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 MEDIUM, CHEMICALLY-DEFINED, ANIMAL COMPONENT-FREE**
Without L-glutamine

Product Code **C 4726**
Storage Temperature 2-8 °C

Synonym: CD CHO Medium, Chemically-defined CHO Medium

## Product Description

CHO Medium, Chemically-defined, Animal Component-free is a complex medium that was developed to achieve optimal recombinant protein expression in CHO cell systems. As more and more recombinant proteins are being employed as therapeutic agents, the methods employed in their production are coming under increasing regulatory scrutiny. One of the areas of regulatory concern is the presence of animal-derived components in the media employed for the growth of cells used for recombinant protein expression. With the utilization of CD CHO Medium, all regulatory concerns associated with animal-derived components have been eliminated. All undefined components that could result in batch-to-batch variability have been eliminated in the development of this product.

## Precautions and Disclaimer

Caution: For manufacturing, processing or repacking. MSDS is available upon request or at **sigma-aldrich.com**. Pluronic® is a registered trademark of BASF Corporation.

## Components

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

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

## Preparation Instructions

This medium is supplied as a sterile 1X liquid. Aseptically add 20 ml of 200 mM L-glutamine (Product Code G7513) 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

Adaptation to CD-CHO Medium

Minimal time is required to adapt CHO cells from serum-containing medium to CD-CHO 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 $2 \times 10^5$ cells/ml in a 1:1 mixture of serum-containing medium and CD-CHO Medium. Allow cells to reach a density of $1 \times 10^6$ cells/ml. Subculture at an initial density of $2 \times 10^5$ cells/ml into medium containing increasing proportions of CD-CHO Medium, first at 1:3 ratio and then 1:7 ratio (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 $1 \times 10^6$ cells/ml. This usually occurs within 7 days after inoculation. The time interval required for adaptation will vary by individual CHO clone. Serum-free CHO cultures in suspension may form clusters of 2 to 10 cells; therefore, it may be necessary to triturate at each subculture to achieve a single cell suspension. All cultures should be incubated at 37 °C in a humidified atmosphere at 5% $CO_2$.

Freezing and Thawing

CHO cells grown in CD-CHO 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 CD-CHO Medium and conditioned CD-CHO 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 CD-CHO Medium. Mix and centrifuge suspension at 200 x g for 5 minutes.
5. Re-suspend the pellet in 1 ml CD-CHO Medium. Add 9 ml additional fresh CD-CHO Medium.
6. Transfer suspension to a T-75 flask containing fresh CD-CHO Medium at a final volume of 30 ml. Suspension culture can be transferred to appropriate spinner culture after 2-3 days.

**Product Profile**

Sigma's CD-CHO Medium (Product Code C4726) was compared to a leading competitor's chemically defined CHO medium (Competitor A) 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 CD-CHO Medium and the competitor's formulation. DME/F12 supplemented with 10% FBS was included as an additional control. **Figure 1** illustrates that Sigma's CD-CHO Medium consistently supported higher cell densities and viability. **Figure 2** shows that CD-CHO Medium achieves higher productivity than both the competitor's chemically defined CHO medium and the serum-containing control medium.

**Fig. 1 Sigma Chemically Defined CHO Media Growth**

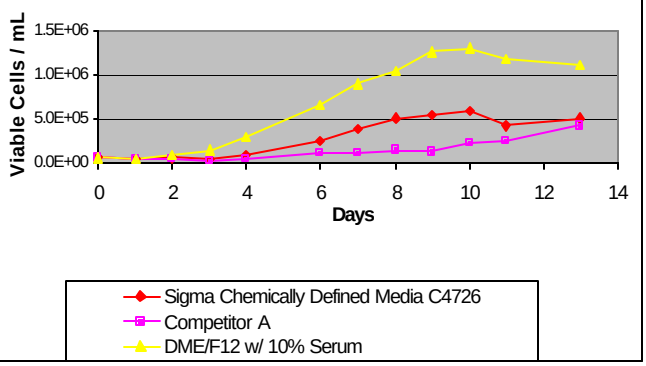


**Fig. 2 Sigma Chemically Defined CHO Media Productivity**