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by life technologies

250X Cholesterol Lipid Concentrate

Description

250X Cholesterol Lipid Concentrate is a protein-free, chemically-defined supplement used for growing NS0 and other lipid dependent cell lines to high densities while supporting growth and protein expression without complicating downstream purification. The supplement can be added to 1X media such as CD Hybridoma and filtered with minimal loss of lipids. 250X Cholesterol Lipid Concentrate does not contain animal derived components.

Product	Catalog no.	Amount	Storage	Shelf Life*
250X Cholesterol Lipid Concentrate	12531-018	100 mL	2°C to 8°C; Protect from light	18 months

* Shelf Life duration is determined from Date of Manufacture.

Product Use

Caution: For manufacturing, processing, or repacking.

Important information

- Medium supplemented with Cholesterol Lipid Concentrate can be filter sterilized immediately after preparation using 0.22 or 0.1 micron low binding filter material (i.e., Millipore® or Durapore® membrane filters) without significant loss of lipid.
- Media supplemented with 250X Cholesterol Lipid Concentrate should be stored at 2°C to 8°C in the dark.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Use

To CD Hybridoma Medium, or other desired medium, add 250X Cholesterol Lipid Concentrate solution to a final dilution of 1:250.

Note: Dilutions of less than 1:250 (e.g., 1:100) have been shown to cause toxicity in NS0 cells. Dilutions greater than 1:250 (e.g., 1:500) should be added aseptically and the supplemented medium should NOT be sterile filtered, because it will result in the loss of lipids on the filtration membrane.

Supplement Media

1. Determine the required volume of desired medium.
2. Aseptically add 250X Cholesterol Lipid Concentrate to the medium. Rinse the pipette tip multiple times to fully disperse the supplement. Swirl to mix.
3. Filter to sterilize (1:250 dilution only) if necessary.

Adapt Cells to 250X Cholesterol 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.

Direct Adaptation

1. Transfer cells growing in current medium to 250X Cholesterol Lipid Concentrate-supplemented medium that 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 1×10^6/mL. Subculture the cells to a viable cell density of $2–3 \times 10^5$/mL in fresh serum-free medium. Subculture in this manner, monitoring cell growth and viability, for 3–5 passages.
3. Once the cells have adapted to 250X Cholesterol 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.

Sequential Adaptation

1. Inoculate cells at double the normal seeding density into a pre-warmed (37°C) 75:25 (v/v) mixture of current medium: 250X Cholesterol Lipid Concentrate-supplemented medium.
2. Monitor the culture until the density reaches 1×10^6 viable cells/mL. Then subculture into a 50:50 (v/v) mixture of current medium: 250X Cholesterol Lipid Concentrate-supplemented medium.
3. Monitor the culture until the density reaches 1×10^6 viable cells/mL. Then subculture into a 25:75 (v/v) mixture of current medium: 250X Cholesterol Lipid Concentrate-supplemented medium.
4. Monitor the culture until the density reaches 1×10^6 viable cells/mL. Then subculture into 100% 250X Cholesterol Lipid Concentrate- supplemented medium.

Note: Multiple passages may be necessary at each step to adapt to each mixture of current medium: 250X Cholesterol Lipid Concentrate-supplemented medium. It is advisable to keep a backup culture in the previous media mixture until the cells have adapted.

Cryopreservation

Prepare desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium from a high viability, mid-log phase culture to prepare cryopreservation medium.

1. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 0.5–1 × 10^7 cells/mL.
2. Prepare the required volume of cryopreservation medium 92.5% Cholesterol Lipid Supplemented-Medium (50:50 ratio of fresh to conditioned media) + 7.5% DMSO and store at 4°C until use (make cryopreservation medium on day of intended use).
3. Harvest cells by centrifugation at 100 × *g* for 5 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
4. Immediately dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
5. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
6. Transfer frozen cells to liquid nitrogen, (vapor phase) storage at –200°C to –125°C is recommended.

Related Products

Product	Catalog no.
Chemically Defined Lipid Concentrate	11905
CD Hybridoma Medium (1X), liquid	11279

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.

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Limited Product Warranty

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For further assistance, email **techsupport@lifetech.com**