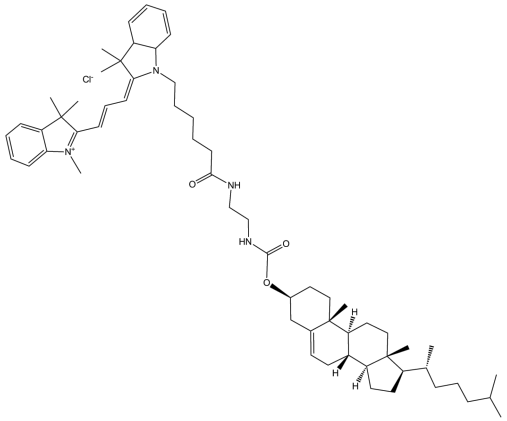
Product Data Sheet

Product Name: Cy3-cholesterol
Cat. No.: GC26723

Chemical Properties

Cas. No.			
Chemical Name	2-((1E,3E)-3-(1-(6-((2-(((((3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)carbonyl)amino)ethyl)amino)-6-oxohexyl)-3,3-dimethyl-3,3a-dihydro-1H-indol-2(7aH)-ylidene)prop-1-en-1-yl)-1,3,3-trimethyl-3H-indol-1-ium chloride		
SMILES	[H][C@@](CC[C@@H]1[C@@H](CCCC(C)C)C)([C@@]1(CC[C@@]2([C@]3(CC[C@@H](CC3=CC4)OC(NCCNC(CCCCCN5C6C(C=CC=C6)C(C)(C)/C5=C\C=C\C7=[N+](C)C8=CC=CC=C8C7(C)C)=O)=O)C)[H])C)[C@@]24[H].[Cl-]		
Formula	$C_{60}H_{89}ClN_4O_3$	M.Wt	949.83
Solubility		Storage	Store at -20°C,protect from light
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

Note: The specific usage methods of Cy3-cholesterol uptake for different cells vary greatly. For specific cells, please refer to the literature or adjust and optimize based on the detection results.

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

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1. Preparation of Cy3-cholesterol uptake working solution.
1.1 Before opening, balance the product to room temperature, briefly centrifuge, and dissolve Cy3-cholesterol in anhydrous ethanol to a concentration of 10mM.
1.2 For 96-well plates and 6-well plates, 100μl and 1ml of Cy3-cholesterol cholesterol uptake working solution (Working Solution) are required per well, respectively. For other culture containers, the amount can be adjusted proportionally. Calculate the volume of the cholesterol uptake working solution needed based on the number of samples. Prepare a 25μM Cy3-cholesterol solution using serum-free working solution.
Note 1: It is recommended to conduct a pre-experiment for different cell samples to determine the optimal cell concentration. A concentration gradient within the range of 1-25μM is suggested.
Note 2: When preparing the Cy3-cholesterol uptake working solution, avoid light. The prepared Cy3-cholesterol uptake working solution must be used up in one go and cannot be frozen.
2. Positive control setup (optional).
Set up separate positive control replicates and add working solution containing 25μM Cy3-cholesterol and 10μM U 18666A. U 18666A can interfere with the cholesterol export pathway, causing cholesterol and other sterols to accumulate in lysosomes.
Note: For most cells, U 18666A treatment for 24 hours can effectively inhibit the outward transport of cholesterol within cells, and the Cy3-cholesterol accumulated in the cells will show strong orange fluorescence; for some cells, the concentration and time gradient need to be explored [1].
3. Microplate Reader assay for cell cholesterol uptake.
a. Cultivation. Place the cells in a 96-well black microplate. The number of cells per well should be controlled within 100-10,000, usually in the range of 2,000-5,000. Perform certain treatments on the cells according to the experimental design.
Note: The number of cells inoculated needs to be considered for the cell count during the detection. If the cells need to be cultured for a period of time or the drug treatment time is relatively long, the number of inoculated cells should be appropriately reduced. Excessive or insufficient cell numbers may affect the final detection result.
b. Washing. For adherent cells, remove the culture medium and wash the cells with PBS once; for suspension cells, centrifuge at 250-1000×g at room temperature for 5 minutes, remove the supernatant, and wash with PBS once.
Note: Whether to use PBS to wash the cells can be determined according to the experimental design.
c. Fluorescent labeling of cholesterol uptake. Add an appropriate volume of Cy3-cholesterol uptake working solution. Usually, 100μl is added to each well of the 96-well plate. Incubate at 37°C in the

dark for 24 hours. The incubation time can be adjusted within 12-48 hours.
Note: If it is the first experiment and the incubation time cannot be determined, it is recommended to first try incubating at 37°C in the dark for 24 hours and observe the fluorescence effect. If the fluorescence of normal cells is too strong, shorten the time appropriately; if the fluorescence is too weak, extend the time appropriately.
d. Detection. After incubation, wash 1-3 times with PBS or HBSS. Use a fluorescence enzyme analyzer directly for detection (Cy3-cholesterol is orange fluorescence, Ex/Em = 554/568nm), with the recommended excitation wavelength of 554nm and emission wavelength of 568nm, with a wavelength width of 5nm. Alternatively, you can optionally remove the liquid and add an equal amount of appropriate lysis solution to lyse the cells for fluorescence detection. By comparing the RFU (Relative fluorescence units) of the control group and the treatment group, the change in cholesterol uptake ability of the treatment group can be quantitatively calculated.
Note 1: Due to the significant differences in cholesterol uptake ability among different cells, the fluorescence enzyme assay for cell cholesterol uptake for different cells must be appropriately optimized to achieve better detection results. For example, the concentration of Cy3-cholesterol, the number of cells during detection, and the incubation time of the Cy3-cholesterol cholesterol uptake working solution may need to be optimized. The top-reading or bottom-reading mode of the fluorescence analyzer may also have some impact on the detection results. It is recommended to prefer top-reading detection.
Note 2: Before the fluorescence enzyme assay, you can also briefly use a fluorescence microscope at low magnification to quickly preliminarily observe the fluorescence intensity of the cells to roughly determine whether the cholesterol uptake experiment is successful. It is not recommended to use high magnification observation, as high magnification observation is prone to fluorescence quenching and interfering with the final quantitative results.
Note 3: If the treatment drug has a significant effect on cell proliferation, it is recommended to use cell quantity calibration for RFU values.
4. Flow cytometry detection of cell cholesterol uptake.
a. Cultivation. Place the cells in a 6-well plate or an appropriate cell culture dish according to the experimental design and perform appropriate treatments on the cells.
b. Cell preparation. Adherent cells are washed once with PBS; suspension cells are centrifuged at 250-1000×g at room temperature for 5 minutes, the supernatant is removed, and washed once with PBS.
Note: Whether to use PBS to wash the cells can be determined according to the experimental design.

c. Fluorescent labeling of cholesterol uptake. For the cells inoculated in the 6-well plates in the previous step, add 1ml of Cy3-cholesterol uptake working solution to each well; for the 1×10^6 cells precipitated from the suspension in the previous step, add 1ml of Cy3-cholesterol uptake working solution and resuspend them to form a single-cell suspension. Incubate at 37°C in the dark for 24 hours. The incubation time can be adjusted within the range of 12-48 hours.
Note: If it is the first experiment and the incubation time cannot be determined, it is recommended to first try incubating at 37°C in the dark for 24 hours and observe the fluorescence effect. If the fluorescence of normal cells is too strong, shorten the time appropriately; if the fluorescence is too weak, extend the time appropriately.
d. Detection. After the incubation is completed, wash the adherent cells once with PBS or HBSS, then digest the cells with trypsin, and then wash once. This can be used for flow cytometry detection; for the suspended cells, wash them 1-3 times with PBS or HBSS, and directly perform flow cytometry detection. Detect the cell suspension with a flow cytometer. Cy3-cholesterol is orange fluorescence, with Ex/Em = 554/568nm. For flow cytometry, it is recommended to use the CY3/PE channel for detection.
Note 1: Due to the significant differences in cholesterol uptake ability among different cells, the flow cytometry detection of cholesterol uptake for different cells must be appropriately optimized to achieve better detection results, such as the concentration of Cy3-cholesterol and the number of cells.
Note 2: Due to the sensitivity of flow cytometry, the concentration of the fluorescent probe used may be lower than that for fluorescence microscopy detection. At this time, the dilution factor of Cy3-cholesterol can be appropriately adjusted according to the cell type and reagent staining conditions.
5. Fluorescence microscopic detection of cholesterol uptake by cells.
a. Cultivation. Place the cells in 96-well plates, cell culture dishes, or cell slides, and treat them appropriately according to the experimental design.
b. Washing. For adherent cells, remove the culture medium and wash the cells once with PBS; for suspended cells, centrifuge at 250-1000×g at room temperature for 5 minutes, remove the supernatant, and wash once with PBS.
Note: Whether to use PBS to wash the cells can be determined by yourself according to the experimental design.
c. Fluorescent labeling of cholesterol uptake. Add an appropriate volume of Cy3-cholesterol uptake working solution. Usually, 100μl is added to each well of a 96-well plate, 250μl is added to each well of a 24-well plate, 500μl is added to each well of a 12-well plate, and 1ml is added to each well of a 6-well plate. Incubate at 37ºC in the dark for 24 hours. The incubation time can be adjusted within 12-

48 hours.
Note: If it is the first experiment and the incubation time cannot be determined, it is recommended to first try incubating at 37ºC in the dark for 24 hours and observe the fluorescence effect. If the fluorescence of normal cells is too strong, shorten the time appropriately; if the fluorescence is too weak, extend the time appropriately.
d. Detection. After incubation, wash 1-3 times with PBS or HBSS, and observe the cholesterol uptake effect under a fluorescence microscope (Cy3-cholesterol is orange fluorescence, Ex/Em = 554/568nm).

References:
[1]. Chen, Lifeng, et al. "Cholesterol-rich lysosomes induced by respiratory syncytial virus promote viral replication by blocking autophagy flux." Nature Communications 15.1 (2024): 6311.

Background

Cy3-cholesterol (λex = 554nm, λem = 568nm) is an orange-fluorescent cholesterol labeled with Cyanine3, which has cholesterol-like binding properties. Cy3-cholesterol can be taken up by cells, enabling the visualization and tracking of intracellular cholesterol distribution and metabolism. Cy3-cholesterol is widely used as a fluorescent probe to monitor cholesterol uptake and intracellular transport between organelles.