# 5(6)-CFDA

**Cat. No.:** HY-D0722

**CAS No.:** 124387-19-5

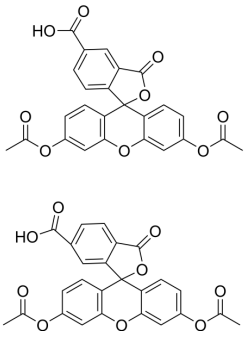
**Molecular Formula:** $C_{25}H_{16}O_9$

**Molecular Weight:** 460.39

**Target:** Fluorescent Dye

**Pathway:** Others

**Storage:** -20°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : 125 mg/mL (271.51 mM; Need ultrasonic)

| Preparing Stock Solutions | Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 2.1721 mL | 10.8604 mL | 21.7207 mL |
| | 5 mM | 0.4344 mL | 2.1721 mL | 4.3441 mL |
| | 10 mM | 0.2172 mL | 1.0860 mL | 2.1721 mL |

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (4.52 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (4.52 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

5(6)-CFDA is a common aliphatic luciferin-line organism. CFDA conducts free diffusion into cells, and then it is hydrolyzed into carboxyl fluorescein (CF) by intracellular non-specific lipase. CF containing portion contains an additional negative charge so that it is better retained in cells, compared to fluorescein dyes[1][2][3].

**In Vitro**

Preparation of 5(6)-CFDA working solution
1. Preparation of the stock solution
Dissolve 1mg 5(6)-CFDA in 0.21 mL DMSO to obtain 10 mM of 5(6)-CFDA.
Note: It is recommended to store the stock solution at -20°C -80°C away from light and avoid repetitive freeze-thaw cycles.
2. Preparation of 5(6)-CFDA working solution
Dilute the stock solution in serum-free cell culture medium or PBS to obtain 1-10 μM of 5(6)-CFDA working solution.
Note: Please adjust the concentration of 5(6)-CFDA working solution according to the actual situation.

Cell staining
1. Cell preparation:
For suspension cells: Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.
For adherent cells: Discard the cell culture medium, and add trypsin to dissociate cells to make a single-cell suspension. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.
2. Add 1 mL of 5(6)-CFDA working solution, and then incubate at room temperature for 30 minutes.
3. Centrifuge at 400 g at 4°C for 3-4 minutes and then discard the supernatant.
4. Wash twice with PBS, 5 minutes each time.
5. Resuspend cells with serum-free cell culture medium or PBS, and then detect by fluorescence microscope or flow cytometer.

Precautions
1. It is recommended to store the stock solution at -20□ or -80□ away from light and avoid repetitive freeze-thaw cycles.
2. Please adjust the concentration of 5(6)-CFDA working solution according to the actual situation.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Theranostics. 2020 Jul 11;10(20):8939-8956.
- Phytomedicine. 2020 Nov;78:153329.
- J Dairy Sci. 2022 Aug 2;S0022-0302(22)00429-5.
- Toxicol Lett. 2020 Jul 1;327:9-18.
- Microbiologyopen. 2022 Aug;168(8).

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## REFERENCES

[1]. Yang T, et al. A novel nonradioactive CFDA assay to monitor the cellular immune response in myeloid leukemia. Immunobiology. 2013 Apr;218(4):548-53.

[2]. Card SD, et al. Assessment of fluorescein-based fluorescent dyes for tracing Neotyphodium endophytes in planta. Mycologia. 2013 Jan-Feb;105(1):221-9.

[3]. Fang X, et al. Bone marrow-derived endothelial progenitor cells are involved in aneurysm repair in rabbits. J Clin Neurosci. 2012 Sep;19(9):1283-6.



Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA