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Pharma & Biopharma Raw Material Solutions

Application Note

Application instructions for incorporating SyntheChol® Synthetic Cholesterol into cell culture medium

Cholesterol is a critical component of many Cell Culture Media (CCM) for biotherapeutic manufacturing, but incorporation into cell culture media can be challenging due to the extremely high hydrophobicity of this raw material.

This document covers application information for incorporating cholesterol into cell culture media for bioprocessing applications and any other cell culture application that requires NAO cholesterol. The recommended cholesterol for these applications is 137672 SyntheChol® Synthetic Cholesterol For Cell Culture Emprove® Expert.

For preparing CCM with concentrations ≤ 2 mg/L cholesterol

For supplementing a hydrated/liquid media: Cholesterol/ethanol solution

This solution must be prepared at 30–35 °C and can be allowed to cool to room temperature (minimum 15 °C) following complete dissolution of the cholesterol. Create a stock solution of cholesterol in ethanol (16.67 mg/mL) by dissolving 1.00 g cholesterol in 60 mL of absolute ethanol, stirring gently in a sealed container with minimal extra headspace to avoid evaporation of the ethanol. If the entire solution will be used immediately, some ethanol evaporation due to using an open container is acceptable.

Supplement liquid cell culture medium with ethanol solution directly. At 2 mg/mL of cholesterol, ethanol concentration in CCM will be 0.0075 % v/v. Cold storage of the cholesterol solution is not recommended, but if necessary, it is recommended to store at –80 °C in an airtight container, and will need to be reheated to 35 °C to redissolve the precipitated cholesterol prior to addition to cell culture.

For incorporating cholesterol into Dry Powder Media (DPM) formulations

For manufacturing DPM formulations containing cholesterol at concentrations lower than 2 mg/L, 137672 SyntheChol® Synthetic Cholesterol is a suitable raw material which can be directly incorporated into the DPM.

Cholesterol-containing media storage recommendation for low cholesterol concentrations

All storage recommendations are for media stored at 2–8 °C which contain cholesterol supplemented as described above. The information below is a guideline only, as the storage stability of any medium is also dependent on the other components in the medium. During indicated storage times, media remain unchanged in transparency and turbidity, with turbidity < 3 NTU.

For cholesterol concentrations ≤ 1 mg/L

After supplementation with cholesterol, media should be used within 4 weeks.

For cholesterol concentrations from 1 to ≤ 2 mg/L

After supplementation with cholesterol, media should be used within 7 days.

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For preparing CCM with concentrations >2 mg/L cholesterol

If the desired cholesterol concentration is above 2 mg/L, the recommended addition strategy is to use a concentrated cholesterol supplement, which can be prepared using the following method, adapted from known literature methods.[1]

Required components:

137672 SyntheChol® Synthetic Cholesterol For Cell Culture Emprove® Expert

C4555 Methyl-β-cyclodextrin

100986 Ethanol absolute Emprove® Expert Ph Eur,BP,ChP,JP,USP

Milli-Q® purified water

Method for making 450 mL of cholesterol supplement (2.22 g/L cholesterol solution)

1. In a sealed container with minimal extra headspace to avoid evaporation of the ethanol, stir 1.00 g SyntheChol® Synthetic Cholesterol in 60 mL ethanol at 30–35 °C at a gentle stirring speed until all the cholesterol has dissolved. Allow the solution to return to room temperature (minimum 15 °C).
2. Stir 35.00 g methyl-β-cyclodextrin in 350 mL of Milli-Q® purified water at high stirring speed until all the cyclodextrin has dissolved (expected time ~20 minutes). If desired, the solution can be heated to 35 °C during dissolution to increase the dissolution speed. Following complete dissolution of the cyclodextrin, allow the solution to return to room temperature (minimum 15 °C).
3. Ensure both solutions are at room temperature (minimum 15 °C). Add the SyntheChol® solution to the cyclodextrin solution portion wise, waiting after each addition for the solution to return to a transparent, clear solution (total addition should take about 15 minutes).
4. Following complete addition of the SyntheChol®/ ethanol solution to the cyclodextrin solution, q.s. with Milli-Q® purified water to obtain a final volume of 450 mL. Stir well to homogenize the solution.
5. Filter-sterilized through a Millipore Express® 0.2 µm PES sterile filter.
6. Store at 2–8 °C for up to 4 weeks.

Supplementing media with the concentrated cholesterol supplement

Add directly to filter-sterilized liquid cell culture media. Media should not be re-filtered. This method is not suitable for cholesterol supplementation of dry powder medium.

Cholesterol-containing media storage recommendation for concentrated cholesterol supplement

All storage recommendations are for media stored at 2–8 °C. The information below is a guideline only, as the storage stability of any medium is also dependent on the other components in the medium. During indicated storage times, media remain unchanged in transparency and turbidity, with turbidity < 3 NTU.

For cholesterol concentrations ≤ 5 mg/L

After supplementation with concentrated cholesterol supplement, media should be used within 14 days.

For cholesterol concentrations 5–10 mg/L

After supplementation with concentrated cholesterol supplement, media should be used within 7 days.

[1] Widenmaier, Scott B., Snyder, Nicole A. et al. (2017) NRF1 Is an ER Membrane Sensor that Is Central to Cholesterol Homeostasis. Cell 171, 1094-1109.

Okonkowski, J., Balasubramanian, U., et al. (2007) Cholesterol Delivery to NS0 Cells: Challenges and Solutions in Disposable Linear Low-Density Polyethylene-Based Bioreactors. J. Biosci. And Bioeng. 103, 50-59

For supplementing liquid EX-CELL® CD Insect Cell Medium (14380C) with SyntheChol® Synthetic Cholesterol

The liquid EX-CELL® CD Insect Cell Medium (14380C) requires SyntheChol® supplementation for optimal growth. To use 137672 SyntheChol® Synthetic Cholesterol for this purpose, it is recommended to formulate the concentrated cholesterol supplement like described above and add the concentrated supplement directly to filter-sterilized media for a fully formulated liquid medium. The user should determine the optimum volume of supplement to use with their cell type experimentally, as this can be variable with cell type. It is advised to test in the range of 3-10 mg/L of cholesterol in the medium, and a recommended concentration to test that is compatible with media preparation instructions for EX-CELL® CD Insect Cell Medium is 3.38 mL of supplement per liter of medium. Do not re-filter after adding the concentrated cholesterol supplement. The shelf life of the medium with this supplement is as described in the EX-CELL® CD Insect Cell Medium product information sheet.

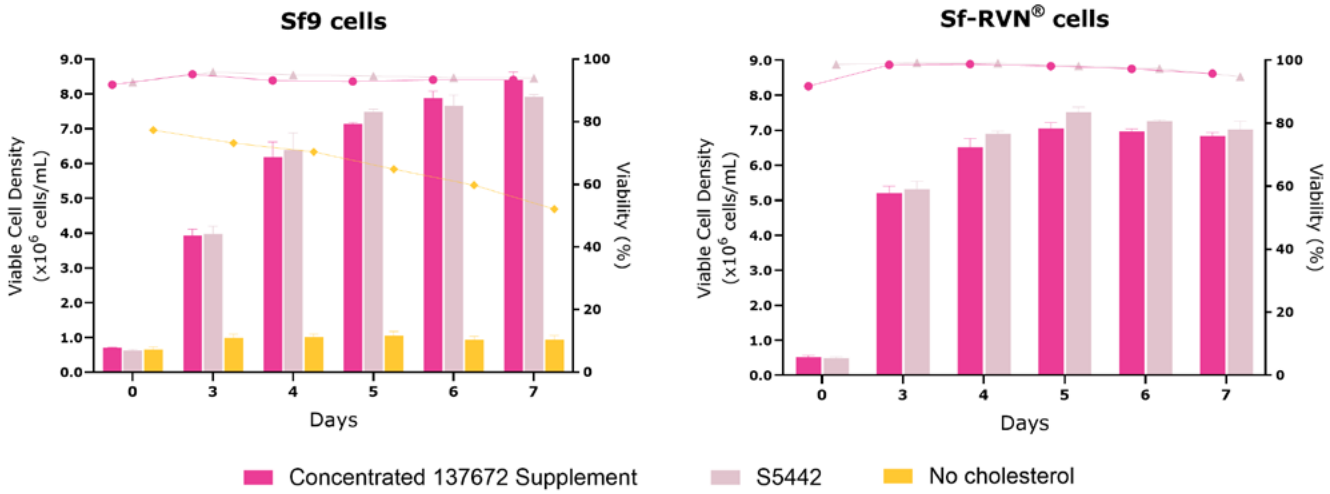

Figure 1: Growth curves of Sf9 and Sf-RVN® (SFRVN-1VL) insect cell lines using liquid EX-CELL® CD Insect Cell Medium (14380C). Cells were adapted to each medium for 2 passages, then seeded at 0.6 x10^6 cells/mL for Sf9 and 0.5 x10^6 cells/mL for Sf-RVN® insect cell lines and monitored for viable cell density and viability for 7 days. The concentrated supplement prepared with SyntheChol® Synthetic Cholesterol (137672) provides the same high growth as the liquid SyntheChol® product (S5442).

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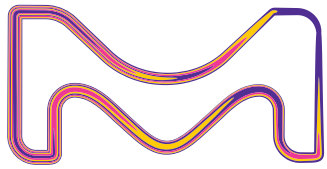

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