cell culture

CHO CD-3 Chemically-Defined Medium Sustains Better Growth Than Hydrolysate-Containing Media in Multiple CHO Recombinant Cell Lines

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Application Notes

- Chemically-defined and animal component-free
- Unsurpassed growth and productivity as compared to hydrolysate-containing formulations
- Quick and efficient adaptation of a wide variety of CHO clones directly into chemically-defined formulation
- Directly scalable to larger stirred-tank bioreactor systems

Introduction

Chinese Hamster Ovary (CHO) cells are the most frequently used expression system for the production of recombinant proteins that require post-translational modification to express full biological function. Recombinant CHO clones can be grown in traditional serum-supplemented medium to the most sophisticated chemically-defined animal component-free medium. As the number of recombinant therapeutic proteins produced in CHO systems increases, the methods used to produce them are facing increased regulatory scrutiny. Consequently, chemically-defined formulations are increasingly preferred by biopharmaceutical clients using CHO cells for drug production.

The most challenging aspect of developing chemically-defined formulations is the replacement of specific components with suitable and cost-effective chemically-defined substitutes, while maintaining optimal protein productivity and minimizing any potential impact to downstream purification processes. By using statistical experimental design and novel analytical methods, we have developed a new chemically-defined CHO medium that achieves these goals.

Meeting the demand for chemically-defined formulations

CHO CD-3 Medium (Product Code C 1490) meets all regulatory concerns for the biopharmaceutical industry by eliminating any animal-derived components in its formula. Additionally, all undefined components such as plant-derived hydrolysates that could result in batch-to-batch variability have been eliminated and other components have been redeveloped for use in this product. CHO CD-3 Medium, chemically-defined and animal component-free, is designed to deliver optimal cell growth and recombinant protein expression in suspension culture. The medium does not contain hypoxanthine and thymidine to permit its use in dihydrofolate reductase (dhfr) gene amplification systems.

Media comparison with competitors' chemically-defined and hydrolysate-containing formulations

Sigma-Aldrich's CHO CD-3 Medium was compared to CHO media from two major competitors (A, B) for growth and productivity in 250-ml spinner flasks. Competitor A medium is a serum-free CHO medium containing plant-derived hydrolysates. Competitor B medium is a chemically-defined CHO medium. For these studies several CHO clones were used – one producing a monoclonal antibody and the second producing a recombinant protein from a CHO-K1-derived parental cell. The clones were adapted to a different non-animal component, serum-free CHO Medium (Product Code C 5467) prior to the start of the experiments. Cells were then inoculated at a density of 5 x 10^4 cells/ml and grown in CHO CD-3 Medium or one of the Competitors' formulations. Figures 1 and 3 illustrate that Sigma's CHO CD-3 Medium consistently supports the highest cell density for CHO cell lines producing recombinant antibody (Cell Lines 1 and 2). Figures 2 and 4 show that CHO CD-3 Medium supports equal or better recombinant protein production as compared to the media of Competitors A and B.

Quick adaptation to CHO CD-3 Medium

The newly developed Sigma-Aldrich CHO CD-3 Medium allows easier adaptation from a protein-free formulation as compared to the two competitors' chemically-defined formulations. Multiple CHO clones have consistently shown transition from protein-free formulations to the newly developed chemically-defined medium with little to no adaptation delay (Figure 5). Most clones have shown immediate adaptability by direct inoculation into Sigma-Aldrich's CHO CD-3 Medium with growth comparable to protein-free formulations. Several competitive formulations need extensive adaptation time, which in some cases can take up to several months.

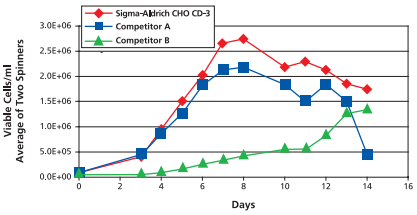

Figure 1. Growth experiment Cell Line 1. *This growth curve indicates that Sigma-Aldrich CHO CD-3 Medium attained a maximum viable cell density of 2.75 x 10^6 cells/ml by day 8 as compared to the CHO media of competitors A and B.*

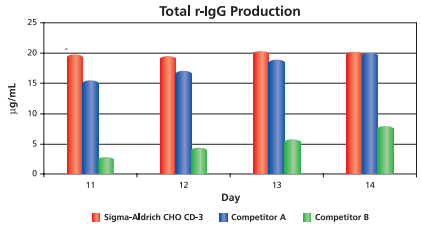

Figure 2. Total r-IgG production Cell Line 1. *By day 14 in this clone, the productivity of Sigma-Aldrich CHO CD-3 Medium is equivalent as compared to the hydrolysate-containing medium of competitor A and superior to the chemically-defined medium of competitor B.*

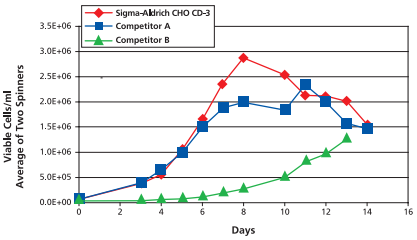

Figure 3. Growth experiment Cell Line 2. *The growth curve indicates that Sigma-Aldrich CHO CD-3 Medium attained a maximum viable cell density of 2.86 x 10^6 cells/ml by day 8 as compared to the CHO media of competitors A and B.*

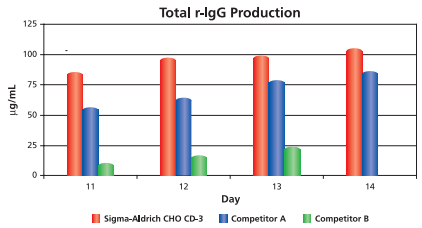

Figure 4. Total r-IgG production Cell Line 2. *By day 14 in this clone, the productivity of Sigma-Aldrich CHO CD-3 is the best as compared to the hydrolysate-containing media of competitor A and the chemically-defined medium of competitor B.*

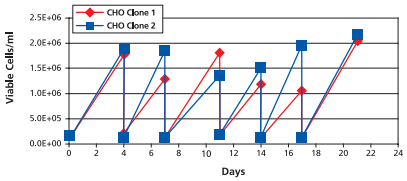

Figure 5. Cell Growth and Adaptation to CHO CD-3 Medium. *Different CHO clones grown in serum-free media were directly inoculated into CHO CD-3 Medium. Cells were multiply passaged every 3-5 days. This graph depicts how quickly the cells adapted and are maintained in CHO CD-3 Medium. CHO clones 1 and 2 are fully adapted within the first week.*

Directly scalable to stirred-tank bioreactors

CHO CD-3 Medium has been designed for the ultimate user goal of scalability to large volume stirred-tank bioreactors. CHO cell cultures can be directly transferred from CHO CD-3 containing spinner flasks to 5 L bioreactors. Under sub-optimized non-fed batch bioreactor conditions, several CHO clones have shown similar cell growth and recombinant protein production as previously seen in the spinner flasks (Figures 6 and 7). The new version of CHO CD-3 makes it easy to scale-up to larger bioreactor systems. All work described here was completed in 125-250 ml spinner cultures with sequential development in 5 L bioreactors for confirmation of scalability. Our studies have shown that direct inoculation into 5 L bioreactors deliver equivalent growth and productivity with no additional formulation adjustments. This allows for quick movement from clonal development to pilot scale with no impedance from the medium. This was repeated with several cell lines to identify any minute differences relative to bioreactor cell culture conditions. This will allow for process development to achieve quick scale-up when this formulation is moved from pilot scale into final production scale.

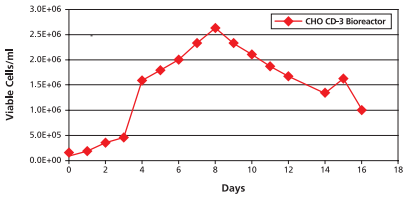

Figure 6. Growth experiment Cell Line 2. *The growth indicates that Sigma-Aldrich CHO CD-3 achieved a maximum viable cell density of 2.65 x 10^6 cells/ml by day 8 in a 5 L stirred-tank bioreactor.*

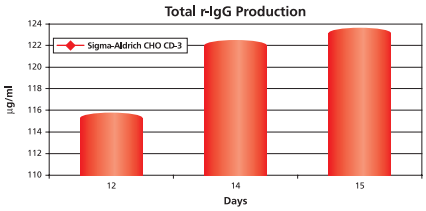

Figure 7. Total r-IgG production Cell Line 2. *The productivity of Sigma-Aldrich CHO CD-3 demonstrates the direct scalability into larger bioreactor systems. Total productivity for Cell Line 2 is equivalent to small-scale spinners.*

Ordering Information

Product	Description	Unit
C 1490	CHO CD-3 Medium, Chemically-Defined, Animal Component-Free	1 L 6 x 1 L

Order: 1.800.325.3010 Technical Service: 1.800.325.5832
SIGMA