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Product Information

NBD C_6-Ceramide

Catalog Number	Unit Size
C049	1 mg

Storage upon receipt:
- -20°C
- Protect from light

Product Description

NBD C_6-ceramide can be used to study sphingolipid transport and metabolism mechanisms. It is also used as a selective stain for the Golgi apparatus in live and fixed cells. The environment sensitive NBD dye is weakly fluorescent in water but increases in aprotic solvents and other nonpolar environments with excitation/emission maxima ~466/536 nm.

Experimental Protocols

Preparation of NBD C_6-ceramide–BSA Complexes

For staining of living and fixed cells, it is efficacious to add NBD C_6-ceramide in the form of complexes with BSA. BSA delivery complexes of NBD C_6-ceramide can be prepared as follows:

1.1 Prepare an approximately 1 mM NBD C_6-ceramide solution in chloroform:ethanol (19:1 v/v).

1.2 Dispense 50 µL of NBD C_6-ceramide stock solution into a small glass test tube and dry, first under a stream of nitrogen, and then under vacuum for at least 1 hour. Redissolve in 200 µL of absolute ethanol.

1.3 Measure 10 mL of serum-free balanced salt solution such as Hanks' buffered salt solution + 10 mM HEPES, pH 7.4 (HBSS/HEPES) into a 50 mL plastic centrifuge tube. Add 3.4 mg (0.34 mg/mL) of defatted BSA.

1.4 Agitate the tube containing the 10 mL of the BSA solution on a vortex mixer. Inject the NBD C_6-ceramide solution in ethanol (200 µL) into the vortex. Store the resulting solution (5 µM NBD C_6-ceramide + 5 µM BSA) in a plastic tube at -20°C.

Staining the Golgi Complex in Living Cells with NBD C_6-ceramide

2.1 Rinse cells grown on glass coverslips in an appropriate medium (such as HBSS/HEPES).

2.2 Incubate the cells for 30 minutes at 4°C with 5 µM NBD C_6-ceramide-BSA complex in HBSS/HEPES.

2.3 Rinse the sample several times with ice-cold medium and incubate in fresh medium at 37°C for a further 30 minutes.

2.4 Wash the sample in fresh medium and examine using a fluorescence microscope. Prominent labeling of the Golgi apparatus and weaker labeling of other intracellular membranes should be seen.

Staining the Golgi Complex in Fixed Cells with NBD C_6-Ceramide

Conditions for staining appear to be generally noncritical. However, detergents and methanol/acetone fixatives should be avoided.

3.1 Rinse cells grown on glass coverslips in HBSS/HEPES and fix for 5-10 minutes at room temperature in 0.5% glutaraldehyde/10% sucrose/100 mM PIPES, pH 7.0 or 2-4% paraformaldeyde in phosphate-buffered saline (PBS).

3.2 Rinse the sample several times with ice-cold HBSS/HEPES, transfer to an ice-bath and incubate for 30 minutes at 4°C with 5 µM NBD C_6-ceramide-BSA complex.

3.3 Rinse in HBSS/HEPES and incubate for 30-90 minutes at room temperature with 10% fetal calf serum or 2 mg/ml BSA to enhance golgi staining.

3.4 Wash the sample in fresh HBSS/HEPES, mount and examine by fluorescence microscopy.