3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

CHO DHFR $^-$ MEDIUM, Animal Component-Free
Without L-Glutamine

Product Code **C 8862**
Storage Temperature 2-8 °C

Synonym: CHO DHFR$^-$ Medium, AF

Product Description

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 production of recombinant proteins that require post-translational modification to express full biological function. Recently, CHO DHFR$^-$ cell clones and the DHFR gene amplification system have been commonly employed in the pharmaceutical industry for the production of recombinant proteins. This system allows one to enhance recombinant protein production through the amplification of recombinant gene copy by addition of methotrexate to culture medium that does not contain nucleosides.

CHO DHFR$^-$Medium (Product Code C 8862) is an animal component-free formulation containing no hypoxanthine and no thymidine. The CHO DHFR$^-$ Medium has been optimized for maximum cell growth and recombinant protein production using the Dihydrofolate Reductase (DHFR) gene amplification system in DHFR$^-$ Chinese Hamster Ovary (CHO) cells.

Although this medium was optimized with multiple DHFR$^-$ clones, it also supports superior cell growth and recombinant protein production with non-DHFR$^-$ derived cell clones tested in-house. It is well known that different CHO recombinant clones require different compositions of nutrients to achieve optimized cell growth and recombinant protein production. Therefore, the development and use of this CHO DHFR$^-$Medium was not intended to replace Sigma's original CHO Animal Component-Free medium (Product Code C 5467), but rather to complement it.

Precautions and Disclaimer

For R&D use only. Not for drug, household or other uses.
MSDS is available upon request or at www.sigma-aldrich.com. Pluronic is a registered trademark of BASF Corporation.

Components

The formulation includes inorganic salts, HEPES, sodium bicarbonate, essential and non-essential amino acids, vitamins, recombinant human insulin, plant hydrolysates, trace elements, and other organic compounds.
It does not contain L-glutamine, phenol red, antibiotics, antimycotics, transferrin, and recombinant peptides. This medium does not contain hypoxanthine or thymidine to allow its use with dihydrofolate reductase (DHFR) gene amplification systems.

Preparation Instructions

This medium is supplied as a sterile 1X liquid. Aseptically add 20-40 ml of 200 mM L-glutamine (Product Code G 7513) to each liter of medium prior to use. The addition of a surfactant (such as Pluronic® F-68) is not required.

Storage/Stability

This medium is stable, when stored 2-8 °C and protected from light, until the indicated expiration date on the label.

Procedure

Freezing and Thawing

CHO cells grown in CHO DHFR$^-$ Medium 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 x 10^6 cells/ml in a 50:50 mixture of fresh CHO DHFR$^-$ Medium and conditioned CHO DHFR$^-$ 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 DHFR$^-$ Medium. Mix and centrifuge suspension at 200 x *g* for 5 minutes.

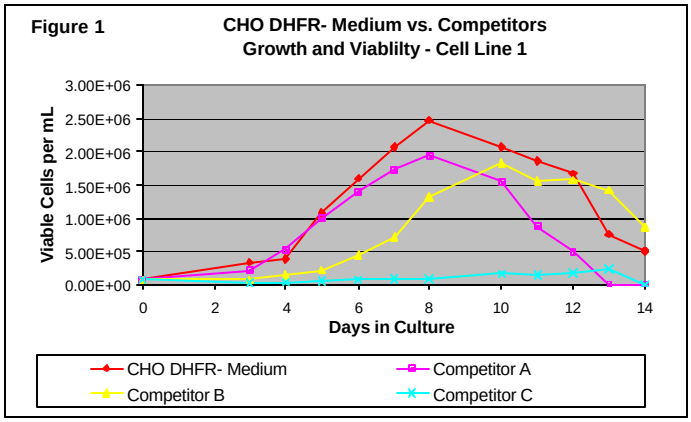
5. Re-suspend the pellet in 1 ml CHO DHFR$^-$ Medium. Add 9 ml additional fresh CHO DHFR$^-$ Medium.
6. Transfer suspension to a T-75 flask containing fresh CHO DHFR$^-$ Medium at a final volume of 30 ml. Suspension culture can be transferred to appropriate spinner culture after 2-3 days.

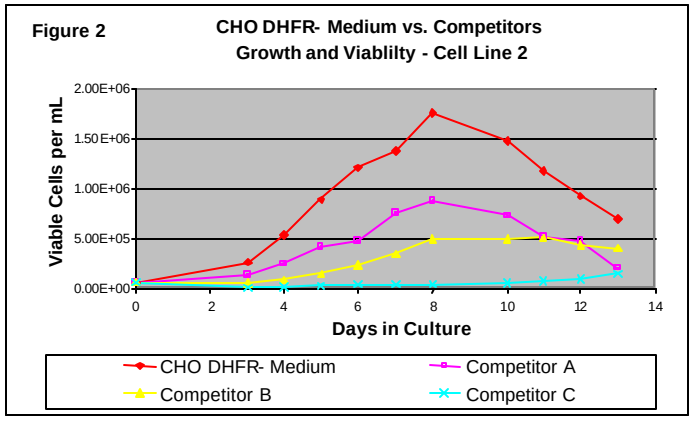
Adaptation to CHO DHFR$^-$ Medium

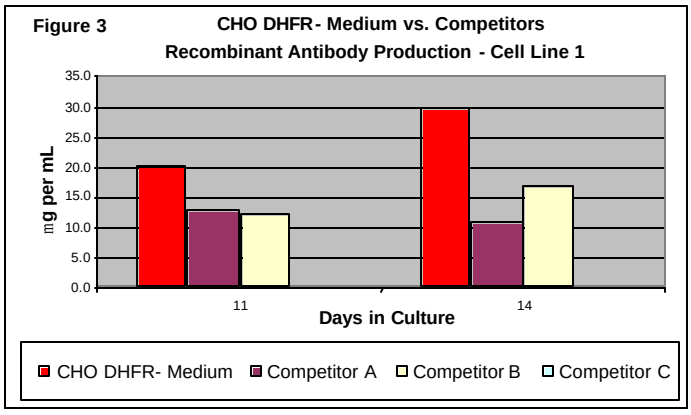
Minimal time is required to adapt CHO cells from serum-containing medium to CHO DHFR$^-$ Medium. For good adaptation, it is critical that cell viability is at least 90% and the cells are in the mid-logarithmic growth phase. Cells grown in serum-containing medium should be inoculated at a viable cell density of 2 x 10^5 cells/ml in a 1:1 mixture of serum-containing medium and CHO DHFR$^-$ Medium. Allow cells to reach a density of 1 x 10^6 cells/ml. Subculture at an initial density of 2 x 10^5 cells/ml into medium containing increasing proportions of CHO DHFR$^-$ 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 x 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.

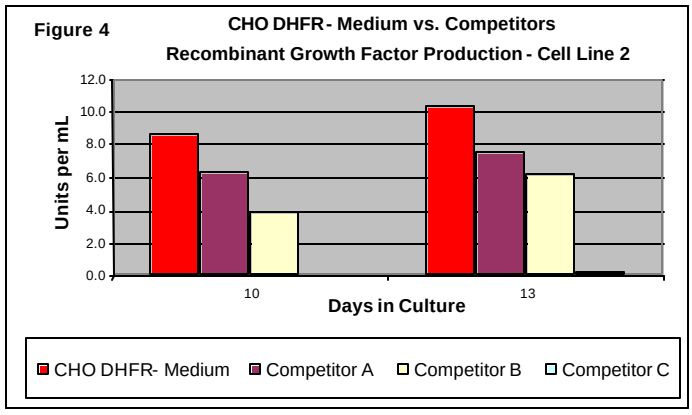
Product Profile

Sigma's CHO DHFR$^-$ Medium (Product Code C8862) was compared to CHO media from three competitors (A, B, C) for growth and productivity in spinner flasks. For these studies, CHO cells were adapted to a different 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 DHFR$^-$ Medium or one of the competitors' formulations. Figures 1 and 2 illustrate that Sigma's CHO DHFR$^-$ Medium consistently supported the highest cell density and viability for CHO cell lines producing a recombinant antibody (Cell Line 1) and a recombinant human growth factor (Cell Line 2). Figures 3 and 4 show that CHO DHFR$^-$ Medium ranks at the top of commercially available products for recombinant protein expression.









References

1. Bjare, U., Serum-free culture. Pharmacol. Ther., **53(3),** 355-374 (1992).
2. Kim, E.J. et al., Development of a serum-free medium for dihydrofolate reductase-deficient Chinese hamster ovary cells (DG44) using a statistical design: beneficial effect of weaning cells. In Vitro Cell Dev. Biol. Animal. **35(4),** 178-182 (1999).
3. Merten, O.W., Safety issues of animal products used in serum-free media. Dev. Biol. Stand., **99**,167-180 (1999).
4. Sinacore, M.S. et al., Adaptation of mammalian cells to growth in serum-free media. Mol. Biotechnol., **15(3),** 249-257 (2000).

Revision date: 2003-10