FT-52493A

CFDA-SE

Product Information

Product name cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$. max. Coupled (nm)	
CFDA-SE FP-52493A, 25mg	557.47	495 / 519	5-(and-6)-carboxyfluorescein Diacetate Succinimidyl ester « mixed isomers » Also called CFSE in the litterature, but this abbreviation should be used for the non diacetate form.
5-CFDA-SE FP-AM496A, 10mg	557.47	495 / 519	5-carboxyfluorescein Diacetate Succinimidyl ester « single isomers »
6-CFDA-SE FP-AM497A, 10mg	557.47	495 / 519	6-carboxyfluorescein Diacetate Succinimidyl ester « single isomers »

Soluble:	In DMF or DMSO
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (coupled hydrophyled) = 495 / 519 nm; pKa=6.4
Storage:	Store at –20°C >1 year. (M) Protect from light and moisture

Introduction

CFDA-SE is the most popularized long term cell tracer in tissues and organisms for survival or redistribution purposes. It allows further tracing of cell becoming during cell division /i.e. in embryogenese studies, cell migration or moving / cancerogenesis, cell transplantation…. It is also used for labeling amine containing probes (antibodies, aminoallyl nucleic acids…).

CFDA-SE is colorless and passively diffuses into cells. After its acetate groups are cleaved by intracellular esterases, it becomes a highly fluorescent amine-reactive fluorophore, labeling covalently intracellular proteins, that keeps the fluorophore inside cells.

The fluorescence is compatible with subsequent fixation with formaldehyde or glutaraldehyde.

CFDA-SE evercomes alternative methods to reduce extracellular fluorescence quenching, including acidification of the extracellular medium, addition of tryptan blue or an antifluorescein antibody.
It also achieves a more stable cell staining than obtained with isothiocyanate derivatives, because the amide linkage formed by the coupling reaction is much more stable than a thiourea linkage.

The litterature reports the advantage of use of CFDA-SE in previous bacterial transport studies. It is efficiently stained bacteria without affect the transport or viability of bacteria.()

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Directions for use

Handling and Storage

Prepare a stock solution of CFDA-SE at a concentration 1000-fold higher than the final usage concentration (for example, 2 mM if the final concentration is 2 µM) in DMSO.

CFDA-SE is EXTREMELY moisture sensitive!

Use dry DMSO. CFDA-SE will hydrolyze quickly at room temperature in the presence of water, and much more slowly at –20ºC under desiccating conditions. You can aliquot and store over desiccant at –20ºC or –70°C but should not be for more than 2 months.

Guidelines for use – on primary cells and cell lines [0]

If your cells show decreased labeling with the same stock of CFDA-SE, hydrolysis is the likely cause.
Generally cells are labeled at a final CFDA-SE concentration of 0.5 to 5 µM.
For best results, do a titration and find the lowest concentration of CFDA-SE that will give effective cell labeling – this will vary from cell type to cell type, and also with the application.
CFDA-SE labeling is somewhat toxic and can induce growth arrest and apoptosis in some cell types – therefore, it is important to find the lowest acceptable labeling concentration and check the viability after labeling.
As a rough guide, 0.5 to 2 uM is usually enough for in vitro experiments – cell tracking and generational analysis in transplanted cells may require 2 to 5 uM.

1. Prepare cells in PBS or HBSS containing 0.1 BSA%. Cell concentrations can range widely from 1 x 10^6 cells/ml (for in vitro experiments) up to 5 x 10^7 cells/ml.
2. Prepare a solution of CFDA-SE from stock solution in PBS/0.1% BSA at 2X the final labeling concentration. For example, if you are labeling at 5 uM, prepare a 10 uM solution. Prepare a volume of CFDA-SE equal to the cell volume above (no more than 2 ml per labeling reaction).
3. Add an equal volume of CFDA-SE solution to the cell suspension. Mix gently and incubate for 5 to 10 minutes at +37ºC.
   Note : titrate to find the minimal effective conditions
4. Immediately fill the labeling tube to the top with the tissue culture media intended for culture (such as RPMI/10% FBS) and centrifuge. Wash the cells three times with tissue culture media at room temperature. To reduce the amount of unbound CFDA-SE in cells, incubate the cells at +37ºC for 5 minutes after the second wash and prior to the third. This allows free unreacted CFDA-SE to diffuse out of the cells and be removed in the final wash.

Guidelines for use – for protein labeling

This method uses carboxyfluorescein succinimidyl ester (CFDA-SE) rather than fluorescein isothiocyanate, resulting in more reliable labeling. Succinimidyl esters are excellent reagents for amine modification since the amide products formed are very stable. CFDA-SE has high reactivity with aliphatic amines, low reactivity with aromatic amines, including tyrosine.

1. Prepare or otherwise obtain pure protein; make sure it is free of other contaminating proteins (e.g. albumin).
2. Ensure protein to be labeled is in a suitable buffer. Buffers containing TRIS are NOT acceptable since the TRIS interferes with labeling. A reasonable buffer to use is PBS, pH 7.4. If necessary, exchange current buffer for PBS using one of three methods:
   a. Gel filtration. Do not use if you have less than 1 mg protein.
   b. Dialysis. Microdialysis is probably the best method if you do not have very much protein.
   c. Centricon microconcentrators.
3. Adjust protein concentration to ~ 1 mg/ml.
4. Prepare CFDA-SE, 1.5 mg/ml in anhydrous DMSO. Refer to "handling" section. Dilute CFDA-SE at desired concentration for labeling in anyhdrous DMSO immediately before use. A starting labeling molar ratio is 3-20 Fluorescein to protein, but should be optimized for each molecule.
5. Add 100 µl CFDA-SE per 1 ml of protein solution.

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6. Incubate for 90 minutes in the dark at room temperature with continuous gentle agitation. Alternatively, incubate overnight in the dark at 4 °C with continuous gentle agitation.

7. Exact conjugation efficiency depends on temperature, length of incubation, concentration of protein, concentration of CFDA-SE, and nature of protein. For critical applications, conjugate and test a small amount first to verify the conditions.

8. Separate labeled protein from free Fluorescein compounds by extensive dialysis versus PBS or by gel filtration. Concentrate with Centriprep and/or Centricon concentrators as necessary.

9. Microfuge on high for 10 minutes and filter through 0.22 µm filter. Optionally add 0.5% azide as preservative.

10. Determine concentration and F/P ratio by measuring absorbance at 280 nm and at 495 nm. For **antibodies**, use the following formulae to get approximate values:

$$\text{Protein concentration (mg/ml)} = (OD_{280} - 0.35 \times OD_{495}) / 1.4$$
$$\text{F/P ratio} = (3.3 \times OD_{495}) / (OD_{280} - 0.35 \times OD_{495})$$

Guidelines for use – on bacteria using microplate spectrofluorometry [0]

Protocol may found in the literature, and in our technical notice NT-Amine reactive

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