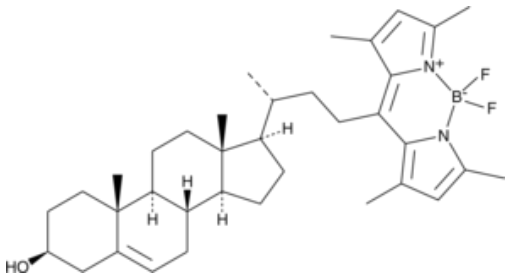
Product Data Sheet

Product Name: BODIPY-Cholesterol
Cat. No.: GC42964

Chemical Properties

Cas. No.	878557-19-8		
Chemical Name	(T-4)-[(3β)-24-(3,5-dimethyl-1H-pyrrol-2-yl-κN)-24-(3,5-dimethyl-2H-pyrrol-2-ylidene-κN) chol-5-en-3-olato]difluoro-boron		
SMILES	O[C@H](C1)CC[C@@]2(C)C1=CC[C@]3([H])[C@]2([H])CC[C@@]4(C)[C@@]3([H])CC[C@]4([H])[C@H](C)CCC(C5=C(C)C=C(C)N5[B-]6(F)F)=C7[N+]6=C(C)C=C7C		
Formula	$C_{36}H_{51}BF_2N_2O$	M.Wt	576.6
Solubility	0.5mg/ml in ethanol; 1mg/ml in DMSO, or in DMF	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Protocol

Evaluation of cholesterol efflux using BODIPY-Cholesterol

This protocol only provides a guideline, and should be modified according to your specific needs

1. Stock and dilute solution preparation for staining of cells

(1) Prepare BODIPY-Cholesterol stock solution (10mM) by dissolving BODIPY-Cholesterol powder in DMSO, which was then aliquoted into appropriate volumes and stored at -20°C.

Product Data Sheet

(2) Dilute the BODIPY-Cholesterol stock solution with PBS to a final 1mM concentration.

NOTE: The working solution must be prepared fresh on the day of the experiment.

2. Preparation of labeling media

(1) Labeling medium containing BODIPY-Cholesterol was prepared by complexing the sterols (unlabeled cholesterol and BODIPY-Cholesterol) with methyl-β-cyclodextrin (CD) at a molar ratio of 1:40 (cholesterol/CD). The BODIPY-Cholesterol represented 20% of the total cholesterol in the labeling medium. Unlabeled cholesterol and BODIPY-Cholesterol working solution were dried under nitrogen in the dark to form a thin film in a round-bottom 100-ml glass bottle.

(2) The cholesterol mixture was solubilized by adding 20mM CD in MEM-HEPES buffer. The suspension was sonicated in a water bath (37°C) for 30min, placed in a shaking water bath for 3h (37°C), and kept at 4°C until use. Before use, the suspension was sonicated for 30min, filtered using a 0.45-μm syringe filter, and diluted with an equal volume of MEM-HEPES buffer containing 2μg/ml acyl-CoA cholesterol acyltransferase (ACAT) inhibitor. There was no loss of fluorescent sterol upon filtration. The final concentrations of BODIPY-Cholesterol, unlabeled cholesterol, and CD in the labeling medium were 0.025mM, 0.1mM, and 10mM, respectively.

3. Efflux of BODIPY-Cholesterol

(1) J774 cells were plated in RPMI supplemented with 10% FBS and gentamicin in 48-well plates at a density of 75000 cells/well for 24h.

(2) Cells were labeled with BODIPY-Cholesterol by incubating the monolayers with 0.25ml of labeling medium containing CD/BODIPY-Cholesterol/unlabeled cholesterol for 1h, followed by washing with MEM-HEPES.

(3) J774 cells were then equilibrated with RPMI containing 0.2% BSA and the ACAT inhibitor, with or without cAMP (0.3mM), for 18h. After this equilibration period, the cells were washed with MEM-HEPES buffer and incubated with MEM-HEPES media containing the cholesterol acceptors for 4h.

(4) At the end of the incubation time, the efflux media were removed, filtered through a 0.45-μm filter, and the fluorescence intensity was recorded using a plate reader (E_x=482nm, E_m=515nm).

(5) After equilibration, the time-zero (t0) cell monolayers (cells not incubated with extracellular

acceptors) were solubilized with 1% cholic acid and mixed well by shaking on a plate shaker for 4h at room temperature; then the fluorescence intensity was recorded.

(6) The fluorescence intensity values obtained at this time were used as the t0 measurements. Fractional efflux of BODIPY-Cholesterol was calculated based on the fluorescence intensity of the media divided by the t0 monolayer fluorescence values measured after cholic acid solubilization.

4. Experimental Precautions

(1) Dissolve the BODIPY-Cholesterol in PBS immediately before use, and ensure that the BODIPY-Cholesterol is adequately protected from light.

(2) This protocol and all reagents are for scientific research use only.

(3) To ensure your safety and health, please wear the laboratory coat and put on disposable gloves during the operation.

References:

[1] Sankaranarayanan S, Kellner-Weibel G, de la Llera-Moya M, et al. A sensitive assay for ABCA1-mediated cholesterol efflux using BODIPY-cholesterol[J]. Journal of lipid research, 2011, 52(12): 2332-2340.

Background

BODIPY-Cholesterol is a lipid-like and cell-permeable cholesterol analogue with a fluorescent BODIPY group (E_x=482nm, E_m=515nm)[1]. The rate of desorption of BODIPY-Cholesterol from monolayers is similar to that of cholesterol upon addition of a water-soluble cyclodextrin to mixed monolayers with sphingomyelin, which endows BODIPY-Cholesterol with the potential to study the interactions between biologically related lipids, the orderliness of lipids, and membrane fluidity[2]. BODIPY-Cholesterol diffuses rapidly and freely in the plasma membrane of living cells without hindrance, complementing cholesterol analogues with intrinsic fluorescence, and is suitable for quantitative live-cell imaging[3]. BODIPY-Cholesterol serves as an effective tool for assessing the distribution of sterols within the body and for evaluating the excretion of cholesterol[4].

References:

Product Data Sheet

[1] Sankaranarayanan S, Kellner-Weibel G, de la Llera-Moya M, et al. A sensitive assay for ABCA1-mediated cholesterol efflux using BODIPY-cholesterol[J]. Journal of lipid research, 2011, 52(12): 2332-2340.

[2] Marks D L, Bittman R, Pagano R E. Use of Bodipy-labeled sphingolipid and cholesterol analogs to examine membrane microdomains in cells[J]. Histochemistry and cell biology, 2008, 130(5): 819-832.

[3] Wüstner D, Lund F W, Röhrl C, et al. Potential of BODIPY-cholesterol for analysis of cholesterol transport and diffusion in living cells[J]. Chemistry and physics of lipids, 2016, 194: 12-28.

[4] Ikonen E, Blom T. Lipoprotein-mediated delivery of BODIPY-labeled sterol and sphingolipid analogs reveals lipid transport mechanisms in mammalian cells[J]. Chemistry and physics of lipids, 2016, 194: 29-36.

Caution: Product has not been fully validated for medical applications. For research use only.
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