GIBCO™
Invitrogen Corporation

Lipid Concentrates

Formula No. 00-0061 - Cholesterol Lipid Concentrate 1000X Aqueous Liquid
Formula No. 01-0025 - Cholesterol Lipid Concentrate 1000X Aqueous Liquid (non-animal source components)
Formula No. 00-0312 - High Density Lipid Concentrate 500X Aqueous Liquid
Formula No. 01-0130 - High Density Lipid Concentrate 500X Aqueous Liquid (non-animal source components)

Introduction

These two Lipid Concentrates were developed using wild-type NS0 (ECACC 85110503) cells grown in suspension culture in CD Hybridoma Medium (Cat. no. 11279). Lipid Concentrates are supplied as aseptically processed concentrates.

Precautions

These Lipid Concentrates contain unsaturated fatty acids that may become oxidized on exposure to air. To minimize potential for this problem, any solution remaining after opening of a bottle should be overlaid with a head of inert gas, or placed in a different container with minimal headspace.

Storage Conditions

Lipid Concentrates should be stored between 2 and 8° C in the dark.

Instructions for Use

Medium Supplementation

In studies conducted by Invitrogen, optimal NS0 cell growth was achieved using a dilution of 1:1000 for the Cholesterol Lipid Concentrate (00-0061, 01-0025). Best results with wild- type NSO cells were obtained using High Density Lipid Concentrate (00-0312, 01-0130) at a 1:500 dilution. A titration of the supplement should be done to establish the optimal dilution for each cell line. It is recommended to use dilutions ranging from 1:100 to 1:1000.

To supplement medium, aseptically pipette the required amount of concentrate into the medium. Thoroughly rinse the pipette tip multiple (10-15) times to fully dispense the supplement. **DO NOT FILTER THE MEDIUM AFTER ADDITION OF THE LIPID CONCENTRATE. FILTRATION OF THE SUPPLEMENTED MEDIUM WILL RESULT IN THE REMOVAL OF THE LIPIDS.** Store the supplemented medium at 2 - 8°C in the dark.

Adaptation of Cells to Lipid Concentrate-Supplemented Medium

A sequential adaptation protocol may be necessary if direct adaptation does not work. In both cases, the cells should be in mid-logarithmic growth phase with high (>90%) viability. Success of the adaptation method will depend upon the particular cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

A. Direct Adaptation

1. Transfer cells growing in current medium to Lipid Concentrate-supplemented medium which has been prewarmed to 37°C. Seeding density should be double the normal seeding density for the cell line. Incubate the cells at 37°C in a humidified atmosphere of 5-10% CO_2 in air.
2. Monitor cell growth until viable cell density reaches 1 x 10^6/mL. Subculture the cells to a viable cell density of 1-2 x 10^5/mL in fresh serum-free medium. Subculture in this manner, monitoring cell growth and viability, for 3 to 5 passages.
3. Once the cells have adapted to Lipid Concentrate-supplemented medium in stationary suspension culture, they can be transferred into agitated suspension culture. It is recommended that backup stationary suspension cultures be maintained until cells have successfully adapted to agitated suspension culture.
4. If the culture fails to maintain acceptable growth and viability over 3-5 passages during direct adaptation, use the sequential adaptation method.

B. Sequential Adaptation

1. Inoculate cells at double the normal seeding density in a 75:25 (v/v) mixture of current medium: Lipid Concentrate-supplemented medium.
2. Monitor the culture until the density reaches 1 x 10^6 viable cells/mL. Then subculture into a 50:50 (v/v) mixture of current medium: Lipid Concentrate-supplemented medium.
3. Monitor the culture until the density reaches 1 x 10^6 viable cells/mL. Then subculture into a 25:75 (v/v) mixture of current medium: Lipid Concentrate-supplemented medium.
4. Monitor the culture until the density reaches 1 x 10^6 viable cells/mL. Then subculture into 100% Lipid Concentrate-supplemented medium.

Note: It may be necessary to subculture more than once into a given mixture of current medium: Lipid Concentrate-supplemented medium until the cells become acclimated. It is advisable to keep a backup culture in the previous media mixture until the cells have adapted.

Cryopreservation

1. Prepare desired quantity of cells, harvesting in mid-log phase of growth with viability > 90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium (50% fresh medium : 50% conditioned medium[1] + DMSO to a final concentration of 7.5%) to give a final cell density of 0.5 - 1.0 x 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium and hold the medium at 4°C until use (make cryopreservation medium on day of intended use).
4. Pellet the cells from culture medium at 100 x g for 5 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (e.g., 4.5 mL in a 5.0 mL vial).
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Frozen cells are stable indefinitely under liquid nitrogen.

[1] Note that conditioned medium should be obtained from a high viability, mid-log phase culture of cells.

Recovery from Cryopreservation

1. Recover cultures from frozen storage by rapid thawing of a vial of cells in a 37°C water bath with shaking just until the medium thaws.
2. Transfer the entire contents of the vial into the appropriately sized vessel so that the cells are seeded at 5 x 10^5 cells/mL of complete growth medium.
3. Incubate the culture in a humidified atmosphere of 5-10% CO_2 in air at 37±0.5°C. **Do not centrifuge the cells as they are extremely fragile upon recovery from cryopreservation.**
4. Maintain the culture between 5 x 10^5 and 10 x 10^5 viable cells/mL for the first two subcultures following recovery; thereafter, returning to the normal maintenance schedule.

For further information on this or other GIBCO™ products, contact Technical Services at the following:
United States TECH-LINE℠ : 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257
Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.
You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

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