NBD C6-Ceramide

Cat: IN3770

Storage: Powder: -20℃, 2 years; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year (protect from light)

Product Introduction

NBD C6-Ceramide is a fluorescent sphingolipid analog that can be used to study sphingolipid transport and metabolic mechanisms. It can also be used to selectively stain Golgi bodies in living and fixed cells. Applications: Cellular sphingolipid transport and metabolism tracking, Golgi stain.

Product Parameter

Ex/Em: 466/530 nm (in MeOH)

CAS: 86701- 10-2

Molecular Formula: $C_{30}H_{49}N_5O_6$

Molecular Weight: 575.7

Solubility: Soluble in Methanol/DMSO

Protocols (*only for reference*)

Dyeing Solution Preparation

1. Remove the NBD C6-Ceramide stored at low temperature from the refrigerator and let it stand until it returns to room temperature.After centrifugation at low speed, take 1 mg of the lyophilized powder from the 173.7 μL anhydrous DMSO dissolution tube and prepare a 10 mM stock solution. Freeze at -20°C in separate units according to single dosage, avoiding repeated freezing and thawing.
2. Preparation of working fluid: Choose a suitable buffer (e.g. serum-free medium, HBSS, HEPES or PBS) and add defatted BSA (to a concentration of 0.34 mg/mL) to prepare a dilution solution. Add a certain volume of NBD C6-Ceramide reservoir solution to the above dilution solution, vortex and shake to make 5-10 μM staining solution.

Note:

a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.

b. If it is found to be difficult to dissolve, it can be properly sonicated to promote dissolution.

Staining of living cells

1. Cells were cultured on sterile coverslips.
2. When the cells were cultured to a suitable density, removed the coverslip from the medium and rinsed the coverslip with a suitable buffer (e.g. serum-free medium, HBSS, HEPES or PBS).
3. Add 100 μL of staining solution (5-10 μM) to one corner of the coverslip, shake gently so that the dye evenly covers all the cells, and incubate for 30 min at room temperature.
4. Drain the staining solution, wash the coverslips 2~3 times with pre-cooled fresh medium at 4℃, then cover all the cells with fresh medium and incubate at 37℃ for 30 min.

5. After washing with fresh medium, observations were made with a microscope.

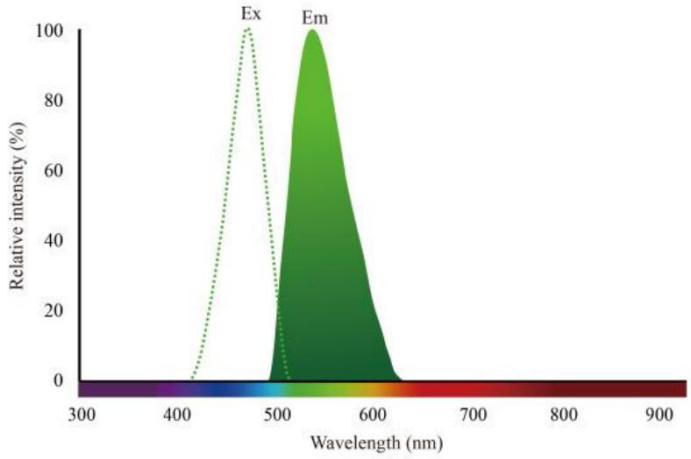
Staining of fixed cells

1. Cells were cultured on sterile coverslips.
2. When the cells were cultured to a suitable density, removed the coverslips from the medium and rinsed the coverslips with a suitable buffer (e.g. serum-free medium, HBSS, HEPES or PBS). Fix the coverslips with 4% paraformaldehyde for 5~10 min at room temperature.
3. Add 100 μL of staining solution (5-10 μM) to one corner of the coverslip, shake gently so that the dye evenly covers all the cells, and incubate for 30 min at room temperature.
4. The staining working solution was aspirated, the coverslips were washed with the same buffer, and then incubated with 10% FBS or 2 mg/mL of BSA for 30~90 min at room temperature to enhance Golgi staining.
5. After washing with the same buffer, observed with a microscope.

Note

1. Fluorescent dyes are subject to quenching, so please avoid light as much as possible to slow down the fluorescence quenching.
2. If Golgi staining is performed on suspended cells, it is recommended that the staining be performed at $2x10^6$ cells/mL.
3. For your safety and health, please wear a lab coat and disposable gloves.

Spectrogram



Note: Spectrum of NBD C6-ceramide dissolved in methanol