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 Protein-Free Medium
Without L-glutamine

Product Code **C 5467**
Storage Temperature 2-8 °C

Synonym: CHO PF-AF Medium

Product Description

CHO Protein-free Medium is an **animal component-free** formulation. It is optimized for use in recombinant protein expression and production 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 recombinant proteins that require post-translational modification to express biological function.

As more recombinant proteins are being employed as therapeutic agents, the methods used in their production are coming under increasing regulatory scrutiny. One of the areas of concern is the use of animal-derived components in media to grow cells for recombinant protein expression. By using CHO PF-AF Medium, regulatory concerns associated with animal-derived components have been eliminated.

Intended Use

Caution: For manufacturing, processing, or repacking.

Components

The formulation includes inorganic salts, HEPES, sodium bicarbonate, essential and non-essential amino acids, vitamins, **recombinant human insulin**, plant hydrolysates, trace elements, Pluronic® F-68, and other organic compounds.

It does not contain L-glutamine, phenol red, antibiotics, antimycotics, or transferrin. This medium does not contain hypoxanthine or thymidine. It can be used with dihydrofolate reductase (dhfr) gene amplification and glutamine synthetase systems.

Precautions and Disclaimer

MSDS is available upon request or at **sigma-aldrich.com**.

Preparation Instructions

This medium is supplied as a sterile 1X liquid. Aseptically add 20 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 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 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 of CHO PF-AF Medium. Add 9 ml of 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

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 x 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 x 10^6 cells/ml. Subculture at an initial density of 2 x 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 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.

References

1. Merten, O.W., Safety issues of animal products used in serum-free media. Dev. Biol. Stand., **99**,167-180 (1999).
2. Sinacore, M.S. *et al*., Adaptation of mammalian cells to growth in serum-free media. Mol. Biotechnol., **15(3),** 249-257 (2000).
3. 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).
4. Bjare, U., Serum-free culture. Pharmacol. Ther., **53(3),** 355-374 (1992).

LCM,MAM 04/05-1

Product Profile

Sigma's CHO PF-AF Medium (Product Code C 5467) was compared to CHO media from three competitors (A, B, C) for growth and productivity. For these studies, CHO cells were adapted to the test media prior to the start of the experiments. Cells were then inoculated at a density of 2 x 10^5 cells/ml and grown in CHO PF-AF Medium or one of the competitors' formulations. DME/F12 supplemented with 10% FBS was included as an additional control. **Figure 1** illustrates that Sigma's CHO PF-AF Medium consistently supported the highest cell density and viability. **Figure 2** shows that CHO PF-AF Medium ranks at the top of commercially available products for productivity.

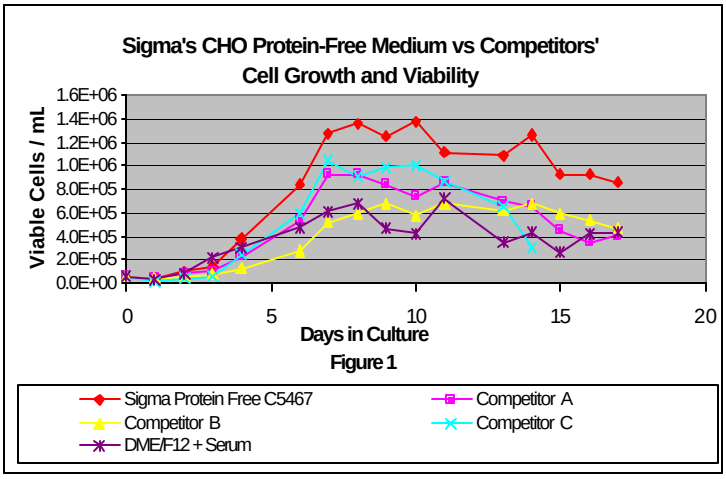

Figure 1

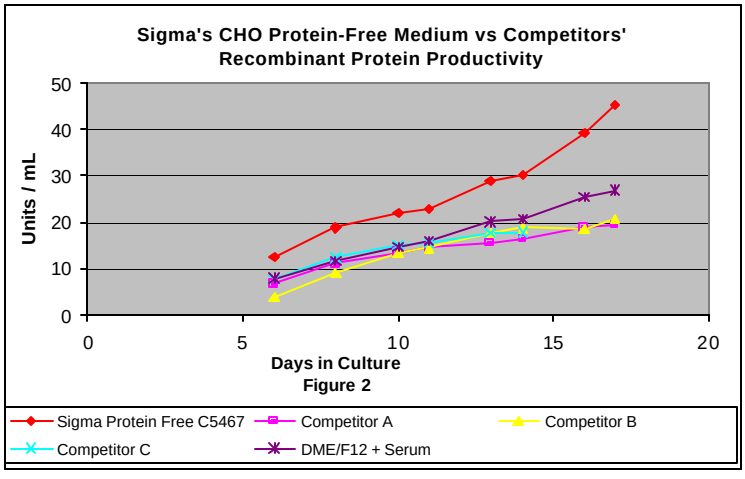

Figure 2