Technical Data Sheet

Ceramide Delivery System

Avanti No.	Description	2 Vials
640001	Ceramide Delivery System	10 mg C6 ceramide 13.8 mg CholPC

Delivery:
Ceramide forms stable, fluid bilayers with cholesteryl phosphocholine, and this formulation has been shown to be more potent than solvent-delivered (DMSO) formulations of C6 Ceramide in inhibiting proliferation, inducing apoptosis, and disturbing calcium homeostasis[1]. It is thought that DMSO formulations of C6 Cer are likely to precipitate into the cell culture medium, where the bioavailability of "crystalline" ceramide is limited[1]. However, the formation of fluid bilayers, which are enriched in C6 Cer, prevent crystallization and allow for the transfer of monomeric ceramide to cell membranes with enhanced bioavailability[1].

Contents:
The kit contains 10 mg of C6 Ceramide and 13.8 mg of Cholesteryl Phosphocholine, individually packaged and ready for use.

Storage:
The kit components should be stored as packaged at -20°C.

Stability:
The kit components are stable for 1 year when stored as packaged at -20°C.

Suggested Reagent Preparation Protocol:
• Prepare 25 mM stock solutions of CholPC and C6-Cer in hexane-isopropanol (3:2 by vol) and methanol, respectively by adding 1 ml of solvent to each vial. These stock solutions may be stored at -20°C until use.
• Prepare a 10 mM dispersion of CholPC/C6-Cer bilayers (1:1 molar ratio) from the stock solutions.

- Add the appropriate amount of each 25 mM lipid stock to a glass tube and dry under a gentle flow of nitrogen at 40°C.

- Re-dissolve the lipid film in chloroform to facilitate proper mixing of the lipid and dry the mixture again using the conditions described.

- Hydrate the dry lipid film to a 10 mM bilayer dispersion using the appropriate amount of HBSS buffer (20 mM Hepes, 118 mM NaCl, 4.6 mM KCl, 1 mM CaCl2, 10 mM glucose, pH 7.4) at 55°C for 20 minutes.

- Sonicate the lipid dispersion for 10 minutes in a bath sonicator.

- The resulting C6 Cer/CholPC lipid dispersion may be stored at room temperature and used within 24 hrs.

• Use the C6 Cer/Chol PC formulation to effectively deliver the desired concentration of C6 Cer to cells in culture.

Important Technical Notes:
• While this formulation has been successfully applied to FRTL-5 and HELA cells, it is important to test for concentration vs exposure time since cell lines are differently sensitive to C6 Cer and to the formulation.
• Asceptic conditions and protocols should be maintained and followed during bilayer preparation.

Reference:
[1]Complexation of C6-Ceramide with Cholesteryl Phosphocholine – A Potent Solvent-Free Ceramide Delivery Formulation for Cells in Culture (2013)
Pramod Sukumaran , Max Lönnfors , Otto Långvik, Ilari Pulli, Kid Törnquist, J. Peter Slotte. PLOS ONE

Application:

Ceramide Inhibits the Proliferation of FRTL-5 and HeLa Cells

The proliferation of FRTL-5 cells has been shown to be inhibited by ceramide. We therefore compared the effects of the different C6-Cer formulations on the rate of [3H]thymidine incorporation into cellular DNA (Fig. 1A). FRTL-5 cells exposed to 0.05 mM of C6-Cer/CholPC or C6-Cer dissolved in DMSO for 48 h had lower [2H]thymidine incorporation compared to control cells (DMSO exposure only), and the effect was much larger in cells exposed to C6-Cer/CholPC. The results were confirmed by counting the number of cells after 48 hrs of treatment (Fig. 1B). Similar data were obtained when HeLa cells were exposed for 12 h to 0.05 mM C6-Cer, either in complex with CholPC, or dissolved in DMSO (Fig. 1C). The antiproliferative effects of C6-Cer was also seen when FRTL-5 cells were exposed to 0.05 mM C6-ceramide made from L-erythro-sphingosine, or from D-*erythro*-sphinganine (Fig. 1D).

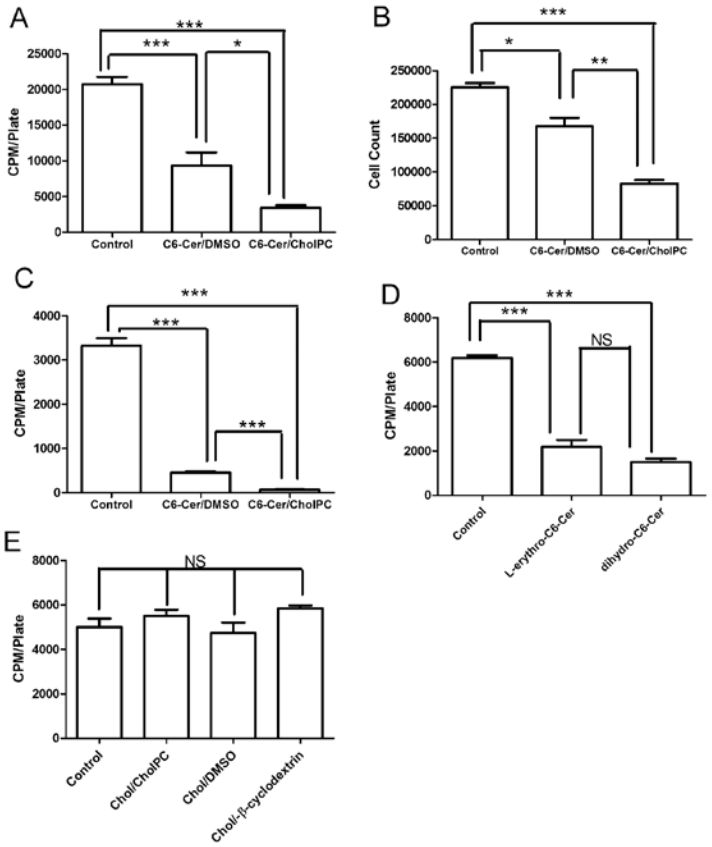

Figure 1: Effect of C6-Cer on cell proliferation.

A. FRTL-5 cells were preincubated for 48 h with 0.05 mM C6-Cer/CholPC or C6-Cer/DMSO. [3H]Thymidine incorporation into cellular DNA during the last 4 h was determined. B. Cell proliferation measurement using cell count. Effect of C6-Cer on cell proliferation on FRTL-5 was measured by counting the cells after preincubation for 48 h with 0.05 mM C6-Cer/CholPC or C6-Cer/DMSO. DMSO alone was used as control. Each value gives the amount of cells per plate. C. HeLa cells were incubated with 0.05 mM C6-Cer/CholPC or C6-Cer/DMSO for 12 h. [3H]Thymidine incorporation into cellular DNA during the last 4 h was determined. D. FRTL-5 cells were exposed to 0.05 mM L-erythro-C6-Cer/CholPC or C6-dihydroCer/CholPC for 24 h after which [3H]thymidine incorporation into cellular DNA during the last 4 h was determined. E. FRTL-5 cells were exposed for 48 h to 0.05 mM Chol/CholPC, Chol/DMSO, or Chol/m-β-cyclodextrin after which [3H]thymidine incorporation into cellular DNA during the last 4 h was determined.

Ceramide Induces Apoptosis in the FRTL-5 Cells

Previous studies have shown that ceramide induces apoptosis in FRTL-5 cells. To test the effects on apoptosis of the different C6-Cer formulations, FRTL-5 cells were exposed to C6-Cer/CholpC or C6-Cer/DMSO (0.05 mM) for 48 h. The proportion of apoptotic cells in controls and C6-Cer exposed cells was measured with FACS, and results calculated using Flowing Software v 2.5 (Fig. 2). Less than 5% of the cells were apoptotic when not exposed to C6-Cer, whereas the fraction of apoptotic cells increased markedly in C6-Cer treated cells (Fig. 2A). C6-Cer/CholPC was significantly more effective in inducing apoptosis in the FRTL-5 cells compared to the C6-Cer/DMSO formulation. As a control experiment the apoptotic cell percentage was also calculated for the cells treated with Chol/Chol-PC for 48 h (Fig. 2B).

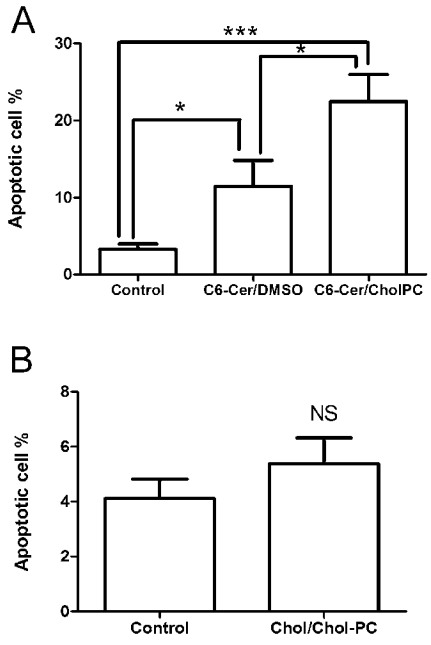

Figure 2: Induction of apoptosis in FRTL-5 cells by C6-Cer.

A. The cells were exposed for 48 hrs to C6-Cer/CholPC or C6-Cer/DMSO (0.05 mM), and the fraction of apoptotic cells was measured. Each bar value is the mean±SEM of 3 different experiments. *p<0.05, ***p<0.001. B. Induction of apoptosis in FRTL-5 cells by Chol/Chol-PC. The cells were exposed for 48 hrs to Chol/Chol-PC (0.05 mM), and the fraction of apoptotic cells was measured. Each bar value is the mean±SEM of 3 different experiments (NS = no significance).

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