

CHO-S-SFM II

Serum-Free Medium for the Growth of CHO Cells and Expression of Recombinant Proteins in Suspension Culture

CAUTION: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Cat. No.: 12052-114 500 mL
12052-098 1000 mL

Component-Deficient Media:
31033-020 500 mL
without hypoxanthine
without thymidine
(Custom packaging available upon request)

Storage Conditions: 2 to 8°C, in the dark.

NOTE: For catalog number 31033 please also refer to Component-Deficient Media Supplementation below.

Background

CHO-S-SFM II is a complete, serum-free, low protein (<100 µg/mL) cell culture medium optimized for the growth and maintenance of Chinese Hamster Ovary (CHO) cells and production of recombinant proteins. This medium is designed for suspension culture of CHO cells in batch, continuous culture and perfusion systems. It is supplied as a complete, ready-to-use liquid medium in a variety of sizes.

CHO cells are the most widely used mammalian cells for the expression of a variety of recombinant proteins owing to their low rate of spontaneous transformation. Serum-free culture of these cells is desirable as it facilitates downstream processing of product and minimizes problems associated with the use of serum, such as lot-to-lot variability, presence of exogenous contaminants and availability.

The early work in serum-free CHO culture was done using adherent monolayers of cells^{1,2,3,4,5}. For large scale industrial applications involving CHO cells^{6,7,8} monolayer culture is impractical. To obtain enough cells to make sufficient quantities of product, some form of suspension culture is necessary. CHO-S-SFM II is designed specifically for growth of CHO cells in suspension culture.

CHO-S-SFM II can be used to grow and maintain CHO cells and to produce recombinant proteins in suspension culture. Typical peak viable cell density in excess of 3 x 10⁶ cells/mL for cells grown in a spinner flask is illustrated in Figure 1. Similar results have been obtained in shaker flasks. This medium has also been used to produce recombinant proteins in a variety of systems both in Invitrogen Corp. R&D laboratories (Figure 2) and in other collaborating laboratories. Superiority of CHO-S-SFM II as compared to other commercially available serum-free media for CHO cells is shown in Figures 3 and 4.

The successful application of CHO-S-SFM II to both batch and continuous culture systems make it the ideal choice for larger perfusion-type systems of the type described by Avgerinos et al⁹.

Quality Control

CHO-S-SFM II is performance tested in a growth and maintenance assay using CHO cells in a dynamic cell culture system. Additional standard evaluations are pH, osmolality, and tests for the absence of bacterial and fungal contaminants. Endotoxin level is specified to be not more than 0.25 EU/mL.

Instructions for Use

Physical Conditions

Standard physical conditions for CHO cells grown in CHO-S-SFM II are 37 ± 0.5°C in a humidified atmosphere of 8% CO₂ in air. Loosen caps of flasks to permit gas exchange. Avoid overexposure of media and cultures to light.

Adaptation of CHO Cells to Serum-Free Culture

Sequential adaptation of CHO cells from serum supplemented medium (or from the original product CHO-S-SFM, Cat. No. 12050) to CHO-S-SFM II may be necessary.* **It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation.** The procedure is as follows:

- 1) Subculture the cell suspension grown in conventional serum supplemented media into a 50:50 ratio (v/v) of serum-free media (SFM) and serum supplemented media at approximately 3 x 10⁵ cells/mL. Incubate culture at 37°C in a humidified atmosphere of 8-10% CO₂ in air. Allow cell density to reach in excess of 5 x 10⁵ cells/mL.
- 2) Subculture the above cell suspension in an equal volume of CHO-S-SFM II to obtain a cell density of approximately 3-5 x 10⁵ cells/mL.
- 3) Continue to subculture the cell suspension in CHO-S-SFM II (at an inoculum of 3 x 10⁵ cells/mL) until the serum concentration is decreased to 0.1% with at least 85% viability, each time allowing the cell density to reach at least 5 x 10⁵ cells/mL.
- 4) Pass the cells in SFM and after 3-4 days post planting, when the cell density has reached 1-3 x 10⁶ cells/mL, subculture again to a density of 3 x 10⁵ cells/mL.
- 5) After several passages, the cell yield should be 1-3 x 10⁶ cells/mL after 4-5 days in culture. At this point, the cells are considered to be adapted to SFM.

Cultures may be grown in spinner flasks with impeller speed set at 75-95 rpm or in shake flasks on an orbital shaker platform rotating at 120-135 rpm.

* NOTE THAT IT IS NOT ADVISABLE TO ATTEMPT TO ADAPT CELLS ALREADY GROWING IN SERUM-FREE FORMULATIONS OTHER THAN GIBCO CHO-S-SFM TO GIBCO CHO-S-SFM II. ADAPTATION OF CELLS GROWN IN DIFFERENT SERUM-FREE MEDIA (OTHER THAN GIBCO) MAY BE AFFECTED BY SELECTION OF SUBPOPULATION(S) TO SPECIFIC COMPONENTS.

Component-Deficient Media Supplementation

When using catalog number 31033, the following supplementation concentrations will be consistent with the original formulation:

31033 hypoxanthine•Na at 0.00166 gm/L
thymidine at 0.00028 gm/L

Cryopreservation

There are two options for freezing cells in serum-free medium. Method A is the preferred method of Invitrogen Development Scientists.

- A. Freeze cells at 0.5 to 1 x 10⁷ cells/mL in a mixture of 92.5% CHO-S-SFM II (50% of conditioned SFM + 50% of fresh SFM) + 7.5% DMSO. Recover cells from cryopreservation by planting directly into shakers or spinners containing SFM at about 3 to 4 x 10⁵ cells/mL.
- B. Freeze cells at 0.5 to 1 x 10⁷ cells/mL in a mixture of 82.5% fresh CHO-S-SFM II + 7.5% DMSO + 5 to 10% of a cell culture grade BSA solution. Recover cells from cryopreservation by planting directly into shakers or spinners containing CHO-S-SFM II.

Use of a controlled rate freezer such as offered by Cryo-Med® is recommended to cryopreserve cells in a controlled and reproducible manner.

Using Cryo-Med® controlled rate freezing preset program #1, the cells are automatically subjected to the conditions described below:

Function (Cooling Rate)	Temperature
WAIT	+4°C COOL+
1°C/MIN	-4°C SAMPLE
25°C/MIN	-40°C COOL+
15°C/MIN	-12°C
1°C/MIN	-40°C
10°C/MIN	-90°C COOL+
END	

If controlled rate cryopreservation equipment is not available, CHO cells in the described cryogenic storage medium may be preserved using the following protocol:

- 1) 1 hour at 4°C
- 2) 2 to 4 hours at -20°C
- 3) overnight at -70°C
- 4) store in liquid nitrogen

Tips on Recombinant Products:

Some recombinant proteins expressed by CHO cells may vary in their susceptibility to proteases. Protease activity may be neutralized by addition of the appropriate protease inhibitors to the culture media prior to or after the harvest of the product.

References

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You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

For research use only.

CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

**GROWTH OF CHO CELLS IN CHO-S-SFM II
IN 500 mL SPINNER FLASK (300 mL VOLUME)**

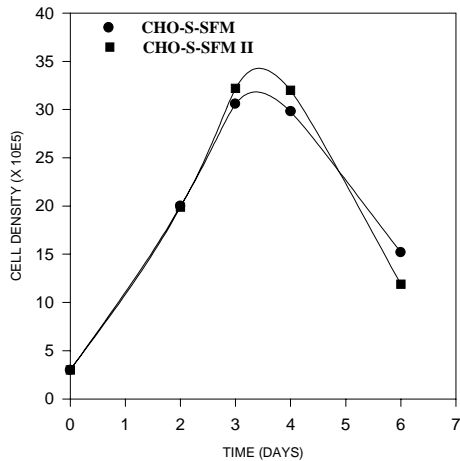


Figure 1. GROWTH OF CHO CELLS IN CHO-S-SFM II in 500 mL SPINNER FLASK (300 mL VOLUME)

CHO cells were planted in 500 mL Corning spinner flasks (300 mL volume) at a density of 3×10^5 cells/mL. Cultures were incubated at 37°C in an humidified atmosphere of 8% CO₂ in air. Impeller speed was 95 rpm. Samples were taken daily for determination of viable cell densities.

PRODUCTION OF rhCG IN CHO-S-SFM II

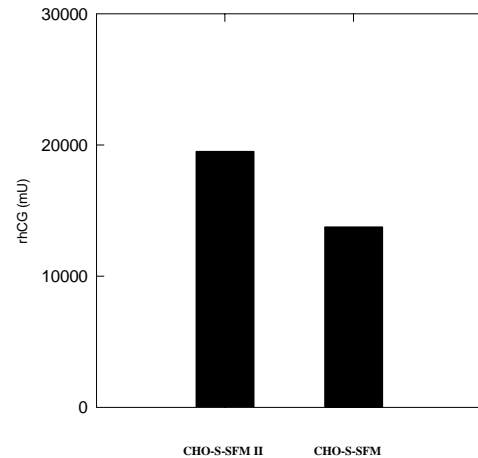


Figure 2. PRODUCTION OF rhCG IN CHO-S-SFM II

Recombinant CHO cells were adapted to suspension culture in serum-supplemented medium (DMEM/F12 + 5% FBS). Cells were then washed 3 times in either CHO-S-SFM or CHO-S-SFM II and planted in 100 mL shake flasks (50 mL volume) on an orbital shaker platform rotating at 135 rpm. Cultures were incubated at 37°C in an humidified atmosphere of 8% CO₂ in air. Samples were taken on day 3 post planting for quantitation of rhCG by RIA

COMPARISON OF CHO-S-SFM II vs. COMPETITORS' SFM

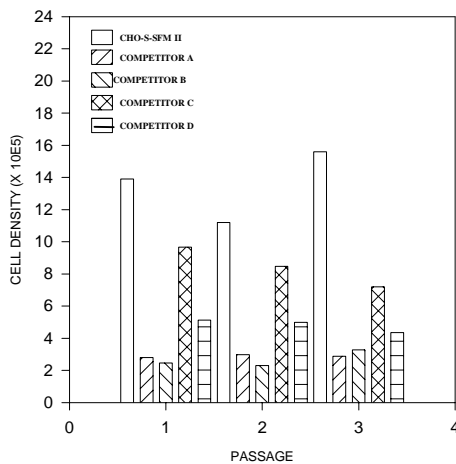


Figure 3. COMPARISON OF CHO-S-SFM II vs. COMPETITORS' SFM

CHO cells growing in EMEM + 1% proline + 5% FBS were adapted to either CHO-S-SFM II or one of four other commercially available serum-free CHO cell media. Cultures were planted in 100 mL shake flasks (50 mL volume) at a density of 3×10^5 cells/mL. Cultures were incubated at 37°C in an humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 135 rpm. Viable cell densities were determined at each of 3 consecutive passages.

COMPARISON OF CHO-S-SFM II vs. COMPETITORS' SFM

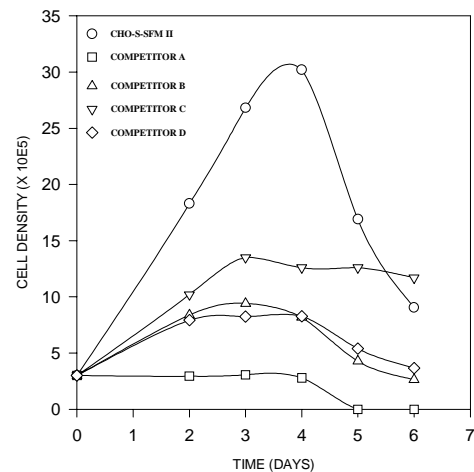


Figure 4. COMPARISON OF CHO-S-SFM II vs. COMPETITORS' SFM (Growth curve)

CHO cells adapted to grow in either CHO-S-SFM II or one of four other commercially available serum-free CHO cell media were planted in 100 mL shake flasks (50 mL volume) at a density of 3×10^5 cells/mL. Cultures were incubated at 37°C in an humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 135 rpm. Samples were taken daily (without refeeding) for determination of viable cell densities.