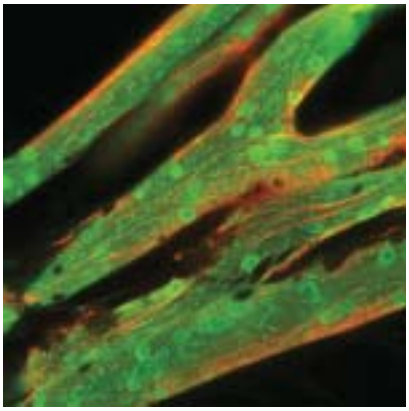


Cell and organelles tracers and markers

Many fluorescent dyes are used for labeling living cells, in order to get morphological data (shape, volume…) or to trace them in tissues and organisms. This section deals with general labels, but not specific labels (i.e. antibodies see chapter A) that generally affect cells too much for cell tracing.

Many derivates from **fluorescein** are widely used, for general purpose labeling and short term labeling.

- **Diacetate derivatives** are the most useful because they are quite non fluorescent and passively diffuse into cells, then become brightly green fluorescent once they are hydrolyzed by intracellular esterases. Some dyes bear an amino group that allows post-fixation.
- **Chemically reactive derivatives** allow the dye to attach to cell proteins. This leads to a long-term cell labeling, that is stable to formaldehyde or glutaraldehyde fixations in IHF techniques. CFSE is the most popular dye.
- **Polyanionic derivates**, with calcein as leading dye, are far less sensitive to pH than other fluorescein derivates. This favored their wide usage, especially for short term labelings in studies of cell structure, function, and many other applications.

Long term cell tracers

Labeling *ex vitro* a specific cell population is a common approach to trace cells then in tissues and organisms for survival or redistribution studies. CFDA-SE and CMTMR have been the most popularized.

Fluorescein based, amine reactive long term tracers

abs em. **CFDA-SE (CFSE, Green Cell Tracker)**

5-(and-6)-carboxyfluorescein diacetate succinimidyl ester "mixed isomers"
$C_{29}H_{19}NO_{11}$ MW : 557.47
Soluble in DMF, DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (after hydrolysis, pH 7.0) : 495/519 nm pKa : 6.4
Several alternatives were proposed to reduce fluorescence quenching, including acidification of the extracellular medium, addition of tryptan blue or an anti-fluorescein antibody. The amide linkage formed by the coupling reaction is much more stable than the thiourea linkage formed by the coupling of an amine with a isothiocyanate.
Main use is labeling cells in-vitro, allowing further tracing of cell becoming (during division / embryogenesis studies, during migration or moving / cancerogenesis, cell transplantation…). It is also used for labeling amine containing probes (antibodies, aminoallyl nucleic acids…). (See also "labeling reagents in chapter B). 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.

Description	Cat.#	Qty
CFDA-SE (CFSE, Green Cell Tracker)	**FP-52493A**	25 mg

abs em. **5-CFDA-SE (5-CFSE)**

5-carboxyfluorescein Diacetate Succinimidyl ester
The pure isomer 5 of CFDA-SE FP-52493.

Description	Cat.#	Qty
5-CFDA-SE (5-CFSE)	**FP-AM496A**	10 mg

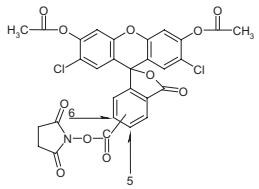
abs em. 6-CFDA-SE (6- CFSE)

6-carboxyfluorescein Diacetate Succinimidyl ester
The pure isomer 6 of CFDA-SE FP-52493A.

Description	Cat.#	Qty
6-CFDA-SE (6- CFSE)	**FP-AM497A**	10 mg

abs em. CDCFDA-SE

5-(and-6)-carboxy-2',7'-dichlolorofluorescein diacetate succinimidyl ester
$C_{29}H_{17}Cl_2NO_{11}$ MW : 626 .36
Soluble in DMF, MeCN ; Store at -20°C anf protect from light
$\lambda_{exc.}/\lambda_{em.}$ (free) : <300 nm/none
Gives after hydrolysis the same fluorescence than FP-46629
Dichloro substitution lowers pKa below CFDA's one, thus making it more useful to follow probes in acidic organelles (vacuoles, endosomes…).



Description	Cat.#	Qty
CDCFDA-SE	**FP-52495A**	25 mg

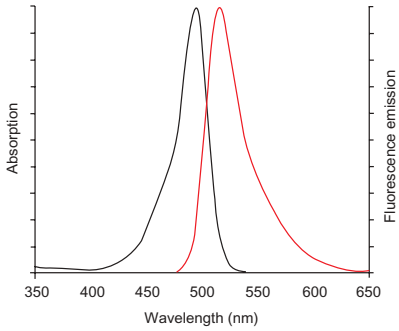
Short term cell tracers

Short term cell follow up can be achieved with the very popular Calcein, but also with various other dyes known for more specific applications.

Calceins

Calcein dye is a polyanionic derivate of fluorescein that exhibits fluorescence that is essentially independent of pH between 6.5 and 12. It is well retained in cells.
These features have made it a popular and versatile dye for various applications, including cell volume changes in neurons and other cells, endocytosis, Gap junctional communication, membrane integrity and permeability, angiography, liposomes…

It is worthy to notice that calcein is strongly quenched by several ions, including Fe^{3+}, Co^{2+}, Cu^{2+} and Mn^{2+} at physiological pH (not by Ca^{2+} or Mg^{2+} ions). Ions levels should thus be monitored.



Fluorescence of calcein at pH 9.0
(note : fluorescence is stable between pH 6.5 and 12).

abs em. Calcein

$C_{30}H_{26}N_2O_{13}$ MW : 622.54
Soluble in DMSO, DMF or pH >6 water
$\lambda_{exc.}/\lambda_{em.}$ (pH 8) : 494/517 nm ; EC : 75 000 $M^{-1}cm^{-1}$
Membrane impermeant.
Can be introduced into cells by microinjection.

Description	Cat.#	Qty
Calcein	**FP-466251**	100 mg

abs em. Calcein-AM

$C_{46}H_{46}N_2O_{23}$ MW : 994.88
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Calcein FP-46625
Enters readily cell membranes. Intracellular esterases converts it into calcein (FP-46625).

Description	Cat.#	Qty
Calcein-AM	**FP-895514**	1 mg
	FP-895515	20 x 50 µg

abs em. Calcein-AM, 1mg/ml solution

This solution in dry DMSO is more convenient (time saving : no solubilization) and increase reproducibility of screening assays.

Description	Cat.#	Qty
Calcein-AM, 1 mg/ml solution	**FP-855422**	1 ml @ 1mg/ml

abs em. Calcein blue-AM

MW : 465.4
Soluble in DMSO
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : 322/437 nm
Blue fluorescent cell viability indicator.

Description	Cat.#	Qty
Calcein blue-AM	**FP-95397A**	1 mg

Acridine Orange

Acridine orange is a prominent marker to study the lysosome-phagosomes fusion, more particularly in macrophages. It traverses biological membranes easily, then diffuse throughout the system, and ultimately accumulate intra- or extracellularly where it is the most efficiently bound. Its presence or absence in phagosomes is therefore not unequivocally indicative of fusion or non-fusion.
Reference : J Leukoc Biol. 1984 Sep ;36(3) :273-92. PuMed

Description	Cat.#	Qty
Acridine Orange	**FP-05920D**	50 g

Fluorescence Reference Standards for liposomes, membranes (and dyeing latex, films) Following dyes are excellent dyes for liposomes and membranes. See also more Fluorescence Reference Standards page B85.

Fluorescence Reference Standards	$\lambda_{abs.}/\lambda_{em.}$ (nm)	Cat. #	Qty
Coumarin 6	458/505	**BS5930**	100 mg
Coumarin 30	412/488	**BS5940**	100 mg
Coumarin 102	389/465	**BS5950**	100 mg
Coumarin 152	397/510	**BS5960**	100 mg
Coumarin 153	423/530	**BS5970**	100 mg
Coumarin 522	410/516	**BS5980**	100 mg
Cresyl violet	601/632	**BS5990**	100 mg

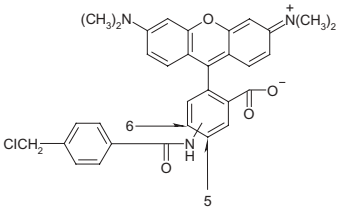
Other Cell biology tracers and markers

Several other dyes can be useful for cell or organelles tracing. This includes :

- Several pH probes, as the popular BCECF
- Fluorescein derivatives, especially their diacetate forms that accumulates inside cells, such as the ubiquitous CFDA-SE, or as Flubida (see below)
- Labeled biotins, for short term tracing
- Membrane probes (amphiphilic, lipidic) with sufficient stability, as used for morphology studies in mitochondria (page E129) or neurology (i.e. Lucifer Yellow CH).
- Most of protein (amine reactive) labeling agents, by fluorescence (page B51) or biotinylation (B88-B97). They typically label cell membrane proteins via amine reactivity, but other targets may be used as our Fluoprobes647-Hydrazide (see below)
- Primary antibodies (pages A1-A254) and antigens (A255-A314).

abs em. CMTMR, Orange Cell Tracking dye

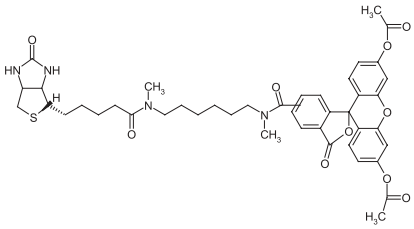
(((4-chloromethyl)benzoyl) amino)tetramethylrhodamine
$C_{32}H_{28}ClN_3O_4$ MW : 554.05
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 541/565 nm ; EC : 91 000 $Mol^{-1}cm^{-1}$
This fluorescent chloromethyl derivative freely enters in living cells, where it reacts mildly with thiol-containing intracellular components and becomes highly fluorescent in cytoplasm at all physiological pH levels (low pKa). It localizes essentially in cytoplasm. Fluorescent cells remain viable for at least 24 hours after loading, and it has been shown to be present in several cell generations up to two weeks.
It also can be fixed in situ with glutaraldehyde.
CMTMR is widely used for long term cell tracing by cytometry, but also in 2 colors imaging microscopy analysis thanks to its stable fluorescence.



Description	Cat.#	Qty
CMTMR, Orange Cell Tracking dye	**FP-12662A**	1 mg

Tel 33 (0)4 70 03 88 55 ♦ Hot line 33 (0)4 70 03 73 06 ♦ Fax 33 (0)4 70 03 82 60

Biotin (Flubida-2)



Flubida-2 is a cell membrane-permeable conjugate of fluorescein diacetate and biotin. The probe has been used to measure cell organelle pH by directing the dye where avidin-chimera proteins are located.

References : Chemistry & Biology 7, 197(2000).

Description	Cat.#	Qty
Biotin (Flubida-2)	**FP-AL667A**	5 mg

Lucifer Yellow Cadaverine Biotin-X, K salt

$C_{17}H_{17}K_2N_3O_8S_2$; MW : 534.
$\lambda_{exc.}/\lambda_{em.}$ 428/536 nm.

Lucifer Yellow Cadaverine can be used as a fixable fluorescent tracer. In addition, the dye can be used to conjugate on carboxylic acid groups.

Reference : Anal. Biochem. 211, 210(1993).

Description	Cat.#	Qty
Lucifer Yellow Cadaverine Biotin-X, K salt	**FP-M1210A**	10 mg

FluoProbes®647-Hydrazide

can be used as extracellular marker.

Reference : Prince L. et al ; Cell surface labeling of CFTR in T84 cells ; Am.J.Physiol.Cell 264 : 491498 (1993)

Description	Cat.#	Qty
FluoProbes®647-Hydrazide	**FP-BP5530**	

CytoRed solution

7-Isobutyloxycarbonyloxy-3H-phenoxazin-3-one
$\lambda_{exc.}/\lambda_{em.}$ (free) : 560/590 nm

CytoRed is cell membrane permeable and accumulates inside of viable cells as Resorufin. CytoRed has much wider spectrum than BCECF or Calcein, so filters for fluoresceins and rhodamine can then be used.

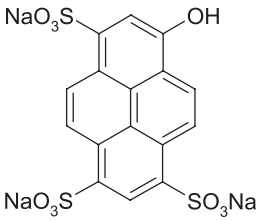
Description	Cat.#	Qty
CDCFDA-SE	**T30820**	1 ml

HPTS

8-hydroxypyrene-1,3,6-trisulfonic acid, also known as pyranine
$C_{16}H_7Na_3O_{10}S_3$; MW : 524.39
$\lambda_{exc.}/\lambda_{em.}$ (pH : 9) : 455/512 nm

A pH-sensitive blue fluorescent dye, used as sensitive tracer for acidic organelles

HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid, also known as pyranine, 982.H348) is a unique pH-sensitive tracer. It fluoresces blue in acidic solutions and in acidic organelles, but fluoresces green in more basic organelles. In addition to its use as a probe for proton translocation, HPTS has been employed for intracellular labeling of neurons and for delineating the cellular pathway of photosynthate transfer in the developing wheat grain. HPTS forms a nonfluorescent complex with the cationic quencher DPX (X1525), and several assays have been described that monitor the increase in HPTS fluorescence that occurs upon lysis or fusion of liposomes or cells containing this quenched complex (Technical Focus : Assays of Volume Change, Membrane Fusion and Membrane Permeability). HPTS has also been used as a viscosity probe in unilamellar phospholipid vesicles.



Description	Cat.#	Qty
HPTS	**FP-46903A**	1 g

4-(4-Hydroxystyryl)pyridinium propylsulfonate

MW : 319.8

Electrochromic membrane probe

Description	Cat.#	Qty
4-(4-Hydroxystyryl)pyridinium propylsulfonate	**BM1940**	1 g

Related products :

Fluorescein biotin conjugates can be used as polar non-fixable probes in cell biology, i.e. to detect membrane or epithelium permeability, intercellular space. We offer several such derivatives, as well versions improved with an extended spacer (FP-95914), an hydrophilic spacer (FT-BT372) or with a more photostable fluorophore (AM5541). Several are described in section labeling (page B50) as a main application is to quantify biotin binding sites and the degree of biomolecules biotinylation.

Description	Cat.#	Qty
Biotin-4-fluorescein	**FP-M1769A**	10mg
Fluorescein-lc-biotin	**FP-959145**	5 mg
Biotin-PEO3-Fluorescein	**BT3721**	5 mg
Biotin-rhodamine 110	**AM5541**	5 mg