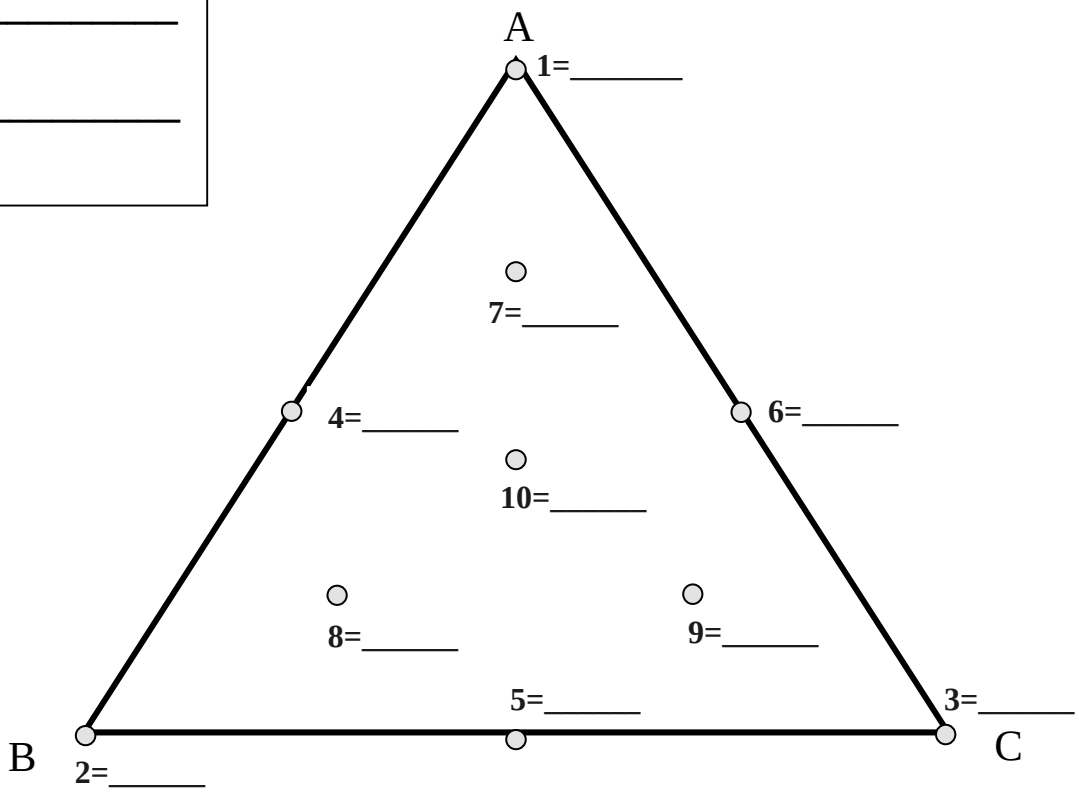
MSDS is availiable upon request or at www.sigma-aldrich.com. Pluronic is a registered trademark of BASF Corporation. Design-Expert is a registered trademark of Stat-Ease, Inc.

Criteria: _____(Growth or Productivity)

A= _____

B= _____

C= _____



Media Mixes:	**Normalized Data**	**Ranking**
1.) 100% A	= _____	= _____
2.) 100% B	= _____	= _____
3.) 100% C	= _____	= _____
4.) 50% A, 50% B	= _____	= _____
5.) 50% B, 50% C	= _____	= _____
6.) 50% C, 50% A	= _____	= _____
7.) 67% A, 17% B, 17% C	= _____	= _____
8.) 67% B, 17% C, 17% A	= _____	= _____
9.) 67% C, 17% A, 17% B	= _____	= _____
10.) 33% A, 33%B, 33%C	= _____	= _____