

Product Information

SyntheChol™ NS0 Supplement

Product Code: S 5442

With synthetic cholesterol

Storage Temperature 2-8 °C

Product Description

SyntheChol™ NS0 Supplement is a synthetic cholesterol supplement that has been developed to specifically meet the demands of the life science and biopharmaceutical industries. This supplement is designed to be added to our chemically defined, animal component-free Hybridoma Medium (Product No. H 4409) and provide a truly animal component-free medium for growing cholesterol-dependent NS0 cells. This resulting supplemented medium supports high cell densities and high antibody productivity in NS0 cells over extended culture periods. The formulation is suitable for use in cloning, fusion, and therapeutic applications. It further minimizes protein levels in downstream production that could interfere with antibody purification. The elimination of animal-derived components reduces the incidence of performance variability in the medium and eliminates safety risks due to adventitious agents associated with these components.

Intended Use

For R&D use only. Not for drug, household or other uses.

Components

This proprietary formulation includes SyntheChol, a synthetic cholesterol. This supplement does not contain L-glutamine, antibiotics or phenol red.

Preparation Instructions

This supplement is supplied as a sterile 500× liquid. Aseptically add 2-6 ml of SyntheChol NS0 Supplement to each liter of medium just prior to use. Note that different recombinant NS0 cell clones may require different concentrations of cholesterol for maximum cell growth and productivity. Once the medium has been supplemented with the SyntheChol NS0 Supplement, we do not recommend re-filtering.

Storage/Stability

This supplement is stable, when stored at 2-8 °C and protected from light, until the date indicated on the label. Supplemented medium stored at 2-8 °C will appear to be cloudy within 72 hours.

Procedure**Thawing Frozen Cultures**

1. Rapidly thaw a 1-ml vial of cryopreserved cells in a 37 °C water bath.
2. Transfer thawed cells to a 15-ml conical centrifuge tube containing 3 ml of Hybridoma Medium, Animal Component-free supplemented with SyntheChol NS0 Supplement.
3. Mix well by gently inverting or swirling the tube.
4. Determine the viable cell density by trypan blue exclusion (Product No. T 8154).
5. Centrifuge at 200 x *g* for 5 minutes.
6. Remove supernatant and re-suspend cells in 2-5 ml of fresh medium.
7. Transfer to a cell culture T-flask and add sufficient medium to bring cells to a density of 3×10^5 viable cells/ml.
8. Place the T-flask in a humidified incubator at 37 °C and 5% CO₂.

Adaptation to medium with SyntheChol NS0 Supplement

Most cells do not require extensive weaning from serum-containing medium prior to inoculation in Hybridoma Medium, Animal Component-free supplemented with SyntheChol NS0 Supplement. Should direct inoculation be unsuccessful, cells should be cultured in basal medium containing 10% FBS to a cell density of 5×10^5 to 1×10^6 cells/ml. Next, harvest and re-seed the cells at 1.5×10^5 cells/ml in the basal medium containing 5% FBS. At subsequent passage, split the cells into a 50:50 ratio of the basal medium

with 2% FBS and Hybridoma Medium, Animal Component-free supplemented with SyntheChol NS0 Supplement. Continue to reduce the ratio of serum-containing medium: hybridoma medium supplemented with SyntheChol NS0 Supplement (25:75) at the subsequent passage and finally to 100% Hybridoma Medium, Animal Component-free supplemented with SyntheChol NS0 Supplement (0:100).

Maintenance of Established Cultures

NS0 cells should be passaged frequently to prevent cells from reaching excessive densities in a T-flask. Generally, they are plated at 3×10^5 cells/ml. Both maximum and minimum densities may vary from cell line to cell line. Most NS0 cell lines should be passaged 3 times per week, but some slow-growing cell lines may

require a more extended culture period between passages.

Cryopreservation

Pellet cells grown in Hybridoma Medium, Animal Component-free supplemented with SyntheChol NS0 Supplement at $200 \times g$ for 5 minutes. Remove the supernatant. Re-suspend in Cell Freezing Medium with DMSO and methyl cellulose (Product No. C 6295) at a density of 1×10^6 to 5×10^6 cells/ml. Dispense aliquots to freezer vials and freeze in liquid nitrogen (1°C decrease per minute).

Precautions and Disclaimer

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

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