

# cell culture

## Creating a New Medium to Meet the Variable Nutritional Requirements of Chinese Hamster Ovary (CHO) Cell Clones

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### Application Notes

- Animal component-free – contains no transferrin or other products of animal origin
- Designed for use with DHFR<sup>-</sup> cell clones – no hypoxanthine or thymidine
- Promotes outstanding cell growth and recombinant protein productivity
- Supports proper glycosylation of recombinant proteins
- Exceptional performance in other selection systems

### Introduction

Chinese Hamster Ovary (CHO) cells are frequently used to express recombinant proteins that require post-translational modification to yield full biological function. Since more and more biopharmaceutical companies are producing their potential therapeutic agents in CHO cells, there has been increased regulatory scrutiny of the medium in which the cells are grown. As a result, animal component-free media have now come to the forefront for use with CHO cells.

It is widely known that different recombinant protein expressing CHO cell clones can have variable nutritional requirements. Sigma has previously developed CHO Animal Component-free Medium (CHO-AF, Product Code [C 5467](#)), to support the suspension culture of CHO cells and to achieve the desired recombinant protein expression. This medium has proven to be the medium of choice for many recombinant protein producing cell lines.

However, not all cell lines perform optimally in this medium. In order to obtain maximal growth and recombinant protein production with those clones for which CHO-AF is not optimal, Sigma has created CHO DHFR<sup>-</sup> Medium, Animal Component-free (Product Code [C 8862](#)). This new medium is designed to give outstanding results in Dihydrofolate Reductase (DHFR) clones. The DHFR system allows one to greatly increase the gene of interest copy number within their specific clone, leading to increased recombinant protein expression.

### The DHFR gene amplification system

The Dihydrofolate Reductase gene amplification system has become very popular for use in developing recombinant CHO cell lines. The DHFR system allows the gene of interest to be amplified many times by gradually increasing the concentration of methotrexate, an inhibitor of dihydrofolate reductase. Utilizing this system can lead to increased recombinant protein expression. CHO DHFR<sup>-</sup> Medium (Product Code [C 8862](#)) is the first commercially available medium designed specifically for DHFR CHO clones. However, this formulation also works quite well for many non-DHFR<sup>-</sup> cell lines.

As previously stated, CHO DHFR<sup>-</sup> Medium was developed in response to data suggesting that our previous CHO-AF medium was not performing as expected with a significant number of recombinant CHO cell clones. This performance difference can be seen in Figure 1. In this experiment (performed in Techne spinner flasks), CHO-AF yields sub-optimal growth and productivity with a proprietary CHO clone that produces a recombinant IgG (CHO-IgG). As is shown, CHO DHFR<sup>-</sup> Medium yields greatly improved results over our CHO-AF Medium with this particular clone.

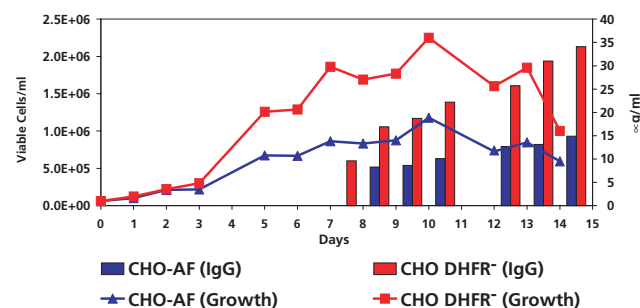
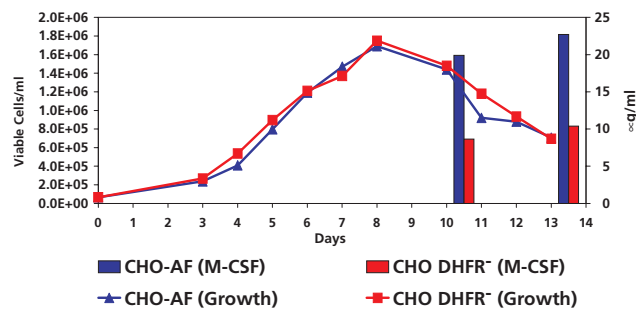


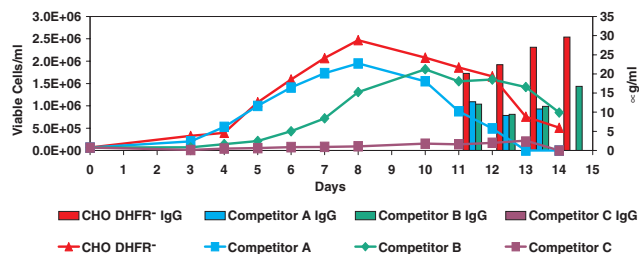
Figure 1. Comparison between CHO-AF and CHO DHFR<sup>-</sup> media for growth and recombinant IgG production. In this experiment CHO-IgG cells, grown in CHO-AF Medium, were seeded in Techne spinner flasks. The results show that CHO DHFR<sup>-</sup> Medium supports much better cell growth and recombinant IgG production.

The variable nutritional requirements of different CHO clones are clearly demonstrated by the results presented in Figure 2. Here the CHO-M-CSF cell line, which produces recombinant human M-CSF and is available from ATCC (CRL-10154), grew similarly in both the CHO-AF and CHO DHFR<sup>-</sup> media. However, rh-M-CSF productivity was much higher in the cells grown in CHO-AF Medium. As such, the development of CHO DHFR<sup>-</sup> Medium is not designed to replace our CHO-AF Medium, but rather to complement it.

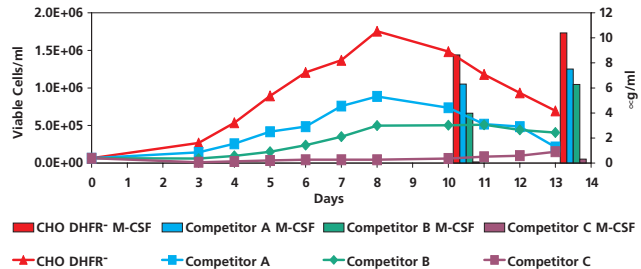


**Figure 2. Comparison between CHO-AF and CHO DHFR<sup>-</sup> media for growth and recombinant M-CSF production.** In this experiment CHO-M-CSF cells, grown in CHO-AF Medium, were seeded in Techne spinner flasks. The results show that the growth in CHO-AF Medium and CHO DHFR<sup>-</sup> Medium were very similar, with a much enhanced production of rh-M-CSF in CHO-AF Medium.

Figures 3 and 4 show how CHO DHFR<sup>-</sup> Medium compares to other commercially available media. With the CHO-IgG cell line (Figure 3), CHO DHFR<sup>-</sup> Medium supported about a 20% higher maximum cell density than the closest competitors. Perhaps more importantly, CHO DHFR<sup>-</sup> Medium greatly surpassed the other media in terms of total IgG production. In Figure 4, CHO DHFR<sup>-</sup> Medium yielded superior performance for both growth and recombinant protein production for the CHO-M-CSF cell line, when compared to the competition.



**Figure 3. Performance Comparison with CHO-IgG clone.** Growth and recombinant IgG production were analyzed in this spinner experiment that compares CHO DHFR<sup>-</sup> Medium to other commercially available media. CHO DHFR<sup>-</sup> Medium yielded the highest for both.



**Figure 4. Performance Comparison with CHO-M-CSF clone.** Growth and recombinant human M-CSF production were analyzed in this spinner experiment that compares CHO DHFR<sup>-</sup> Medium to other commercially available media. Again, CHO DHFR<sup>-</sup> Medium yielded the highest for both cell growth and productivity.

Taken together, the data demonstrates that the nutritional requirements for different CHO clones can vary significantly. Therefore Sigma's new product, CHO DHFR<sup>-</sup> Medium, along with our CHO-AF Medium, can be a potent combination for scientists growing recombinant CHO cells.

### Ordering Information

Product	Description	Unit
<a href="#">C 8862</a>	CHO DHFR Medium, Animal Component-Free	1 L 6 x 1 L