

Product Data Sheet

Product Name: CFDA-SE

Cat. No.: GC14056

Chemical Properties

Cas. No. 150347-59-4

Chemical Name 5-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-diyl diacetate

SMILES O=C1N(OC(C2=CC=C(C3(C(C=CC(OC(C)=O)=C4)=C4OC5=C3C=CC(OC(C)=O)=C5)OC6=O)C6=C2)=O)C(CC1)=O

Formula C₂₉H₁₉NO₁₁

M.Wt 557.46

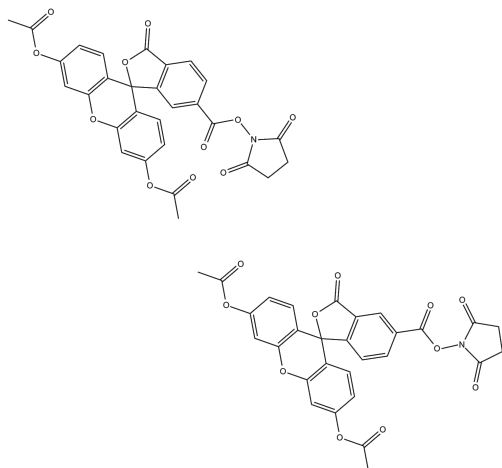
Solubility ≥ 37.2mg/mL in DMSO with ultrasonic

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

1. Preparation of staining solution

(1) Preparation of stock solution: dissolve CFDA-SE in DMSO to prepare a stock solution, typically at

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a concentration of 10mM. Stock solution are recommended to be aliquoted and stored at -20°C or -80°C protected from light. Avoid repeated freeze-thaw cycles.

(2) Preparation of working solution: dilute the stock solution with serum-free medium or PBS to prepare a working solution at 1-10 μ M. Since CFDA-SE hydrolyzes rapidly in aqueous solutions, prepare the working solution immediately before use. Proteins present in serum and culture media compete with CFDA-SE for binding, leading to a substantial decrease in labeling efficiency. The preparation of working solutions and the labeling process must be strictly performed under serum-free conditions.

2. Staining of Suspension Cells

(1) Cell collection: centrifuge at 1000 $\times$ g for 3-5min at 4°C, discard the supernatant, and wash the cells twice with pre-warmed (37°C) serum-free PBS, 5min each time.

(2) Cell density adjustment: resuspend the cells in serum-free PBS and adjust the density to 1 $\times$ 10⁶-1 $\times$ 10⁷ cells/mL.

(3) Staining: add CFDA-SE working solution (ensure the final concentration reaches the working concentration) and mix quickly and gently.

(4) Incubation: incubate the cell suspension at 37°C in the dark for 10-30min. The optimal incubation time varies among different cell types and should be determined empirically based on specific experimental requirements.

(5) Washing and resuspension: after incubation, centrifuge at 1000 $\times$ g for 5min, discard the supernatant, and wash the cells twice with PBS, 5min each time, to remove residual free dye.

3. Staining of Adherent Cells (Suspension Labeling Method)

(1) Cell collection: remove the culture medium, wash the cells twice with PBS, and detach the cells using trypsin. After digestion, centrifuge at 1000 $\times$ g for 3-5min.

(2) Follow steps (2) through (5) under "Staining of Suspension Cells".

4. Staining of Adherent Cells (In Situ Labeling Method)

(1) Plating: culture cells on plates until they reach approximately 60%-80% confluence.

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(2) Washing: remove the old medium and wash the cells twice with pre-warmed serum-free PBS.

(3) Incubation: add CFDA-SE working solution to just cover the cells, according to experimental requirements. Incubate at 37°C in the dark for 10-30min. The optimal incubation time varies among different cell types and should be determined empirically.

(4) Washing: aspirate the staining working solution and wash the cells twice with PBS.

5. Detection

Depending on the experimental purpose, detach the cells if needed, and observe under a fluorescence microscope or by flow cytometry at Ex/Em 488/518nm.

Notes:

(1) Intracellular esterase activity varies among different cell types, which may result in differential staining efficiency.

(2) Fluorescent dyes are susceptible to photobleaching. Protect from light throughout the staining procedure.

Background

CFDA-SE is a cell-permeable fluorescent dye that undergoes irreversible covalent binding to free amine groups of intracellular cytoskeletal proteins and releases green fluorescence upon hydrolysis^[1,2]. The fluorescence of CFDA-SE is evenly distributed to daughter cells upon cell division, with the fluorescence intensity halving at each division, and its attenuation is proportional to the number of cell divisions^[3,4]. CFDA-SE can be detected at an Ex/Em wavelength of 488/518nm and is commonly used for cell proliferation assays and live-cell tracing^[5,6,7]."

Reference:

[1] Morales-Kastresana A, Welsh J A, Jones J C. Detection and sorting of extracellular vesicles and viruses using nanoFACS[J]. Current protocols in cytometry, 2020, 95(1): e81.

[2] Ender F, Zamzow P, von Bubnoff N, et al. Detection and quantification of extracellular vesicles via FACS: Membrane labeling matters![J]. International journal of molecular sciences, 2019, 21(1): 291.

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- [3] Wu C X, Zou Q, Zhu Z Y, et al. Intrahepatic transplantation of hepatic oval cells for fulminant hepatic failure in rats[J]. World journal of gastroenterology: WJG, 2009, 15(12): 1506.
- [4] Duan X M, Huang J, Ma H X, et al. A flow cytometric assay for simultaneously measuring the proliferation and cytotoxicity of cytokine induced killer cells in combination with carboxyfluorescein succinimidyl ester (CFSE) labeling[J]. African Journal of Biotechnology, 2011, 10(65): 14598.
- [5] WANG X Q, DUAN X M, LIU L H, et al. Carboxyfluorescein diacetate succinimidyl ester fluorescent dye for cell labeling[J]. Acta biochimica et biophysica Sinica, 2005, 37(6): 379-385.
- [6] Urbani S, Caporale R, Lombardini L, et al. Use of CFDA-SE for evaluating the in vitro proliferation pattern of human mesenchymal stem cells[J]. Cytotherapy, 2006, 8(3): 243-253.
- [7] Ujiie M, Dickstein D L, Carlow D A, et al. Blood–brain barrier permeability precedes senile plaque formation in an Alzheimer disease model[J]. Microcirculation, 2003, 10(6): 463-470.

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