PRODUCT INFORMATION

CytoTrace™ Red CFDA

Item No. 20697

FW: 652.4
Purity: ≥90%
Supplied as: A solid
Storage: -20°C
Stability: ≥4 years
Special Conditions: Light sensitive

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

CytoTrace™ Red CFDA is a fluorescein-based dye used as a long-term cell tracer. It is nonfluorescent but cell-permeant and, upon entering the cell, is converted to a fluorescent compound by intracellular esterases.[1] CytoTrace™ Red CFDA is retained by living cells and displays excitation/emission spectra of 560/574 nm, respectively. It remains fluorescent for at least 24 hours and is retained through cell division for several cycles. The fluorescence survives fixation, allowing for immunocytochemistry techniques to be applied as well.

Assay Protocol

NOTE: The following is a recommended protocol and only provides a guideline. It should be modified according to specific lab needs.

1. Prepare a 2-10 mM DMSO stock solution.
   NOTE: The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at -20°C. Avoid repeated freeze-thaw cycles and protect from light.
2. Prepare a dye working solution.
   Prepare a 1-20 µM dye working solution immediately before use by diluting the DMSO stock solution from Step 1 with Hanks' and 20 mM HEPES buffer (HHBS), or the buffer of your choice, at pH 7.0. Mix well by vortexing.
3. Analyze cells with a flow cytometer or a fluorescence microscope:
   a. Treat cells with test compounds for a desired period of time.
   b. Centrifuge cells to obtain 2-10 × 10^5 cells per tube.
   c. Resuspend cells in 500 µl of the dye working solution (from Step 2).
   d. Incubate cells with dye solution at room temperature or 37 °C for 15 to 30 minutes, *protected from light*.
   e. Remove the dye working solution from the cells and wash the cells with HHBS or buffer of your choice.
   f. Resuspend cells in 500 µl pre-warmed HHBS or medium to get 2-10 × 10^5 cells per tube.
   g. Monitor the fluorescence at excitation and emission wavelengths of 560 and 574 nm, respectively, with a flow cytometer (FL1 channel) or a fluorescence microscope.

NOTE for bacterial cell staining: *Staining is most efficient when the stock dye solution is diluted 1:800 in nutrient broth preconditioned by overnight growth of the test bacteria, but fresh nutrient broth or PBS may also be used. Bacterial suspensions should be diluted with PBS to 10^5-10^7 organisms per ml. Bacteria may be stained by applying one ml of solution to a 0.45 µm filter (25 mm), vacuum filtering to remove the solution, then adding 1 ml of dye solution and incubating 5 to 10 minutes at room temperature.*

Reference

1. Beem, E., and Segal, M.S. Evaluation of stability and sensitivity of cell fluorescent labels when used for cell migration. *J. Flouresc.* **23(5)**, 975-987 (2013).

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