Fluorescent pH Indicators

Fluorescent pH indicators show increased sensitivity and flexibility over conventional chromogenic indicators (phenolphtalein, phenol red…) and microelectrode techniques. Intracellular pH measurements are performed in many cell viability assays and cell physiological studies. It is essential to match the indicator's pKa to the pH of the studied medium.

*For **neutral pH studies**, **BCECF,** and **calcein** are now preferred to popularly used Fluorescein derivatives (CFDA…) that undesirably leak from cells. Many other Fluorescein derivatives have also been used. Applications concern various physiological and pathological processes, including cell proliferation, apoptosis, muscle contraction, malignancy, multidrug resistance, ion transport and homeostasis, endocytosis and Alzheimer's disease. Fluorescent pH indicators can also be used as pH sensors in cell-free media.

*For **acidic pH studies**, we provide **DCFDA** , that elicits a low K_d suitable to acidic organelles like endosomes and some vacuoles.

BCECF

BCECF is the most widely used fluorescent pH indicator. BCECF shows excellent retention in cells, and can detect small pH changes above pH 7 with higher sensitivity than fluorescein derivatives because its (ideal) pKa (6.97) is close to physiological cytosolic pH of most cells. At low pH, the dye is weakly fluorescent but becomes more fluorescent with increasing pH. The excitation spectrum of the dye undergoes a slight shift during pH change, while the wavelength of the emission maximum remains unchanged. The pH is determined ratiometrically by the relative fluorescent intensities at 535 nm when the dye is excited at 439 nm and 505 nm respectively.

Applications are widely documented in the literature, including study of ionic exchanges like $Cl^-/HCO3^-$, K^+/H^+ , Na^+/H^+, Na^+/Ca^{2+}, lactate transport and metabolism, NH^{4+} transport, cell proliferation assays, apoptosis, cytotoxicity, multidrug resistance, cell volume changes and cytosolic pH regulation in osteoblasts and osteoclasts, pH measurements in intercellular spaces of epithelial cell monolayers, interstitial spaces of normal and neoplastic tissue and isolated cell fractions intracellular pH changes monitoring in mammalian fibroblasts, gastric cells, lymphocytes, myocytes, and distal convoluted tubules and Phagocytosis.

Reference :
Kermis HR, et al. (2003). Rapid method for the preparation of a robust optical pH sensor. Analyst 128, 1181-6. Marechal X, et al. (1999). In vivo application of intestinal pH measurment using 2",7"-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) fluorescence imaging. Photochem Photobiol 70, 813-9. Weinlich M, et al. (1998). Simultaneous detection of cell volume and intracellular pH in isolated rat duodenal cells by confocal microscopy and BCECF. Res Exp Med (Berl) 198, 73-82
Carrero J and Voss EW, Jr. (1996). Temperature and pH dependence of fluorescein binding within the monoclonal antibody 9-40 active site as monitored by hydrostatic pressure. J Biol Chem 271, 5332-7.

abs em. BCECF-AM

Soluble in DMSO
Store at -20°C and protect from light
MW : 615-821 (mean)
Spectra after hydrolysis : see BCECF free acid (FP-45441A)
Cells in suspension or adherent are easily loaded by simple incubation in a 1–10 µM solution.

Description	Cat.#	Qty
BCECF-AM	**FP-45440A**	1 mg
	FP-45440B	10 x 100 µg
	FP-45440C	20 x 50 µg
	FP-72629A	1 ml in DMSO

abs em. BCECF free acid

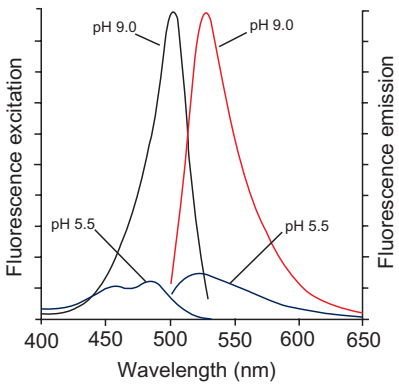
$C_{27}H_{20}O_{11}$ MW : 520.45
Soluble in methanol or water (pH > 6)
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 503/528 nm ; EC : 90 000 $M^{-1}cm^{-1}$
Membrane impermeant ; can be loaded into cells via microinjection, electroporation, or scrape loading. The net anionic charge is also higher, resulting in a slower leakage from cells. Useful in cell proliferation assays.

Description	Cat.#	Qty
BCECF free acid	**FP-45441A**	1 mg

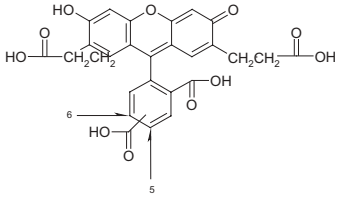
Technical tip

AM and DA derivative dyes
Many salt form dyes contain COO^- groups which anionic charges prevent membrane crossing and thus cell loading ("cell impermeant"). FluoProbes® provides the **AcetoxyMethyl** (AM) esters and/or **diacetate** (DA)derivatives for cell biology applications. These lipophilic blocking groups neutralize anionic charges. As a result, AM and DA modified dyes permeate cell membranes. Once inside the cell, AM and DA groups are cleaved by nonspecific esterases, restoring COO^- groups, hence membrane-permeation blocage, that reduce greatly undesirable leakage of the dye out of cells. In some cases, (e.g., calcein AM), the AM ester is colorless and non-fluorescent until hydrolyzed: this reduces eventual background due to unloaded dye, and help to control if spontaneous hydrolysis eventually occured during storage. For using, solubilization in anhydrous organic solvent, usually DMSO, is required. Loading is facilitated by low dispersing agents like Pluronic F-127 (FP-37361) or cyclodextrins (ADVASEP, FluoCD™). Concentrations of AM or acetate derived dyes should be kept as low as possible, usually 1-10 µM, to reduce artifacts generated by overloading such as incomplete hydrolysis or toxic effect of by-products. Loading times usually range from 15 minutes to 1 hour at room temperature, up 3-4 hours for some dyes as SBFI-AM and PBFI-AM.

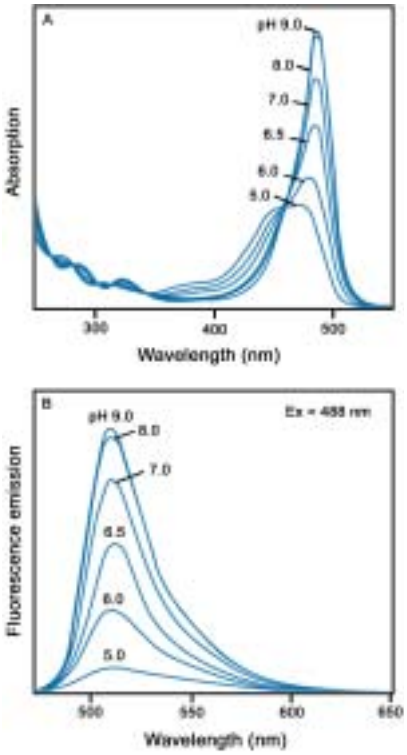
Related products : SQI dyes



Fluorescence excitation (detected at 535 nm) and emission (excited at 490 nm) spectra of BCECF in pH 9.0 and 5.5 buffers.



Fluorescent pH Indicators



Absorption and emission of Fluorescein (FP-19365) in aqueous buffer pH 9, 8, 7 and 6.

Fluorescein derivatives

Fluorescein and many of its derivatives exhibit multiple ionic equilibria, that make fluorescence dependent on pH. This section highlights selected derivatives for general use in pH measurements. Other fluorescein derivatives formerly used for that purpose can be found in section "labeling/fluoresceins" page B61.

*As compared to fluorescein, **carboxyfluorescein diacetate** (CFDA) contains extra negative charges and is therefore better retained in cells. It is mainly used as a mixture of 5- and 6-isomers for intracellular pH measurements, as both isomers exhibit essentially quite identical pH-dependent spectra with a pKa ~6.5.
CFDA and its AM ester are used for cell viability, apoptosis and cell adhesion monitoring.

***carboxy-2',7'-dichlorofluorescein** (CDCF) elicits modified spectral properties with a low pKa. It is frequently used as a selective probe for the relatively acidic yeast vacuole, and for viability assays.

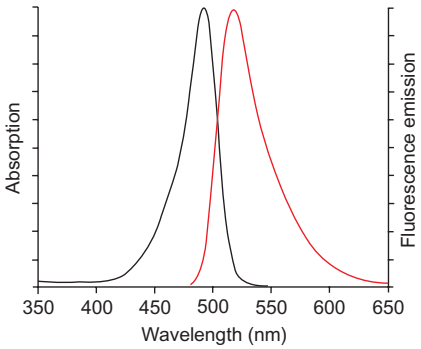
***Others dyes** are listed below because they were used for pH measurements, but show generally lower performances or are rather dedicated to other applications. Descriptions are given in other sections.

Note : Several approaches have been proposed to quench low levels of fluorescence of extracellular fluorescein, including hemoglobin, anti-fluorescein, and tryptan blue.

CFDA

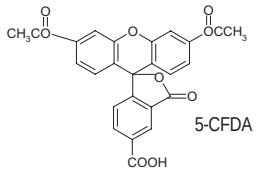
CFDA has been used as a viability probe for a variety of cells, including bacteria, fungi (e.g., Saccharomyces cerevisiae), spermatozoa, natural killer (NK) cells and tumor cells.

Fluorescence properties of the final product (FAM, FP-46641)
$\lambda_{exc.}/\lambda_{em.}$: 492 nm/517 nm
EC (pH9) : 80 000 $M^{-1}cm^{-1}$
Extinction coefficients and fluorescence quantum yields decrease significantly at pH <7.



Absorption and emission spectra of FAM (final product for CFDA) at pH.9.

abs em. **CFDA**
5-(and-6)-carboxyfluorescein diacetate
$C_{25}H_{16}O_9$ MW : 460.40
Soluble in DMSO
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see FAM (FP-46641A)
Hydrolysis of acetate group by intracellular esterases yields to FAM. Still used for pH measurements, and also commonly employed as a polar tracer. Mixed isomers are generally used, as they elicit same fluorescence pattern. However, if your application requires a single isomer of CFDA, we have it!



Description	Cat.#	Qty
CFDA	**FP-33953A**	100 mg

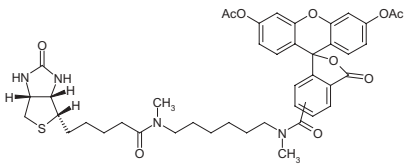
abs em. **5-CFDA**
5-carboxyfluorescein diacetate
characteristics : see CFDA (FP-33953A)
Single 5-isomer of CFDA.

Description	Cat.#	Qty
5-CFDA	**FP-M1162A**	100 mg

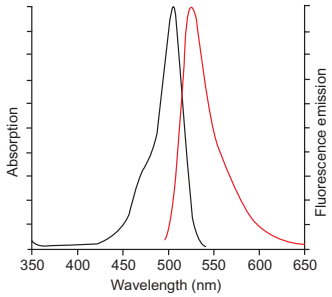
abs em. **6-CFDA**
6-carboxyfluorescein diacetate
characteristics : see CFDA (FP-33953A)
Single 6-isomer of CFDA.

Description	Cat.#	Qty
6-CFDA	**FP-M1163A**	100 mg

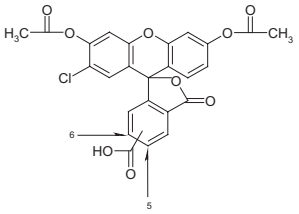
abs em. **Fluorescein diacetate-Biotin**
Spectra after hydrolysis : see Fluorescein (FP-19365A)
Cell membrane-permeable conjugate of fluorescein diacetate and biotin. Localizes a biotin label in cell compartments, for further detection with avidin. Useful to measure cell organelle pH.
Reference : Chemistry & Biology 7, 197(2000).

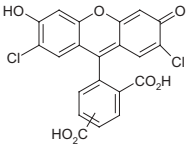


Description	Cat.#	Qty
Fluorescein diacetate-Biotin	**FP-AL667A**	5 mg



Absorption and emission spectra of CDCF in pH 9.0 buffer (FP-46629A).





CDCFDA/carboxydichlorofluorescein diacetate

CDCF has a low pKa. It is a useful probe for viability and cytotoxicity assays, and for studies of cell compartments having an acidic pH, like endocytosis and phagocytosis vacuoles, as well as other vacuoles, acrosome of spermatozoa...
It is available as a diacetate derivative for more convenient loading, and as a Succinimidyl ester for biomolecules in *vitro* or in *vivo* labeling, that can be followed up in living cells (see CFDA-SE FP-52495 page E97).

abs em. **CDCFDA**

5-(and-6)-carboxy-2' ,7'-dichlorofluorescein diacetate
$C_{25}H_{14}Cl_2O_9$ MW : 529.29
Soluble in DMSO
Store at -20°C and protect from light
Spectra after hydrolysis : see CDCF (FP-46629A)
Membrane-permeant and thus can be loaded into cells via incubation. The diacetate is readily hydrolyzed to CDCF (FP-46629A) by intracellular esterases.

Description	Cat.#	Qty
CDCFDA	**FP-46630A**	100 mg

abs em. **CDCF**

5-(and -6)-carboxy-2',7'dichlorofluorescein
$C_{21}H_{10}Cl_2O_7$ MW : 445.21
Soluble in DMF or pH >6 ; pKa : 4.8
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$ (pH 4) : 495/529 nm ; EC : 38 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (pH 8) : 504/529 nm ; EC : 107 000 $M^{-1}cm^{-1}$
Does not readily enter cells but can be loaded by injection. May be useful for acidic pH measurements, i.e. as fluid phase marker for endocytosis.

Description	Cat.#	Qty
CDCF	**FP-46629A**	100 mg

abs em. **5-CDCF**

5-carboxy-2',7'-dichlorofluorescein
$C_{21}H_{10}Cl_2O_7$ MW : 445.21
Store at 4°C
Spectra similar to CDCF (FP-46629A)
Single 5-isomer of CDCF (FP-46629A), used for specific applications

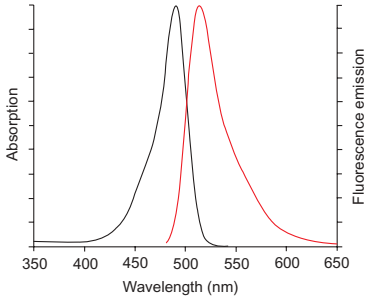
Description	Cat.#	Qty
5-CDCF	**FP-AM3021**	100 mg

Related products :
- See reactive oxygen dyes (H2DCFDA...)
- See Cell Tracers (CDCFDA-SE...)

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

Other pH indicators

Other **Fluorescein derivatives** (as FDA, FAM) have also a pH-dependent spectral response very similar to that of fluorescein. They were and are still used in many assays, but in a lot of applications are profitably replaced by other dyes, as BCECF (see above). Several miscellaneous dyes may be helpful for acidic measurements (as CRF).



abs em. FDA

$C_{24}H_{16}O_7$ MW : 416.39
Fluorescein diacetate
Soluble in DMSO
Store at 4°C
Spectra after hydrolysis : see fluorescein (FP-19365A)
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : 492/517 nm ; EC : (pH9) : 80 000 $M^{-1}cm^{-1}$ *
Hydrolysis of diacetate group releases Fluorescein FP-19365A. Still occasionally used to measure intracellular near neutral pH, to study cell adhesion , to determine cell viability (in combination with propidium iodide), or for cell tracing.
* EC and fluorescence QY decrease markedly at pH <7.

Description	Cat.#	Qty
FDA	**FP-29403A**	1 g

abs em. FAM

5-(and-6)-Carboxyfluorescein
$C_{21}H_{12}O_7$ MW : 376.32
Soluble at pH>6 in DMF
$\lambda_{exc.}/\lambda_{em.}$: 492/517 nm ; EC : (pH9) : 80 000 $M^{-1}cm^{-1}$
See section B3

Description	Cat.#	Qty
FAM	**FP-46641A**	100 mg

abs em. 5-FAM

5-Carboxyfluorescein
$C_{21}H_{12}O_7$ MW : 376.32
Soluble at pH>6 in DMF
$\lambda_{exc.}/\lambda_{em.}$: 492/517 nm ; EC : (pH9) : 80 000 $M^{-1}cm^{-1}$
See section B3

Description	Cat.#	Qty
5-FAM	**FP-34426A**	100 mg

6-FAM

abs em. boxyfluorescein
$C_{21}H_{12}O_7$ MW : 376.32
Soluble at pH>6 in DMF
$\lambda_{exc.}/\lambda_{em.}$: 492/517 nm ; EC : (pH9) : 80 000 $M^{-1}cm^{-1}$
See section B3

Description	Cat.#	Qty
6-FAM	**FP-84858A**	100 mg

Related products : FAM-SE, see page B64