Labels, used essentially in detection assays, come in main following categories :

- **Biotin**, an indirect label popularized thanks to is versatility for detection, purification, and amplification systems.
- Enzymatic labels that allowed notably the development of immuno-assays with greater sensitivity than previous colorimetric assays. Main enzymes are **peroxidase**, **alkaline phosphatase**, then glucose oxidase and other osidases, acetylcholine esterase.
- Fluorescent labels that have become very popular in detection technologies, thanks to exceptional features (sensitivity, multicolour, ...) and to the wide spreading of suitable instruments. Main fluorophores are **Fluorescein** compounds, but also **Rhodamines**, Coumarins and Cyanines....
  Now, fluorophores are available to cover not only the green light region, but also the entire visible light spectrum until the infrared region.
- Other markers or probes, more or less properly referred as labels, are presented in sections "as Antigens", "Immunologicals", "tags"...

The conjugation of labels to biomolecules (proteins, as well as other biomolecules), is based on the same chemistry as cross-linking, (please refer above pages B11-B36)

Biotinylation Labeling

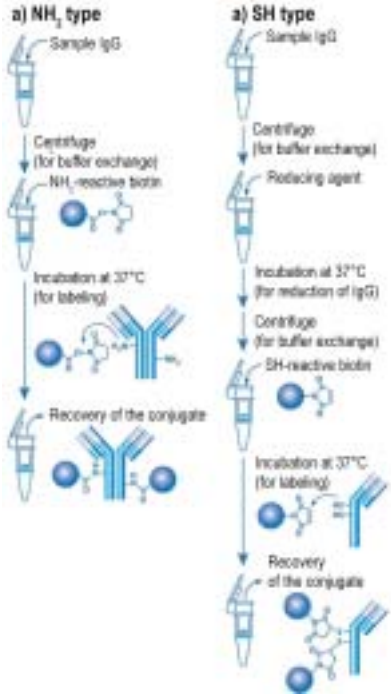
The biotinylation was popularized with the succinimidyl esters of biotin when amine targeting is desired (general purpose protein labeling), and with maleimides derivatives when sulfhydryl are available, or hydrazide biotin to label carbohydrates.

More technical information about general coupling strategies, chemical reactivities and spacers is given in section 'crosslinking/technical tips' page B11.

For general purpose use, we recommend to use NHS-, Maleimide, or Hydrazide- PEO4-Biotins. However, several other considerations may lead to prefer to use :

- shorter or longer spacer, or cleavable one (NHS-SS-Biotin)
- more classical sulfoNHS derivatives. They are extensively used as topological probes to label proteins in the outer membrane surface (Marmorstein 1998),
- NHS derivatives: they are extensively used for soluble proteins if organic solvents are acceptable, because hydrolysis can be better controlled, beside cost reasons. A modification preliminar of the molecule may be needed for special labelings, i.e. sulfhydryl introduction with the useful SATA reagent (UP84235 see page B36)

Also, complete biotinylation kits are proposed for non experienced investigators, as well as for convenient labeling (spin format) while avoiding to buy separate reagents.



Biotinylation kits

Microspin biotinylation labeling kits-NH_2 and -SH

- Quick : only 1 hour (/NH_2) or 3 hours (/SH) to get conjugates
- Easy : all processes in a single Filtration tubes
- Reliable : high recovery of conjugates
- Efficient : applicable for 50-200 µg IgG

Biotin Labeling Kits are primarily used for the preparation of biotin-labeled IgG for immunoassays. We offer kits with a very convenient format : spin filters where reaction and washes take place, that are available with 2 coupling strategies.

The kit BG767 biotinylates **on amines**, the most standard strategy. It uses a succinimidyl ester activated biotin, and contains all necessary reagents for labeling 3 samples of IgG antibody (10 µg to 200 µg). It can also be used to biotinylate any protein greater than MW 50 000 Da.
The labeling process is simple. Just add the NH2-reactive biotin to IgG solution on a filter membrane, and incubate at 37 ºC for 10 min. On the average, 5 to 8 biotin molecules conjugate to each IgG molecule. Exceeding biotin molecules can be removed using a Filtration tubes included in this kit.
The kit BT3591 biotinylates **on sulfhydryls** to obtain oriented and defined biotinylation. It uses a maleimide-activated biotin. Features are similar to kit BG7670, except 1/ there is an additional step to create a free sulhydryl in those protein (IgG) that do not have one, without loss of affinity ; 2/maleimide incubation occurs at 37 ºC for 30 min.

Protein labeling

Description	Cat.#	Qty
Microspin biotinylation labeling kit-NH_2	**BG7671**	1kit* (3 rxn/100µg)

Contains :
NH_2-reactive biotin (3 vials) - Wash. Storage buffer - Reaction buffer - Filtration tubes (3 tubes)
*The kit allow for labelings of 50-200 µg IgG,
The kit exists in greater size:
#BG7672 for 1 labeling of 1mg ;
#BG7673 for 1 labeling of 10 mg, or 2 labeling of 5mg.

Microspin biotinylation labeling kit-SH	**BT3591**	1kit* (3 rxn/100 µg)

Contains :
SH-reactive peroxidase (3 vials) - Reducing agent (3 vials) - Solution A - Solution B
Reaction buffer - Storage buffer - Filtration tubes (3 tubes)
*The kit allow for labelings of 50-200 µg IgG

Biotinylation labeling kit-NH_2

1 hour procedure
Flexible for IgG/proteins from 100 µg to 2 mg quantity

This kits uses a succinimidyl ester activated long spacer biotin, and contains all of the necessary reagents for labeling from small to 2mg of IgG antibody in one or several runs. Compared with kit #BG7670, reactions occur in standard vials and desalting is performed by dialysis (membrane is provided). Gelfiltration desalting (UP84874) may also be used for small volume samples (quicker operating).

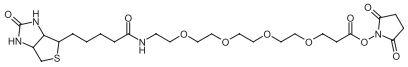
Description	Cat.#	Qty
Biotinylation labeling kit	**I98710**	1 kit (/2 mg)

Contains :
10 mg NH_2-reactive Biotin - Reaction buffer - Stabilizing buffer - Dialysis bag 10 000Da MWCO

Biotinylation of amines - reagents

NHS-PEO4-Biotin

$C_{25}H_{40}N_4SO_{10}$; MW : 588.67



See page B.11 for more information (labeling strategies, reactivities,spacers,...)

Description	Cat.#	Qty
NHS-PEO4-Biotin	**UPR20279**	4 x 5 mg
	UPR2027A	50 mg
	UPR2027C	1 g

This amine reactive biotin replaces advantageously NHS-x-Biotins in most applications :

- Highly water-soluble
- Spacer length optimal for rapid and tight avidin/streptavidin binding
- Prevents conjugates aggregation
- Amine labeling through NHS reactivity in less than 1H

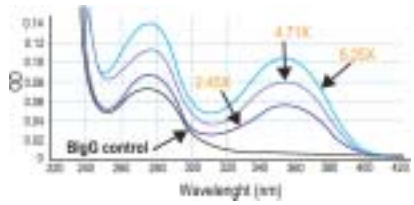
The spacer length (19.2 Å) is same as 'lc-lc' spacer (bis hexanoate) but presents several benefits (see the technical tip "PEO spacers" page B13)

Chromalink-Biotin

A unique biotinylation reagent for direct measure of biotinylation coupled ratio.

- Reactive to amines through a succinimidyl group
- Allows for direct spectrophotometric quantitation of **total** biotin incorporation
- Preserve biotin/avidin affinity as well as increase solubility

The spacer includes a chromophore (λabs : 354 nm ; EC : 29 000) along with a long chain PEO_4 and a succinimidyl ester. The spacer acts as a tag that allows quantitating accurately total coupled biotin, and not only available biotin as determined by the HABA method.



Incubation ratio	HABA ratio	CLS354 ratio
5X	1.03	2.45
10X	1.60	4.71
15X	2.22	6.25

Bovine IgG (bIgG) reacted with different biotin ratios and subsequent determination of coupled ratio by Chromalink method and HABA method. The streptavidin binding efficiency of ChromaLink Biotin coupled protein was proved to be similar to Biotin-PEO4-NHS labeled protein (not shown).

Biotin Chromalink

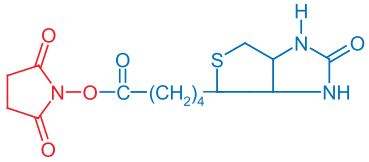
$C_{38}H_{50}N_8O_{10}S$
MW : 810.92
*Contains (Kit #BT3611) :
Biotin Chromalink reagent : 5 x 0.5 mg (BT3601) or 5 x 1 mg (BT3602).
DMF (anhydrous) - 10X modification buffer - 4 X 5K MWCO diafiltration apparatus
Biotin Chromalink is available as a single reagent, and in a biotiny lotion kit.

Description	Cat.#	Qty	Kit Cat.#	Qty
Biotin Chromalink	**BT3601**	5 x 0.5 mg	**BT3611**	Kit1*
	BT3602	5 x 1 mg	**BT3612**	Kit2*

NHS-Biotin

N-hydroxySuccinimido-Biotin
MW : 341.4
A classic for amines biotinylation

- Reacts with primary amines at pH7-9
- Short spacer
- Ideal for antibody and DNA biotinylation
- Economic

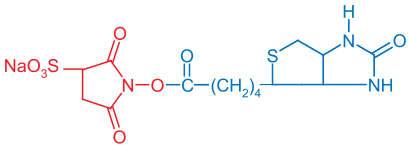


Description	Cat.#	Qty
NHS-Biotin	**UP39044A**	100 mg
	UP39044B	50 mg

SulfoNHS-Biotin

Sulfo-Succinimido-Biotin Acid
MW : 443.4
The water-soluble analog of #UP39044

- Directly soluble in aqueous buffer (no DMSO needed)
- Does not cross biological membranes / label inside cells



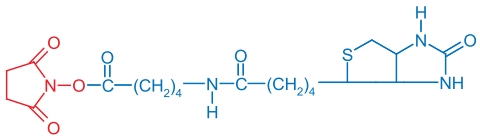
Description	Cat.#	Qty
SulfoNHS-Biotin	**UP52117A**	100 mg
	UP52117B	50 mg

NHS-lc-Biotin

Succinimidyl-6-(biotinamido)-hexanoate
MW : 455
Extended spacer arm than NHS-Biotin to improve biotin availability

- Reacts with primary amines at pH7-9
- Extended spacer
- Ideal for antibody and DNA biotinylation
- Economic

Applications :
Solid support labeling
Hydrophobic molecules labeling
Receptor studies
In Situ labelling
Immunoassays : immunoglobulins



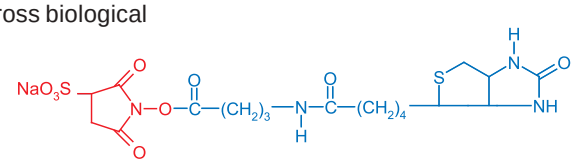
Description	Cat.#	Qty
NHS-lc-Biotin	**UP85262A**	100 mg
	UP85262B	50 mg

SulfoNHS-lc-Biotin

Sulfo Succinimidyl-6-(biotinamido)-hexanoate
MW : 556.6
The water-soluble analog of #UP85262

- Directly soluble in aqueous buffer (no DMSO needed). Does not cross biological membranes / label inside cells

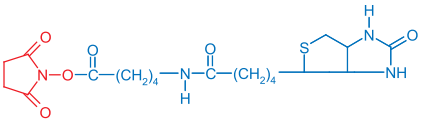
Applications :
Cell membrane studies
In vivo targeting



Description	Cat.#	Qty
SulfoNHS-lc-Biotin	**UP54398A**	100 mg
	UP54398B	50 mg

Protein labeling

NHS-lc-lc-Biotin



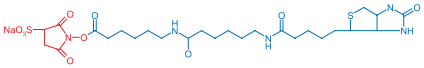
6-Biotinamidocaproylamido)caproic acid N-hydroxysuccinimide ester
MW : 567.7 - spacer length : 30.5A - Soluble 25mg/ml DMF.
The longest spacer version of the NHS-x-Biotins

- 14 atom extended spacer !
- Water-soluble, does not cross biological membranes

Applications :
Special applications where NHS-lc-Biotin gives low sensitivity of detection when buried labeling sites are suspected.

Description	Cat.#	Qty
NHS-lc-lc-Biotin	**UP29847A**	50 mg
	UP29847B	100 mg

SulfoNHS-lc-c-Biotin



MW : 669.75
The water-soluble analog of # UP29847

- Directly soluble in aqueous buffer (no DMSO needed). Does not cross biological membranes / label inside cells

Description	Cat.#	Qty
SulfoNHS-lc-c-Biotin	**UP37924A**	50 mg

NHS-imino-Biotin

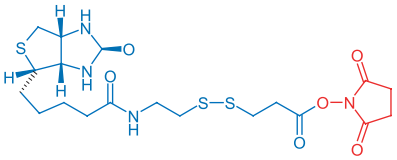
N-HydroxySuccinmide imino-Biotin
MW : 340.4
Perfect for further biotin-affinity purification (and immunoprecipitation)

- Lower affinity for (strept)avidin products than normal biotin
- Binds at alkaline pH
- Dissociates at pH4

Applications :
Recovery of biotinylated molecules after biotin-affinity separation from complex mixtures : plasmatic proteins bound to membrane cells, in-vivo biotinylated proteins…

Description	Cat.#	Qty
NHS-imino-Biotin	**UP35329A**	100 mg
	UP35329B	50 mg

NHS-SS-Biotin



MW : 504.65
Spacer 24.3 Å
The reversible biotinylation agent

- Reacts with primary amines at pH7-9
- Extended spacer and cleavable by reducing agents (DTT, DTE, TCEP)

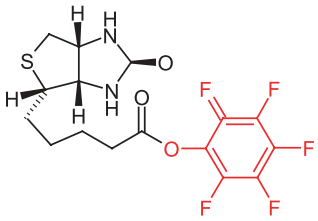
Applications :
Conjugates for in-vivo release
Recovery of non-biotinylated molecules after biotin affinity purification

Han, J.C., et. al. (1994) Anal. Biochem. 220, 5-10.1.
Moulton, C.A., et. al. (1982) Arch. Biochem. Biophys. 218, 101-108.
Lomant, A.J., Fairbanks, G. (1976) J. Mol. Biol. 104, 243-261.

Description	Cat.#	Qty
NHS-SS-Biotin	**UPS0738A**	100 mg

PFP-Biotin

Biotin PentaFluorophenyl Ester
MW : 410.37

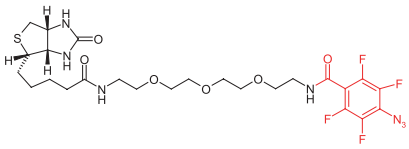


- More reactive and stable than corresponding NHS ester.
- Highly electrophilic leaving group enables direct labeling of nucleotide bases. PFP It reacts at pH7.9 with primary amines (as NHS), creating an amide bond, as well as with secondary amines.

Description	Cat.#	Qty
PFP-Biotin	**UP88364A**	100 mg

Biotin-PEO4-TFPA

MW : 635.63



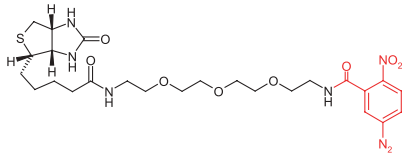
- Water-soluble !
- Labeling occurs by photolysis at 320 l.
- Tetrafluorophenyl stabilized nitrene gives higher yields of substrate-insertion products.1

1. Keana J. F. W., Cai, S. X. (1990) J. Org. Chem. 55, 3640-3647.

Description	Cat.#	Qty
Biotin-PEO4-TFPA	**BT3621**	10 mg

Biotin-PEO4-ANB

MW : 608.68



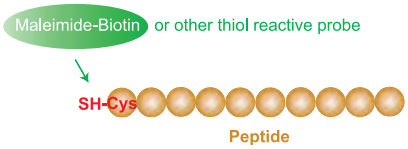
- Water-soluble !
- Labeling occurs by photolysis at λ : 320-350 nm on amines (non specific)

Description	Cat.#	Qty
Biotin-PEO4-ANB	**BT3741**	10 mg

Biotinylation of Thiols

Maleimido PEO_x Biotins

Replace the popular maleimido-biotins with the advantages of PEO spacers.



- Maleimide reacts specifically with free sulfhydryls at pH6.5-7.5
- Water-soluble (see associated benefits of PEO page B.13)

Description	Cat.#	Qty
Maleimide-PEO_3-Biotin	UP87284A	50 mg

MW : 525.62 ; 29.1 Å spacer

Maleimido PEO_4 Biotin

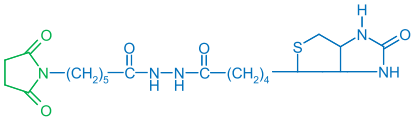
Maleimido PEO_4 Biotin	**UPR2028A**	25 mg
	UPR2028A	50 mg

MW : 505.63 ; 38 Å spacer

Maleimido-biotin

MW : 451

Biotinylates proteins on specific sites



- Maleimide reacts specifically with free sulfhydryls at pH 6.5-7.5
- Allows more defined labeling of proteins
- Avoids undesired amine modification on proteins

Applications :
Biotinylation of F(ab')2 Ig fragments
Biotinylation of SH of enzyme catalytic site

Description	Cat.#	Qty
Maleimido-Biotin	**UP48198A**	100 mg

Associated Products
SATA #UP84235A, Iminothiolane #UP42425A

Protein labeling

Biocytin-lc-maleimide

N-Biotinyl-N-(3-MaleimidoPropionyl)-L-Lysine
MW : 523.6
A long spacer analog of Maleimido-Biotin

- Maleimide reacts specifically with free sulfhydryls at pH 6.5-7.5
- Biocytin binds to avidin with lower affinity than biotin does

Applications :
Protein blotting and immunoassays : detection of SH groups on dot blot in the femto range
Protein immobilization
Cytochemical studies

Description	Cat.#	Qty
Biocytin-lc-maleimide	**UP99687A**	25 mg

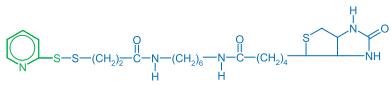
BMCC-Biotin

4,'4-MaleimidoMethyl)cyclohexane Carboxyamido)-butane
MW : 533.7
An original alternative to maleimido biotin.

- Reacts with free -SH at pH5-7 giving a thio-ether bond
- More specific and works at lower pH than iodoacetyl
- Extended 32.6Å spacer arm
- Iodinatable

Description	Cat.#	Qty
BMCC-Biotin	**UP27443A**	100 mg
	UP27443B	50 mg

HPDP-Biotin



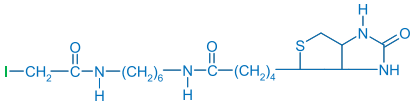
N-(6-(Biotinamido)Hexyl)-3'-(2'-Pyridylthio)propionate
MW : 510.4
A reversible thio-biotinylation reagent

- Reacts with free -SH at pH7-9 giving a stable -S-S- bond
- Released pyridine-2-thiol, measured at 343 nm, monitors the reaction
- Extended 29.2 Å spacer, and cleavable by reducing agents

Applications :
Functional and structural studies of Cys-containing proteins (receptors, enzymes…)
Immunoprecipitation with immobilized avidin then purification

Description	Cat.#	Qty
HPDP-Biotin	**UP83035A**	100 mg
	UP83035B	50 mg

Iodoacetyl-lc-Biotin



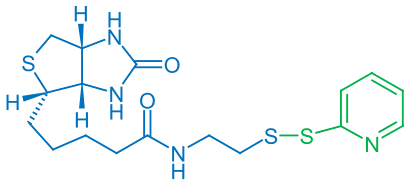
N-iodoacetyl-N-biotinylhexylenediamine
MW : 510.4

- Reacts with free -SH at pH 7.5-8.5 giving a thioether bond
- Extended 27.1 Å spacer

Applications :
Functional and structural studies of Cys-containing proteins (receptors, enzymes…)

Description	Cat.#	Qty
Iodoacetyl-lc-Biotin	**UP55533A**	100 mg
	UP55533B	50 mg

Biotin-PDA



MW : 412.6

- Sulfhydryl reactive biotinylation reagent
- Quantitate biotinylation by release of 2-thiopyridine, λmax 343 nm, Ec : 8, 080 M^{-1} cm^{-1}
- Biotin conjugation through an -SS- linkage enables cleavage of the adduct by mild reducing reagents, i.e. DTT, TCEP, etc.

Description	Cat.#	Qty
Biotin-PDA	**BT3631**	100 mg

MTSE-Biotin

2-((biotinoyl)amino)ethyl MethaneThioSulfonate
MW : 381.52

This reagent would fit inside a cylinder about 0.6nm in diameter and 1nm in length (Akabas 1992). Half-life (pH7.0, 20°C) : ca 12 min, Half-life (pH6.0, 20°C) : ca 92 min, Half-life (pH7.0, 4°C) : ca 116 min (Karlin 1998)

Description	Cat.#	Qty
MTSE-Biotin	**UPR5752**	10 mg

Biotinylation of Aldehydes and others groups (Carbohydrates, Nucleic acids)

Aldehydes generated by periodate oxidation of viccinal diols can be biotinylated using biotin-hydrazides, in glycoproteins, polysaccharides and sialic acids, steroids, glycolipids, LDL, and nucleic acids. An alternative approach is to activate carboxyls by EDAC (UP52005), and use an aminated biotin (page B.48).

Hydrazide-PEO$_x$-Biotin

MW : 505.63
20.6 Å spacer
Replace conventional hydrazide-biotins with the benefits of PEO spacers :

- Reacts with aldehydes and ketones to give stable hydrazones in a single step.
- Benefits of PEO spacer (hydrophilicity, eliminates non-specific binding, aggregation and precipitation, non-immunogenic...) see page B13

Description	Cat.#	Qty
Hydrazide-PEO$_x$-Biotin	**BJ008A**	50 mg

Hydrazide-X-Biotins

A classic carbohydrate reactive biotinylation reagent

- Reacts with aldehydes at pH 4-6 giving a stable CH=N-NH- bound
- Allows the glycoproteins labeling through their glycan
- Reacts also with carboxyls in presence of EDAC
- Available with extended spacer lengths improving greatly biotin availability

Applications :
Biotinylation of glycoproteins, lipopolysaccharides[2], hyaluronan[3]
Biotinylation of Immunoglob ulins in the Fc region for better orientation / activity
Biotinylation of nucleic acids though sugar ring
Functional and structural studies of glycosylation biomolecules
Detection of glycosylated proteins in membranes, leukocyte surface proteins[1]

1. Kahne, T., et .al. (1994) J. Immunol. Meth. 168, 209-218.
2. Luk, J.M., et. al. (1995) Anal. Biochem. 232, 217-224.
3. Yu, Q., et. al. (1995) Biotechniques 19(1), 122-129.

Hydrazide-Biotin - MW 371.5	**UP36466A**	100 mg
	UP36466B	50 mg
Hydrazide-lc-Biotin - MW 258.3	**UP78631A**	100 mg
	UP78631B	50 mg
Hydrazide-lc-lc-Biotin - MW 441.6	**BT3671**	50 mg

Hydrazide-biocytin

MW : 386.52

- Carbohydrate-reactive
- More water-soluble than Biotin-lc-Hydrazide
- Forms hydrazone bond

Description	Cat.#	Qty
Hydrazide-biocytin	**FP-22772A**	25 mg

See also Aminated Biotins (section B48)
Amine bearing biotins (i.e. BAPA UP84961) offer a unique way to biotinylate proteins, DNA or sugars : through their COOH.

See also damaged DNA assay kits #Q9506 page E182

AMCH-Biotin

N'-Aminoxymethylcarbonylhydrazino D-biotin
MW : 341.40
Aldehyde reactive biotin specific for a basic site of DNA

- Aldehyde-specific
- Labeling and detection of the abasic sites (AP sites, depurine/depyrimidine sites) of DNA

It has been reported that less than one abasic site in $1x10^4$ nucleotides of DNA can be detected.

Description	Cat.#	Qty
AMCH-Biotin	**UPR0756A**	10 mg

Psoralen-PEO_4-Biotin

MW : 688.79
Great for one-step labeling of all nucleic acids, and notably dsDNA, but also RNA, cDNA, PCR products, oligonucleotides, and even proteins and peptides !

- Psoralen intercalates between thymine and other pyrimidine containing bases.
- DNA/RNA probe modification does not interfere with hybridization.
- Labeling occurs by photolysis at 350 nm, 10-30min.
- PEO spacer confers excellent water-solubility (see page B13)

Description	Cat.#	Qty
Psoralen-PEO_4-Biotin	**UPL7784A**	10 mg

Biotin, aminated biotin and other biotin building blocks

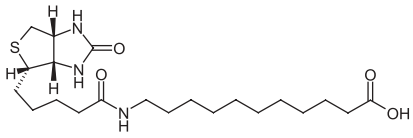
Biotin is used in biochemistry as well as in many other biotech applications (inhibitions in immunoassays, elution during purifications with avidin affinity supports, tissue/cell assays, controls,...).

Aminated-biotins can be used to label DNA and oses in presence of carbodiimides (i.e. EDAC UP52005). They are also used in organic chemistry (synthesis) to create biotinylated peptides or nucleotides. We also provide also other biotin derivates (Fmoc, tboc protected biotins) for this later application.

Biotin

D-biotin
MW : 244.31

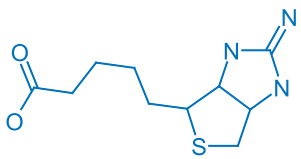
- Highest purity
- High affinity for (strept)avidin products
- High Biological activity (vitamin H)
- N-terminal biotinylation of peptides using EDC·HCl
- Useful for custom, synthetic modification of substrates
- Available as standard Biotin and 3 versions with longer spacer.



Description	Cat.#	Qty
Biotin - MW 244.31	**UP10685D**	200 mg
	UP10685E	1 g
Biotin-lc-COOH	**BT3641**	100 mg
6-((biotinoyl)amino)hexanoic acid - MW 357.47	**BT3643**	1g
Biotin-lc-lc-COOH - MW 427.6	**BT3651**	100 mg
Biotin-PEO_4-COOH - MW 491.6	**BJ007A**	50 mg
	BJ00AB	500 mg

IminoBiotin

2-iminobiotin, GuanidinoBiotin
MW : 243.33
Lower affinity for (strep)avidin products than Biotin



Description	Cat.#	Qty
IminoBiotin	**UP39375A**	100 mg

Biotin-PEO$_x$-Amine

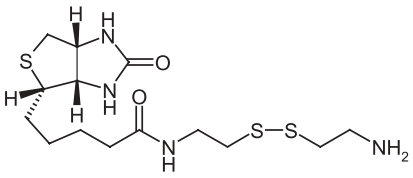
Used with carbodiimides (EDAC UP52005) to biotinylate carboxyls

- Available with various lengths of spacer.
- PEO arm confers improved features to conjugates (see page B.13)

Description	Spacer	MW	Cat.#	Qty
Biotin-PEO$_3$-Amine	20.4 Å	374.50	**UP77872A**	100 mg
Biotin-PEO$_4$-Amine	22.9 Å	418.56	**UP91577A**	100 mg

Aminoethyl-SS-Biotin

MW : 382.97



- Amino-biotin with cleavable tether (with DTT, DTE, or TCEP.[1])
- C-terminal biotinylation of peptides

1. Han, J.C., et. al. (1994) Anal. Biochem. 220, 5-10.

Description	Cat.#	Qty
Aminoethyl-SS-Biotin	**BT3761**	50 mg

Biotin-Amino-PentylAmine

(BAPA)
MW : 328.48

Applications :
Colorimetric assays for site-carboxyl-containing enzymes
Biotinylation of carboxyls with carbodiimides

Description	Cat.#	Qty
Biotin-Amino-PentylAmine	**UP84961A**	50 mg
	UP84961B	100 mg

Biotin-X-Cadaverin

MW : 555.67
Used as a polar tracer in cell study.

Description	Cat.#	Qty
Biotin-X-Cadaverin	**FP-83882A**	

Biotin Dimers

A biotin dimer cross-links avidin molecules, generates linear avidin oligomers.
Useful in immunodiagnostics as a signal enhancers.

Description	Spacer	MW	Cat.#	Qty
Biotin-PEO$_6$-Biotin	43.4 Å	732.97	**UPQ7467A**	50 mg
Biotin-PEO$_{12}$-Biotin		637.81	**UPT5046A**	50mg

Related products :
See also other biotin derivatives are used as probes for detection purpose (detection of biotin binding sites, proteins biotinylation degree, avidin and streptavidin measurement in crude biofluids, polar tracer for cell biology study (page E99).

Biotinylated and Fluorescent Biotins

Fluorescein biotin conjugates can detect and quantify biotin binding sites and the degree of biotinylation of proteins and detection of biotin binding sites (avidin). 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).The single isomer Biotin-4-fluorescein (FP-M1769) has been shown to binds quicker to avidin and thus improves results for quantitating biotin binding sites.These fluorescent biotins, and others, are also used for measurement of avidin and streptavidin in crude biofluids, polar tracer for cell biology.... See page Dxxx.

Fluorescein-lc-Biotin

MW : 831.03
Soluble in DMF, pH > 6
λabs./λem. : 494/518 nm ; EC : 75 000 $M^{-1}cm^{-1}$ (pH9)(greatly reduced < pH7)

- Used to detect and quantify biotin binding sites and the biotinylation degree of proteins, and also as a non-fixable
- Polar probe in cell biology

References : Biochim Biophys Acta 1381, 203 (1998).
Gruber HJ, et al ; Accurate titration of avidin and streptavidin with biotin-fluorophore conjugates in complex, colored biofluids ; Biochim Biophys Acta 1381, 203-212 (1998).
Buranda T et al ; Peptides, antibodies, and FRE on beads in flow cytometry : A model system using fluoresceinated and biotinylated beta-endorphin ; Cytometry 37, 21-31 (1999).

Description	Cat.#	Qty
Fluorescein-lc-Biotin	**FP-959145**	5 mg

Biotin-4-fluorescein

MW : 644.71
λabs./λem. : 494/523 nm
Soluble in DMF, DMSO, pH >7

- Improves results for quantitating biotin binding sites (binds quicker to avidin).

References : Biochim Biophys Acta 1427, 44 (1999).
Kada G et al; Rapid estimation of avidin and streptavidin by fluorescence quenching or fluorescence polarization ; Biochim Biophys Acta 1427, 44-48 (1999).

Description	Cat.#	Qty
Biotin-4-fluorescein	**FP-M1769A**	10 mg

Biotin-PEO_3-Fluorescein

λabs./λem. : 492 nm, EC=76 600 M-1 cm-1[1]
PEO tether provides improved water solubility! that was helpful in diagnostic applications.

1. Rinderknecht, H. (1962) Nature 193, 167.

Description	Cat.#	Qty
Biotin-PEO3-Fluorescein	**BT3721**	5 mg

Biotin-rhodamine 110

MW : 802.94
Soluble in DMF or DMSO
λabs./λem. : (MeOH) = 502/524 nm
Biotin-rhodamine 110 replaces advantageously Fluorescein-biotins, and is a better choice for studies where prolonged exposure to light may be necessary.

- Similar absorption and emission wavelengths
- Spectra and fluorescent quantum yield relatively unaffected by pH change (pH 4-9), whereas the fluorescence of fluorescein is significantly reduced at acidic pH.
- Much more photostable than fluorescein

Description	Cat.#	Qty
Biotin-rhodamine 110	**FP-AM5541**	5 mg

Fluorescence Labeling

Fluorescence, with its unique properties, improved greatly the detection sensitivity and possibilities of analysis over conventional technologies as colorimetry, UV and infrared analysis. Remarkable developments are achieved with multiparametric, single molecule, and interactions detections. This is supported by the continuous development of new fluorescent molecules (also called fluorochromes and fluorophores), labeling methods, and applications to tag biomolecules of interest with a fluorescent moiety.

Fluorochromes are natural compounds (i.e. Phycobiliproteins) or small synthetic molecules. They can be conjugated chemically to virtually any biomolecule, including small molecules (as peptides, nucleotides, drugs, steroids…) and big ones (as antibodies, DNA probes, biopolymers…).

This chapter presents an extended list of fluorescent labels (dyes), beginning with our superior FluoProbes® labels (section B51-B57), then standard labels by structure type (phycobiliproteins, fluoresceins, coumarins, rhodamines,...). You will find available derivatives for each label, starting with the most useful as amine reactivity (NHS, IC), sulfhydryl reactivity (Maleimide, MTS), aldehyde reactivity (Hydrazide) and other building blocks (Amino- and Carboxyl- derivatives). Please refer to pages B11, B60 for more information about chemical reactivities.

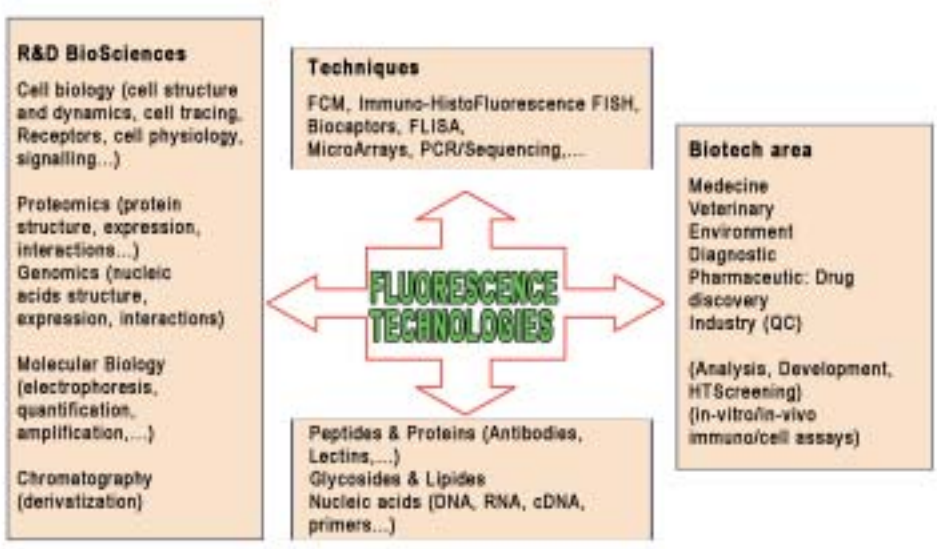
FluoProbes® labels

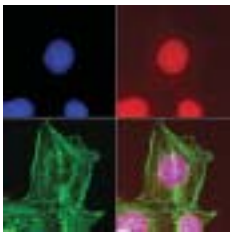
FluoProbes® developed many fluorochromes to improve properties over conventional fluorochromes in labeling experiments, but you also may find others and new applications.

Superior fluorescent features include :

- High photostability
- High molar absorbance
- Excellent solubility and stability in water and/or organic solvents
- pH-independent fluorescence between 5 and 9
- Weak fluorescence if not bound / high fluorescence if bound to target
- Large Stokes shift
- Choice to cover the visible and far red spectrum
- Available as free acid form, amine- or thio-reactive derivates (1)

(1) A molecule can be obtained labeled with our FluoProbes® dyes on a custom basis. Please inquire at interbiotech@interchim.com





Technical tip

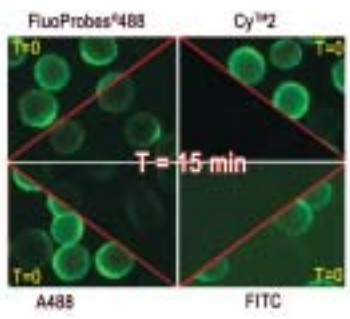
Preparing the Optimal Bioconjugate
Fluorescent labeling is usually rather easy to achieve, but it can become tricky when un-adapted choices (fluorochrome, chemistry, coupling ratio...) have been made for your specific application. For optimal assays, preparing the optimal conjugate may require extensive experimentation (comparison of different dyes, coupling ratio calibration, removal of un-reacted fluorochrome and reagents...). Protocols are available in technical sheets or on request.

Don't waste time to set up labeling ! We may have already done it or will do it for you: Already labeled specific probes are available in chapter E (i.e. labeled toxins) or (fluorescent II antibodies), or Genomics (fluorescent oligonucleotides). With these commercially available reagents, you can save time to focuse on your research, and take benefits from our documentation and reagent qualities. We also offer custom services for labelings, including for example FITC, SR101 and our proprietary FluoProbes® dyes.

QUIZ :
Have you a problem with a definite fluorochrome ?

- Low fluorescence ?
- Important background with some samples ?
- Undesired highly fluorescent aggregates on sample microscope slides ?
- Dye does not dissolve readily, or precipitates during labeling ?
- Dye/probe does not load or distribute properly in cells or organelles ?
- Fluorescence is fading ?
- Insufficient match in excitation or emission between a dye, your light source and filters ?
- Search fluorochromes to design better FRET tandems, and multiple labeling ?

...
>>> Try our FluoProbes® dyes ! FluoProbes® improves your results, and solves your problems.

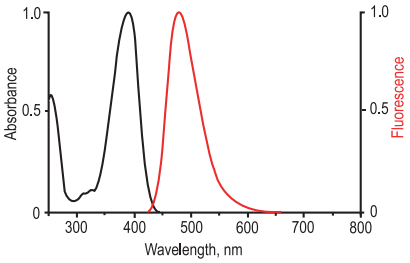


See pages B9-B11 for more information about these reactivities and labeling strategies.

Description of Selected FluoProbes® - labeling reagents

FluoProbes® labels are supplied with :

- Free carboxy group : can be used for labeling through conventional biochemistry, i.e. to amines by the carbodiimides activation method.
- Succinimidyl ester : suits direct labeling of amino groups in proteins, peptides, and any other molecules (DNA, RNA...)
- Maleimide group : suits labeling of thiol groups, i.e free cysteines natively present or introduced in proteins and other molecules.



FluoProbes®390

$\lambda_{abs}/\lambda_{em}$: 390/479 nm
EC : 24 000 M^{-1} cm^{-1}

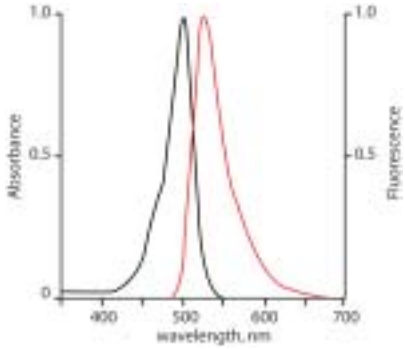
FluoProbes390 has high fluorescence quantum yield, large Stokes Shift and high stability at physiological pH-values. It is moderately hydrophilic. Its NHS-ester and maleimide show excellent solubility in polar solvents like DMF, DMSO or acetonitrile. Its fluorescence can be excited efficiently in the range 360 – 410 nm. A useful excitation source is, e.g., a Mercury Arc Lamp with its lines at 365 nm and 405 nm, making it a potential alternative to AMCA .

Description	Cat.#	Qty
FP390- COOH	**FP-BS5610**	1 mg
FP390- NHS-ester	**FP-BS5620**	1 mg
FP390- maleimide	**FP-BS5630**	1 mg
FP390- biotin	**FP-BS5640**	1 mg

FluoProbes® 488

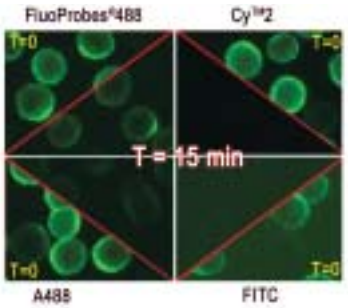
$\lambda_{abs}/\lambda_{em}$: 593/517 nm
EC : 90 000 M^{-1} cm^{-1}
The new standard of green fluorescent labels!

- Bright green fluorescence [1]
- Ultimate photostability, hence minimal fading [2]
- pH-independent fluorescence between pH 5 and 9
- Very high hydrophilicity
- Compatible with standard filters for FITC/Cy™2...
- Superior alternative / Compatible with filters FITC, Cy™2 [3]



[1] FP®488 shows elevated extinction coefficient,and excellent QY and can usually be coupled at high ratios without quenching.
[2] FP®488 elicits the better photostability amongst all tested green dyes, including references FITC, Cy2, A488. Consequently, longer integration of signal in digital imaging can be achieved to gain detection of low abundance molecules, signal amplification method may be avoided, re-analysis of samples remain as quantitative you do not need to use (additional price and time) antifading agents or their additives with correlated cyto-toxicity.
[3]FP488 suited filter sets include Zeiss #09.10.16.17.38.44, Nikon #FITC-HVQ, Olympus U-MNIBA2 and Omega # XF100-2.

Description	Cat.#	Qty
FP488-COOH	**FP-BA6790**	1 mg
FP488-NHS	**FP-BA6800**	1 mg
FP488-Maleimide	**FP-BA6810**	1 mg
FP488-Hydrazine	**FP-B3882A**	1 mg
FP488-Biotin	**FP-BA6820**	1 mg
FP488-Streptavidin	**FP-BA2221**	1 mg
FP488-Protein labeling kit	**FP-BE3750**	1 kit (see page B.55)
FP488-Annexin V	**FP-BH4140**	500 µl
FP488-II Abs	table page A324-A345	
Any other on inquire		

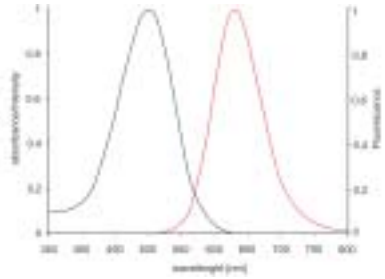


See more information and comparative photostability page A319.

FluoProbes®480XXL

$\lambda_{abs}/\lambda_{em}$: 500/630 nm
EC : 50 000 $M^{-1}cm^{-1}$
Our bright extra large stokes shift dye

- Excited in far blue
- Infra-red fluorescence
- Multi-color use with FluoProbes®488
- pH-independent fluorescence
- High brightness, pH-independent



Description	Cat.#	Qty
FP480XXL-COOH	**FP-BA6070**	1 mg
FP480XXL-NH2	**FP-BA6080**	1 mg
FP480XXL-NHS	**FP-BA6100**	1 mg
FP480XXL-Maleimide	**FP-BA6090**	1 mg
FP480XXL-Streptavidin	**FP-BA6340**	1 mg

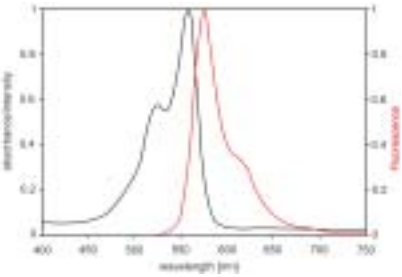
Any other on inquire

FluoProbes®547

$\lambda_{abs}/\lambda_{em}$: 557/574 nm
EC : 150 000 $M^{-1}cm^{-1}$
Our great orange dye

- Bright, orange fluorescence [1]
- Compatible with standard filters for Cy™3, Rhodamine TRITC
- High brightness

[1] FluoProbes® 547 is a superior alternative to Rhodamine TRITC, A546, Cy™3. It works in tandem with FP647.



Description	Cat.#	Qty	
FP547- COOH	**FP-BA3460**	1 mg	
FP547- NH2	**FP-BA3470**	1 mg	
FP547- NHS	**FP-AK7730**	1 mg	
FP547- Maleimide	**FP-BA3480**	1 mg	
FP547- Hydrazine	**FP-BP5530**	1mg	
FP547- Streptavidin	**FP-AX1460**	1 mg	
FP547- Protein labeling kit	**FP-BC0900**	1 kit	See page B55

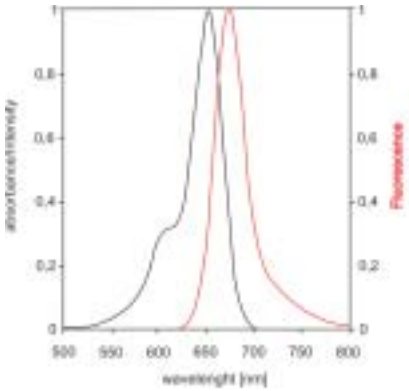
Any other on inquire

FluoProbes®647

$\lambda_{abs}/\lambda_{em}$: 652/673 nm
EC : 250 000 $M^{-1}cm^{-1}$
Our brighter red dye

- Brightest red fluorescence
- Alternative / suits light sources and standard filters for Cy™5 [1]
- Great for proteins labeling, and ideal for nucleic acids labeling [2]

[1] FluoProbes® 647 is a superior alternative to Cy™5 and A647, and work well invtandem with FP547.
[2] Better labeling of RNA has been reported compared with A647.

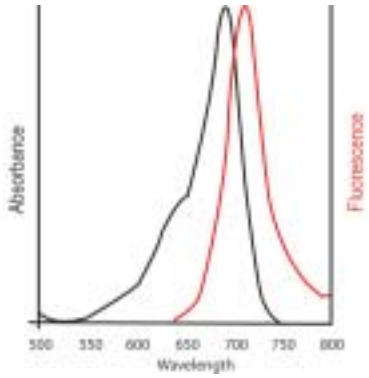


Description	Cat.#	Qty	
FP647- COOH	**FP-BA3830**	1 mg	
FP647- NH2	**FP-BA3840**	1 mg	
FP647- NHS	**FP-AK7740**	1 mg	
FP647- Maleimide	**FP-AZ5280**	1 mg	
FP647- Hydrazine	**FP-BP5530**	1 mg	
FP647- Streptavidin	**FP-BA1270**	1 mg	
FP647- Protein labeling kit	**FP-BA0310**	1 kit	See page B55

Any other on inquire

See also II antibodies conjugated to our orange FluoProbes 546 and red FluoProbes 642 pages A324-A345.

Protein Labeling

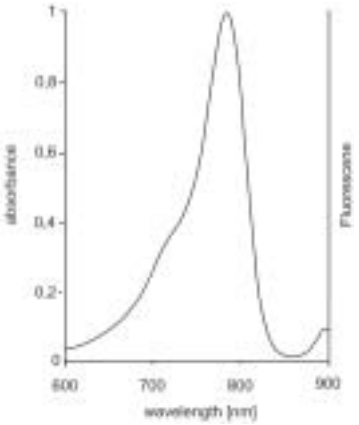


FluoProbes®682

$\lambda_{abs}/\lambda_{em}$: 690/709nm
EC : 140 000 M^{-1} cm^{-1}

- Exceptional emission duration
- Suits standard filters for CY™5.5, IRD700, A680
- Recommended for in vivo study, Western Blot, and confocal microscopy.

Description	Cat.#	Qty	
FP-682- NHS	**FP-BE6200**	1 mg	
FP-682- Maleimide	**FP-BE8230**	1 mg	
FP-682- Streptavidin	**FP-BE8050**	1 mg	
FP-682- Goat anti-Mouse IgG(H+L)	**FP-BE7250**	1 mg	
FP-682- Goat ant Rabbit IgG(H+L)	**FP-BF1690**	1 mg	
FP-682- Protein labeling kit.	**FP-BE8280**	1 kit	See page B55

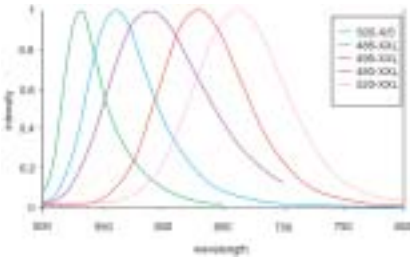


FluoProbes® 781

$\lambda_{abs}/\lambda_{em}$: 783/800 nm
EC : 170 000 $M^{-1}cm^{-1}$

- Excellent signal in the near infrared region
- Reduced background (bears a negative charge)
- Used routinely on Li-Cor® sequencer

Description	Cat.#	Qty
FP781- COOH	**FP-BA4370**	1 mg
FP781- NH2	**FP-BA4380**	1 mg
FP781- NHS	**FP-AP2200**	1 mg
FP781- Maleimide	**FP-BA4390**	1 mg



FluoProbes® XXL dyes

Multiple color detection with a single excitation source.
Several FluoProbes® dyes have close excitation maxima, but different Stokes shifts, that can be taken to good account for multiple detections. The following selection of FluoProbes® dyes can be efficiently excited with a same light source at 488 nm£, and thanks to extra large Stokes shifts, they emit over a wide wavelength range. They allow 2 or 3 colors detection with direct conversion of excitation light into emission without using a FRET mechanism. All have small size, at the opposite of phycoerythrin (see technical tip page B58). Applications include DNA sequencing IHF/FISH microscopy, flow cytometry.

FluoProbes® dye	MW (NHS ester)		$\lambda_{abs.}/\lambda_{em.}$ (nm)	EC ($M^{-1}cm^{-1}$)	Cat.# (NHS ester)	Qty
FluoProbes® 480XXL	611.68	£	500/630 nm	50 000	**FP-BA6080**	1 mg
FluoProbes® 481XXL	727.75	£	515/650 nm	50 000	**FP-BT2940**	1 mg
FluoProbes® 485XXL	502.6	£	485/560 nm	50 000	**FP-BA6140**	1 mg
FluoProbes® 505-X/5	523.97	£	505/530 nm	80 000	**FP-BA3420**	5 mg
FluoProbes® 510XXL	554.7	£	509/590 nm	50 000	**FP-BA6240**	1 mg
FluoProbes® 520XXL	514.6	£	520/664 nm	50 000	**FP-BA6280**	1 mg
FluoProbes® 521XXL	630.67	£	523/668 nm	35 000	**FP-BB1970**	1 mg
FluoProbes® 661XXL			661/725 nm	110 000	**FP-BB2020**	1 mg

£ : co-excitable

A Starter kit is available (FP-BA2021), that contains 1mg each of FluoProbes480XXL, 485XXI, 500XXL, 510XXL, and 520XXL

FluoProbes® labeling kits

FluoProbes® labeling kits are designed for the easy-to-use and efficient labeling of protein with molecular weights greater than 25 kD, including especially antibodies. They use a succinimidyl ester of fluorescent labels that form a covalent stable linkage. Up to 15 nmol of protein (~ 2.2 mg IgG for example) can be labeled in a 1h30 procedure. They are available with many of our FluoProbes® labels. Following is a list of selected and popular ones (others on inquire).

Description	Cat.#	Qty
FluoProbes®FITC-X Protein labeling kit	**FP-AX1350**	1 kit (5 runs)
594/519 nm (Fluorescein with an extended spacer for improved fluorescent properties)		
FluoProbes®488 Protein labeling kit	**FP-BE3750**	1 kit (5 runs)
593/517 nm (compatible with standard filters for FITC, Cy™2)		
FluoProbes®547 Protein labeling kit	**FP-BC0900**	1 kit (5 runs)
557/574 nm (compatible with standard filters for TR Cy™3)		
FluoProbes®647 Protein labeling kit	**FP- BA0310**	1 kit (5 runs)
652/673 nm (compatible with standard filters for Cy™5)		
FluoProbes®682 Protein labeling kit	**FP- BE8280**	1 kit (5 runs)
690/709 nm (compatible with standard filters for Cy™5.5)		

These kits are available on inquire for conventional labels. Other kits are described in following sections :

- FITC labeling kit (#BT951)
- PE, APC labeling kits (page B59)
- Label IT® Nucleic acid labeling kits, available with Rhodamine, Cy™3, 5, and other labels : see section DNA/RNA labeling pages D121, D134.

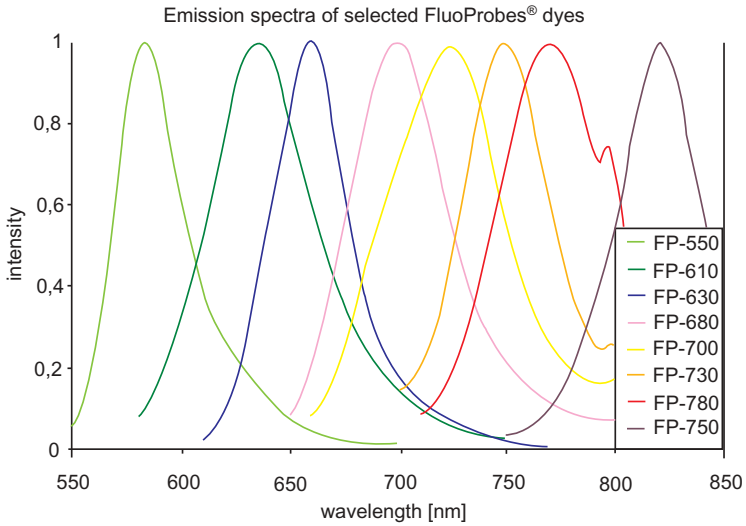
Antibodies fluorescent labeling methods
If you need to label antibodies, you may like :
1-direct labeling : this chapter
2-**indirect labeling** : use your **unlabeled Antibodies** then our superior **FluoProbes® labeled secondary antibodies** (see page A324). This method offers signals amplification and simplifies detection procedures of several Antibodies from same species.
3-**indirect biotin labeling :** biotinylate your I Antibody (see **biotinylation reagent** page B41) and use our superior **FluoProbes® labeled streptavidin** (page A350). This approach as been shown to be even more effective than direct labeling of I Antibodies : 1/ the biotinylation is quicker, easier to calibrate, and cheaper 2/ the biotinylated Antibody can be labeled in less than 30 min by FluoProbes®-streptavidin and used as a direct-FluoProbes®-labeled Antibody ; 3/ biotinylated Antibodies are more versatile for other uses including changing of label, multicolor labeling, and immunoprecipitations.

Table of FluoProbes® labeling reagents

This section, and following table, presents all our FluoProbes® Dyes. Each elicits interesting fluorescent properties that might be interesting in several applications. For example FluoProbes® 488, 547, 647, 731 and 480XXL show superior features for labeling applications in bioanalysis (see detailled information pages B52-B54). It is however not possible to document all fluorescence properties for all of the dyes in several applications. So it is recommended to **select,** thanks to the specifications/ datas given in the table below, the dyes that might suit at the best the specific requirements of your application.

FluoProbes® will be pleased to give inputs for your selection, because we aim at serving the research community with our expertise, and discover new applications! But there is no general rule to find the best dye, so testing selected dye(s) in your application may finally be the best way to get the optimal results.

How to choose a label ?
You may consider first, λ_{abs} and $\lambda_{em.}$ to match your instrument, light source and filters, but also suitable Stokes shift (multicolor analysis), elevated EC if highest sensitivity is required...also, photostability (confocal microscopy), hydrophobicity concerns depending on samples...!
Ask for FluoProbes experience !



Our FluoProbes dyes are listed by excitation wavelength order. Selected dyes are depicted on green background (see detailed description pages B52-B54).

Isolation/Modification/Labeling

Protein Labeling

label activated forms,	λ abs.* [nm]	EC* [L mol^{-1} cm^{-1}]	λ emis.* [nm]	Comments	COOH (free acid)	NH2 (amine reactive)	NHS (sulfhydryl reactive)	Maleimide conjugates, and custom labeling	Other
FluoProbes® 390A	**390**	**24 000**	**479**	Alternative to AMCA, MB®	**FP-BS5610**		**FP-BS5620**	**FP-BS5630**	& page B52
FluoProbes® 415	418	34 000	467	Alternative to A430, DEAC	**FP-BS5510**	**FP-BU5000**	**FP-BS5540**	**FP-BU5010**	& Streptavidin - FP415
FluoProbes® 444	424	-	473	(Lipophilic : A/E:424/479)	**FP-BB1920**				&
FluoProbes® 425A	436	45 000	484 #		**FP-BA6700**		**FP-BA6720**	**FP-BA6730**	&
FluoProbes® 465A	453	75 000	508 #		**FP-BA6750**		**FP-BA6760**	**FP-BA6770**	&
FluoProbes® 482	482	15 000	502	dependent on pH	**FP-BB1930**				&
FluoProbes® 485XXL	485 £	50 000	560 #		**FP-BA6120**	**FP-BA6140**	**FP-BA6130**	**FP-BA6150**	&
FluoProbes® 488	**493** £	**90 000**	**519** £	Alternative to FITC, Cy™2	**FP-BA6790**		**FP-BA6800**	**FP-BA6810**	II Abs FP488, Hydrazide Streptavidin-FP488, B52
FluoProbes® 495	493	70 000	521	FITC with extended spacer	**FP-BA3390**	**FP-BA3400**	**FP-AX1760**	**FP-BA3410**	SAV
FluoProbes® 494XXL	494	20 000	628 #		**FP-BB1940**		**FP-B44530**		&
FluoProbes® 495C	495	-	605 #	(lipophilic : A/E:545/583)	**FP-BB1950**		**FP-B44540**		&
FluoProbes® 495A	495	80 000	527		**FP-BA6850**		**FP-BA6860**	**FP-BA6870**	&
FluoProbes® 495D	495 £	70 000	520	Fluorescein with long spacer	**FP-BA3390**	**FP-BA3400**		**FP-BA3410**	&
FluoProbes® 480XXL	500 £	50 000	630 #		**FP-BA6070**	**FP-BA6080**	**FP-BA6100**	**FP-BA6090**	Streptavidin-FP480XXL&
FluoProbes® 505-X/5505	£ **80 000**		**530**	Alternative to FITC, A488 Cy™2	**FP-BA3420**	**FP-BA3430**	**FP-AX1720**	**FP-BA3440**	Streptavidin-FP505-X/5 tandem with FP648 & FP556
FluoProbes® 510XXL	509 £	50 000	590 #		**FP-BA6240**	**FP-BA6260**	**FP-BA6250**	**FP-BA6270**	&
FluoProbes® 520C	512		632		**FP-BB1960**		**FP-BU4550**		&
FluoProbes® 481XXL	515	50 000	650 #	see page B46	**FP-BV0800**	**FP-BT0810**	**FP-BT2940**	**FP-BV0820**	&
FluoProbes® 520XXL	520 £	50 000	664 #		**FP-BA6280**	**FP-BA6300**	**FP-BA6290**	**FP-BA6310**	&
FluoProbes® 521XXL	523	35 000	668 #		**FP-BB1970**	**FP-BU4560**			&
FluoProbes® 520A	525	110 000	545		**FP-BA6900**		**FP-BA6910**	**FP-BA6920**	&
FluoProbes® 532A	532	115 000	553	Alternative to A532	**FP-BA6940**		**FP-BA6950**	**FP-BA6960**	&
FluoProbes® 546	545	110 000	561	Alternative to Cy™3, A532, A642	**FP-BB1980**	**FP-BV2330**			Antibodies-FP546 Streptavidin-FP546&
FluoProbes® 555	547	100 000	572		**FP-BA3550**	**FP-BA3560**	**FP-AK7720**	**FP-BA6440**	SAV
FluoProbes® 556	548	100 000	573		**FP-BU4950**	**FP-BU4960**	**FP-AK7820**	**FP-BU4940**	Streptavidin-FP556&
FluoProbes® 550D	550	90 000	570	Alternative to Cy™3, A546			**FP-AM4290**	**FPAM4350**	&
FluoProbes® 554	551	100 000	572		**FP-BA3550**	**FP-BA3560**	**FP-AK7720**	**FP-AZ5270**	SAV
FluoProbes® 550	553	120 000	578	Alternative to Cy™3, A546	**FP-BA3490**	**FP-BA3520**	**FP-BA3510**	**FP-BA3540**	&
FluoProbes® 550A	554	120 000	576		**FP-BA6980**		**FP-BA7000**	**FP-BA7010**	&
FluoProbes® 555	555	100 000	580	Alternative to TMR, A546	**FP-BA3550**	**FP-BA3560**	**FP-AK7720**	**FP-AZ5270**	Streptavidin-FP555&
FluoProbes® 547	**557**	**150 000**	**574**	Alternative to Rhodamine	**FP-BA3460**	**FP-BA3470**	**FP-AK7730**	**FP-BA3480**	Streptavidin-FP547&, B53
FluoProbes® 548	558	150 000	572		**FP-BV5090**	**FP-BV5420**	**FP-BT2900**	**FP-BT2910**	&
FluoProbes® 565A	563	120 000	592	Alternative to TMR, SR101	**FP-BA7030**		**FP-BA7040**	**FP-BA7050**	&
FluoProbes® 568XXL	570	20 000	678 #		**FP-BB1990**				&
FluoProbes® 590	580	120 000	599	RT-X, SR101-X			**FP-R14040**	**FP-BL1370**	&
FluoProbes® 590A	594	120 000	624	Alt. to RT, SR101	**FP-BA7070**		**FP-BA7080**	**FP-BA7090**	&
FluoProbes® 594A	601	120 000	627	very hydrophilic	**FP-BV6050**		**FP-BU7160**	**FP-BU7190**	&
FluoProbes® 610	609	80 000	629	Alternative to SR101, A610	**FP-BA3580**	**FP-BA3600**	**FP-BA3590**	**FP-BA3610**	&
FluoProbes® 610A	615	150 000	634	pH sensitive	**FP-BA7110**		**FP-BA7140**	**FP-BA7150**	&
FluoProbes® 620A	619	120 000	643		**FP-BA7180**		**FP-BA7190**	**FP-BA7200**	&
FluoProbes® 615	621	200 000	641	Alternative to light cycler, A633	**FP-BA3620**	**FP-AN1160**	**FP-BA3630**	**FP-BA3640**	&
FluoProbes® 635A	635	120 000	659	pH sensitive	**FP-BA7220**		**FP-BA7230**	**FP-BA7240**	&
FluoProbes® 637A	635	120 000	659		**FP-BT9070**		**FP-BT9080**	**FP-BT9090**	&
FluoProbes® 630	636	200 000	657	Alternative toCy™5, A633	**FP-BA3650**	**FP-BA3670**	**FP-AM7580**	**FP-BA3680**	Streptavidin-FP630&
FluoProbes® 631	637	200 000	658		**FP-BA3690**	**FP-BA3710**	**FP-BA3700**	**FP-BA3720**	&
FluoProbes® 633	637	200 000	657		**FP-BA3730**	**FP-BA3750**	**FP-BA3740**	**FP-BA3760**	&
FluoProbes® 641	641	-	659	Lipophilic	**FP-BB2000**				&
FluoProbes® 642	642	170 000	660	Alternative to Cy™5, A647 FRET with FP546	**FP-BB2010**		**FP-BV2210**		Antibodies FP642 Streptavidin-FP642&
FluoProbes® 647N	644	150 000	669		**FT-BT9100**		**FT-BY7020**	**FT-BT9120**	&
FluoProbes® 647A	645	120 000	669	pH sensitive	**FP-BA7260**		**FP-BA7270**	**FP-BA7280**	&
FluoProbes® 636	645	200 000	671	Alternative to Cy™5	**FP-BA3800**	**FP-BA3810**	**FP-AZ6480**	**FP-BA3820**	&
FluoProbes® 635	647	200 000	671		**FP-BA3770**	**FP-BA3780**	**FP-AZ6490**	**FP-BA3790**	&
FluoProbes® 647	**652**	**250 000**	**673**	Alternative to Cy™5, A647	**FP-BA3830**	**FP-BA3840**	**FP-AK7740**	**FP-AZ5280**	Streptavidin-FP647&, B53
FluoProbes® 648	653	250 000	674	Tandem with FP556 and FP505	**FP-BU4970**	**FP-BU4980**	**FP-BT2040**	**FP-BU4990**	Streptavidin-FP648&
FluoProbes® 650	653	220 000	674		**FP-BA3850**	**FP-BA3860**	**FP-AZ6470**	**FP-BA3870**	&
FluoProbes® 651	653	220 000	678		**FP-BA3880**	**FP-BA3890**	**FP-AK7830**	**FP-BA3900**	&
FluoProbes® 661XXL	661 #	110 000	716 #	Page B46	**FP-BB2020**				&
FluoProbes® 655A	663	125 000	684	Alter. to Cy™5 (more photostable)	**FP-BA7300**	**FP-BA3930**	**FP-BA7310**	**FP-BA7320**	&
FluoProbes® 677	673	180 000	694		**FT-BU8540**	**FP-BU8550**	**FT-BU8520**	**FT-BU8530**	&
FluoProbes® 675	674	180 000	699		**FP-BA3950**	**FP-BA3970**	**FP-BA3960**	**FP-BA3980**	&
FluoProbes® 676	674	180 000	699	Alternative to Cy™5.5	**FP-BA3990**	**FP-BA4010**	**FP-BA4000**	**FP-BA4020**	&
FluoProbes® 680A	680	125 000	700	Alternative to Cy™5.5	**FP-BA7350**		**FP-BA7360**	**FP-BA7380**	&
FluoProbes® 681	690	140 000	708		**FP-BA4060**	**FP-BA4070**	**FP-AP2150**	**FP-BA4080**	&
FluoProbes® 680	690	140 000	709		**FP-BA4030**	**FP-BA4040**	**FP-AP1210**	**FP-BA4050**	&
FluoProbes® 682	**690**	**140 000**	**709**	Alternative to Cy™5.5	**FT-BV0900**	**FT-BV0910**	**FT-BE6200**	**FT-BE8280**	& see page B54
FluoProbes® 700A	700	120 000	719		**FP-BA7400**		**FP-BA7420**	**FP-BA7440**	&
FluoProbes® 701	706	140 000	731		**FP-BA4130**	**FP-BA4150**	**FP-BA4140**	**FP-BA4160**	&

Protein Labeling

abel	λ abs.* [nm]	EC* [L mol^{-1} cm^{-1}]	λ emis.* [nm]	Comments	COOH (free acid)	NH2	NHS (amine reactive)	Maleimide (sulfhydryl reactive)	Other activated forms, conjugates, and custom labeling
FluoProbes® 700	707	140 000	730		**FP-BA4090**	**FP-BA4110**	**FP-BA4100**	**FP-BA4120**	&
FluoProbes® 725	729	120 000	752		**FP-BT9130**		**FP-BT2120**	**FP-BT9150**	&
FluoProbes® 730	732	240 000	738		**FP-BA4170**	**FP-BA4190**	**FP-BA4180**	**FP-BA4200**	&
FluoProbes® 731	736	240 000	759	Alternative to Cy™7	**FP-BA4210**	**FP-BA4230**	**FP-BA4220**	**FP-BA4240**	Streptavidin-731&
FluoProbes® 740	740	120 000	764		**FP-BT9160**		**FP-BT2110**	**FP-BT9180**	&
FluoProbes® 750	747	270 000	776	Alternative to Cy™7	**FP-BA4250**	**FP-BA4270**	**FP-BA4260**	**FP-BA4280**	&
FluoProbes® 751	751	270 000	779	Alternative to Cy™7	**FP-BA4290**	**FP-BA4300**	**FP-AZ3520**	**FP-BA4310**	&
FluoProbes® 776	771	240 000	801		**FP-BA4320**	**FP-BA4340**	**FP-BA4330**	**FP-BA4360**	&
FluoProbes® 781	**783**	**170 000**	**800**	**Great for sequencing**	**FP-BA4370**	**FP-BA4380**	**FP-AP2200**	**FP-BA4390**	Streptavidin-FP781&, B54
FluoProbes® 831	844	220 000			**FP-BU5020**	**FP-BU5040**	**FP-BU5030**	**FP-BU5050**	

Our FluoProbes dyes are listed by excitation wavelength order. Selected dyes are depicted in orange (see detailed description pages B52-B54).
EC : molar absorbance * ; λ_{abs} : maximum apsorbtion wavelenght * ; λ_{em} : maximum emission wavelenght * ; LT : LifeTime
* : The given values may change depending on the environment of the label (nature of conjugate and solvent).
# : Large Stockes shift (>50nm)
£ : can be excited and read with FITC filters
& : All our FluoProbes® dyes are available for custom labeling :
- Streptavidin (SAV) conjugates are presented page A350
- secondary antibody conjugates are presented pages A324-A345

[505] FluoProbes®505 is more photostable then FITC, Cy™2, and suits confocal microscopy with FITC filters but do not suit FCM.

Technical tip

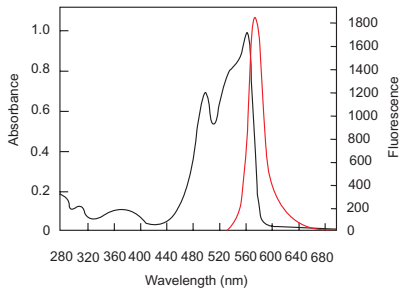
PE limitations

The FITC/PE tandem is widely used in FCM, but quite impossible in microscopy.
PE intense fluorescence is appreciated, but several reasons limit its use :

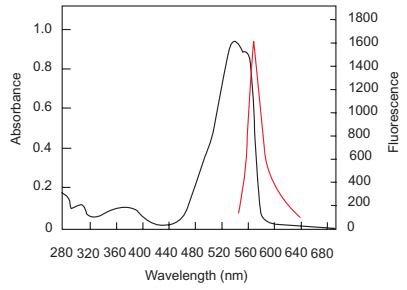
- Considerable spectral overlaps between phycoerythrin and FITC need to be corrected by compensation.
- Phycoerythrin big size (240Kda) prevents cell internal detections, or requires permeabilization procedures (if possible!).
- Phycoerythrin is prone to self quenching, notably with high density antigens.

An alternative approach is to adopt a synthetic (smal) fluorophore with an adequate excitation/emission/stocke's shift, i.e. FluoProbes® 494XXL.

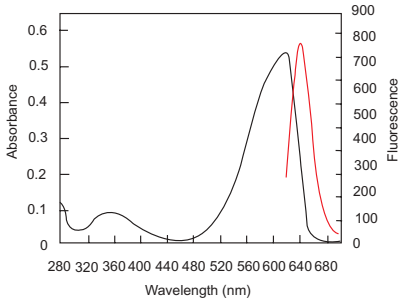
Related product :
Streptavidin-RPE #**FP-77776**



Absorption and emission spectra of R-Phycoerythrin.



Absorption and emission spectra of B-Phycoerythrin.



Absorption and emission spectra of C-Phycocyanine.

Phycobiliproteins (PE, APC,...)

Phycobiliproteins, are protein complexes purified from cyanobacteria and algae. They include Phycoerythrins (PE) and Phycocyanines (APC, CPC). They elicit exceptional fluorescent properties for labeling techniques compared to organic fluorophores, especially when high sensitivity or multicolor detection is required :

- **Broad and high absorption of light** suitable with many light sources
  So it is easier to select appropriate excitation wavelength in order to record fluorescence emission with efficiency
- **Very intense emission of light**
  Thanks to highest absorption and quantum yield, fluorescence, phycobiliproteins are 10-20 times brighter than organic fluorophores.
- **Relative large Stokes shift** gives low background, and allows multicolor detections.
  Excitation and emission spectra do not overlap compared to organic dyes. They allow too simultaneous use with conventional chromophores (i.e. PE and FP488/FITC, or APC and SR101 with the same light source).
- **Photostable** :
  Fluorescence emission is not easy to quench, because fluorescence retention period is longer.
- Very **high water solubility**
  Stable even after multiple sites conjugation

As a result, phycobiliproteins allow higher detection sensitivity, and can be used in **various fluorescence based techniques** (Fluorimetry in microplate, Flow Cytometry, FISH, two or multicolor detections…).
Both R-PE and B-PE suit classic applications with Kr/Ar and Ar/He (best) lasers, while APC has higher wavelength applications. These fluorochromes, especially R-PE, have also been used to detect and measure antioxidants as peroxyl radicals.

R-PhycoErythrin (R-PE)

Max λ_{abs} : 498 ; 546 ; 565 nm
Max λ_{em} : 576 nm
EC (565 nm) : 1.53 x 10^6 M^{-1} cm^{-1}
QY : 0.86

A566/A280 : 5.5
A566/A498 : 1.5
A620/A566 : 0.005

Can be excited with Kr/Ar and Ar/He (best) lasers
Ideal for double labeling with FITC or any other alternative fluorochrome

Description	Cat.#	Qty
R-PhycoErythrin (R-PE)	**FP-28310A**	2 mg

B-PhycoErythrin (B-PE)

MW : 240 000
Max λ_{abs} : 546 ; 566 nm
Max λ_{em} : 576 nm
EC (545 nm) : 2.4 x 10^6 M^{-1} cm^{-1}
EC (563 nm) : 2.33 x 10^6 M^{-1} cm^{-1}
QY : 0.98

A545/A280 : 5.5
A565/A545 : 0.95
A620/A545 : 0.005

Description	Cat.#	Qty
B-PhycoErythrin (B-PE)	**FP-17885A**	2 mg

C-PhycoCyanine (C-PC)

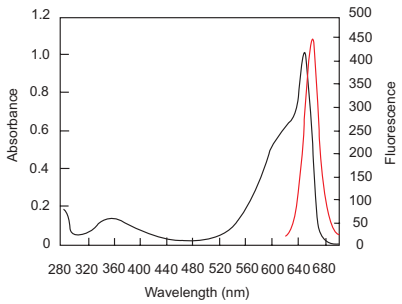
MW : 232 000
Max λ_{abs} : 620 nm
Max λ_{em} : 642 nm
EC : 1.54 x 10^6 M^{-1} cm^{-1}
QY : 0.81

C-PC accepts quanta from phycoerythrin by fluorescent energy transfer. Also, its red fluorescence can be transferred to allophycocyanin.

Description	Cat.#	Qty
C-PhycoCyanine (C-PC)	**FP-35191A**	0,5 mg

AlloPhycoCyanine XL (APC)

(Stabilized by cross-linking)
MW : 105 000
Max λ_{abs} : 650 nm
Max λ_{em} : 661 nm
EC : 7.3 x 10^5 M^{-1} cm^{-1}
QY : 0.68
A651/A280 : 5,
A651/A620 : 1.4



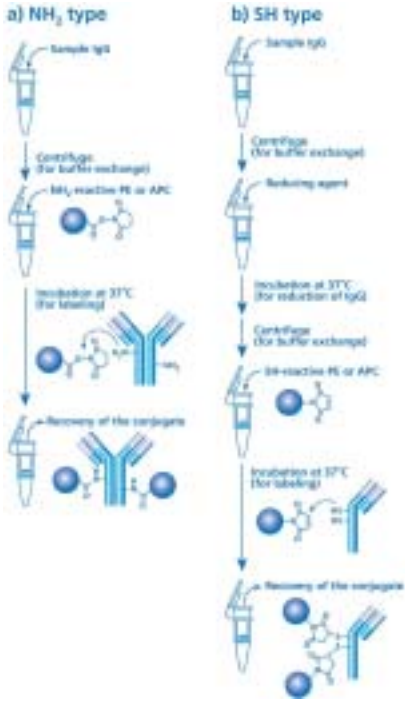
Absorption and emission spectra of AlloPhycocyanine.

Ideal for He/Ne laser, double labeling with Sulfo-Rhodamine 101 or any other equivalent fluorochrome.
Its near-infrared fluorescence is relatively free of interference from the auto fluorescence of cellular components and other biological materials. It is > 10 times more sensitive than conventional organic fluorophores and has been used in applications such as flow cytometry, homogeneous FRET assay, immunofluorescent staining and other immunoassays. It is provided as a cross-linked product to stabilize the most fluorescent form (trimer).

Description	Cat.#	Qty
AlloPhycoCyanine XL (APC)	**FP-35298A**	2 mg

Phycobiliproteins Labeling kits

- Quick : only 3 hours to get conjugates
- Easy : all processes in a single filtration tube
- Reliable : high recovery of conjugates
- Efficient : applicable for 50-200 µg IgG



PE and APC Labeling Kits have a very convenient format, spin filters where reaction and washes takes place, that are available with 2 coupling strategies.

Note : please refer above for the characteristics of fluorophores R-PE, B-PE and APC.

The kit –NH2 uses a succinimidyl ester activated PE or APC to perform conjugates suitable for most applications. Since all amino groups of NH_2-reactive the fluorochrome are blocked, no oligomerization is possible. The labeling process is simple. Just add the NH2-reactive biotin to IgG solution on a filter membrane, and incubate at 37ºC for 10 min. On the average, 1 to 2 fluorochrome conjugate to each IgG molecule. Excess fluorochrome molecules can be removed using a filtration tube included in this kit.

The kit –SH biotinylates on sulfhydryls to obtain oriented and defined labeling, and usually greater sensitivity. It uses maleimide activated PE or APC. Features are similar to kit –NH2, except 1/ there is an additional step to create a free sulfhydryl in those protein (IgG) that do not have one, without loss of affinity; 2/maleimide incubation occurs at 37 ºC for 30 min.

PE and APC Labeling Kits are primarily used for the preparation of labeled IgG for immunoassays, but can be applied to any biomolecule bearing amino or sulfhydryl groups, with a MW greater than 50 000 or lower than 5 000.

Description	Cat.#	Qty
B-PE Labeling kit-NH_2	**BT3801**	1 kit /3 rxn*
B-PE Labeling kit-SH	**BT3811**	1 kit /3 rxn*
R-PE Labeling kit-NH_2	**BT3821**	1 kit /3 rxn*
R-PE Labeling kit-SH	**BT3831**	1 kit /3 rxn*
APC Labeling kit-NH_2	**BT3841**	1 kit /3 rxn*
APC Labeling kit-SH	**BT3851**	1 kit /3 rxn*

- All kits provide all necessary reagents for labeling 3 samples of IgG antibody (50µg to 200µg). This include
- for NH2 kits: NH2-reactive phycobiliprotein , Reaction buffer, Wash/Storage buffer, and 3 filtration tubes
- for SH kits: SH- reactive phycobiliprotein, reducing agent, Reaction buffer, Wash/Storage buffer, RA solution, and 3 filtration tubes

Synthetic fluorophores

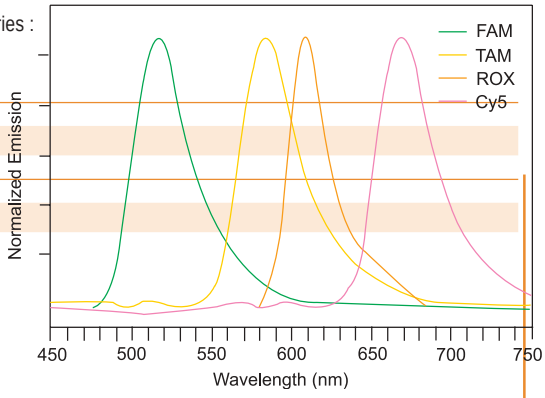
Overview - Synthetic fluorophores & their reactive derivatives

Synthetic fluorochromes are continuously developed to always cover better fluorescence technology needs. Each has fluorescence performance (absorption/emission wavelength, signal intensity, photostability...), and other physical features, as hydrophilicity that renders them more or less suitable for the different fluorescence detection applications (microscopy, fluorimetry, FCM...). They are usually classified on their chemical structure. Fluorescein based fluorophores were popularized, supplemented by Rhodamines and cyanine based fluorophores for longer wavelengths emission and multiple color detection. However, criteria of choice are always multiple and complexe.

So Interchim developed and selected outstanding dyes, FluoProbes, for the new requirements from general to demanding labeling applications (coverage of blue, green, orange, red, IR spectrum, high signal to noise ratio, photostability, low tendency to aggregate...).

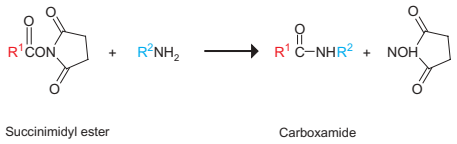
Conventional and FluoProbes® fluorochromes are thus presented in this catalog by following categories :

Fluorophore family	Main spectrum coverage	Products descriptions
Coumarins	blue to green	page B67
Fluoresceins	green to orange spectrum	page B61
Rhodamines	green to red spectrum	page B70
Cyanines	green to infra Red	
Other fluorochromes (eosin, pyrene, furan... based)		page B81
FluoProbes®	bleu to infraRed	page B51



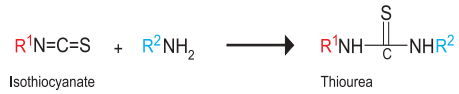
They are provided under several derivatives, that can be conjugated to virtually any chemical group via standard chemistry (with cross-linkers for example) or even, that can react directly in appropriate conditions. Some derivatives elicit multiple reactivities (sulfonyl chloride, thiocyanate), others are highly selective of definite groups (including the classic succinimidyl ester and maleimide derivatives).

Succinimidyl (SE, NHS) is an excellent and convenient reagent for labeling purposes. It reacts efficiently at pH 7.5-9 with aliphatic amines but has very low reactivity with aromatic amines, alcohols, phenols (including tyrosine) and histidine. A peptidic bond is formed, that confers many benefits compared to other chemistries. It is stable when dessicated (for storage). One limitation relies on its susceptibility to hydrolysis that competes with amine reaction. Another one is its weak solubility.These drawbacks are usually limited, or acceptable, or can be compensated by increased ratios for most applications.



Sulfonyl chloride (SC) derivatives are highly reactive. Their use for amine conjugation is limited by poor stability at the required pH 8.5-9. So alternative succinimidyl esters are recommended general manner. But SC stronger reactivity is useful for difficult molecules/chemistry.

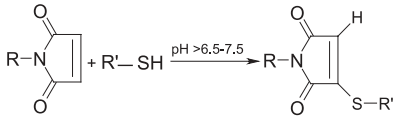
Isothiocyanates (IC) form with amines less stable bond than succinimidyl or sulfonyl halides and it can be converted into guanidine by concentrated ammonia. Selectivity is also lower, as reaction might occur with free H+. Despite these drawbacks, there may be useful in specific applications, and FITC or TRITC remains popular reagents.



MethaneThioSulfonate (MTS) reacts selectively and rapidly with thiols to form a disulfide bond. It is more efficient than pyridylthiol group. Our following reagents can be used for various biochemistry works (protein and peptide labeling), and for channel studies (SCAM method).

Substitued Cystein Accessibility Method

Maleimide reacts quickly and specifically with thiols in mild conditions. In most proteins, the reaction site is on cystein residues that are either intrinsically present or result from cysteines reduction, or introduced chemically or by genetic engineering. Unlike iodoacetamides, maleimides do not react with histidines and methionines under physiological conditions. This promoted maleimides among the most frequently used reagents for thiol modification. It is however a rigid structure and immunogenic.



Carboxylic acid (COOH) group of fluorochromes can also be used to conjugate amines with the help of NHS (Succinimidyl), or STP (4-sulfo-2,3,5,6-tetrafluorophenol) chemistries. I.e. Sulfosuccinimidyl esters can generally be prepared in situ simply by dissolving the carboxylic acid dye with Sulfo-N-Hydroxysulfosuccinimide (sNHS, FP-544225) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC, FP-52005D). This chemistry can be a good strategy to overcome aggregation, precipitation or quenching problems observed with succinimidyl ester dyes. However, burried amine might not react. The carboxylic acids may also be useful to modify aromatic amines and alcohols through the preparation of acid chlorides and anhydrides.

Main available reactive groups in **proteins and peptides** are amines, carboxyls and secondarily sulfhydryl (often involved in 3D structure, and biological activity.

Main available reactive groups in **glycoproteins, glycolipides, nucleotides and nucleoside** are Carboxyls and Aldehydes. Besides respective coupling strategies described above, alternative methods include the famous amidites, which find their main application in nucleotide synthesis, and some intercalating agents (psoralen derivates, AMCH-Biotin).

For more information about these reactivities and protein, nucleic acids, lipids conjugation strategies (conditions of reaction, stability of formed bond...), please refer to cross-linking section.

Fluorescein reagents are available as many different derivatives of the basic fluorescein structure and with reactive groups :

- **Carboxyfluorescein** (FAM) : carboxamidation improves the yield and stability of conjugates page B63.
- **Chlorinated fluoresceins** (JOE, HEX, TET...) : chlorine substitution shifts emission red page B65.
- **Diacetate** derivatives : the acetate groups, that block reversibly the carboxyls of fluorescein, are useful in cell applications, favoring cell loading and intracellular retention (see technical tip page E65).
- Different **reactive groups** containing derivatives : **Carboxyls** and **amino** groups are mainly used for conjugations with conventional chemistry (organic synthesis); More convenient reactivities are available toward amino groups (**NHS**, **Thiocyanates**...), thiol groups (**Maleimide**, **MTS** and carbonyls (**Hydrazide**...). More information about theses reactivities is given page B60 and in the technical tips of section cross-linking.

MicroSpin Fluorescein labeling kit-NH_2

- Quick : only 1 hour to get conjugates
- Easy : all processes in a single filtration tube
- Reliable : high recovery of conjugates
- Efficient : applicable for 50-200 µg IgG

Fluorescein Labeling Kit-NH_2 is mainly used for the preparation of fluorescein-labeled proteins such as IgG for immunostaining and cellular proteins for tracing. It uses fluorescein ($\lambda_{abs.}/\lambda_{em.}$: 495/520 nm) activated by succinimidyl ester: NHS) that reacts with an amino group of proteins or other molecules. This kit contains all of the necessary reagents for labeling, including storage buffer. Each tube of NH_2-reactive fluorescein can label 10 µg to 100 µg, up to 200 µg of IgG, conjugating about 4 to 6 fluorescein molecules per IgG molecule. The labeling process is simple. Add the NH_2-reactive fluorescein to IgG solution on a filter membrane, and incubate at 37 ºC for 10 min. A filtration tube can remove the excess of fluorescein molecules.

Fluorescein Labeling Kit-NH_2

Description	Cat.#	Qty
Fluorescein Labeling Kit-NH_2	**BT9511**	1 kit

Contains :
- NH_2-reactive fluorescein (100 µg x 3 tubes)
- WS buffer (4 ml x 1 bottle)
- Reaction buffer (0.5 ml x 1 tube)
- Filtration tube (3 tubes)

See also the excellent alternative FluoProbes488 (more photostable)

Fluorescamine

MW : 278.27
$\lambda_{abs}/\lambda_{em}$ (unbound) : 315 nm/none
$\lambda_{abs}/\lambda_{em}$ (NH_2 bound) : 385/486 nm

Fluorescamine is non-fluorescent but readily reacts with primary aliphatic amines (from peptides, proteins…) to a blue-green fluorescent compound that can be excited by UV light. The amine adduct has maximum absorption at 385 nm and maximum fluorescence at 486 nm.
Applications: a popular fluorogenic reagent to assay protein concentrations in solutions and on gel analysis of low molecular weight amines (amino acids, peptides) by TLC, HPLC and capillary electrophoresis-FRET assays (3,4), up to femto mole sensitivity on beads with a cytometer [2]

References: 1.Bartzatt R, et al. (2003) ; Biotechnol Appl Biochem. 2. Buranda, T, et al. (2001) ; 2.Anal Biochem 298, 151-162; 3.Li, Y and AN Glazer (1999) ; Bioconjug Chem 10, 241-245 ; 4. Yang, M, et al. (1998) ; Anal Biochem 259, 272-274.

Description	Cat.#	Qty
Fluorescamine	**FP-12631E**	100 mg
Fluorescamine, Fluograde	**FP-R1246A**	100 mg

Technical tip

Fluorescein based labels

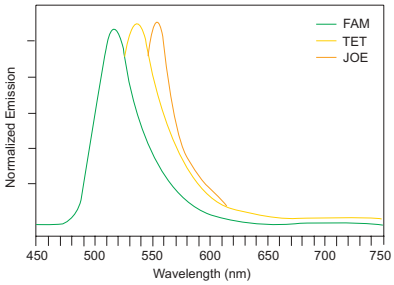
Fluorescein is surely the most popular fluorochrome since the first ages of fluorescence biotechnologies. Many derivatives were developed to modify its fluorescence and conjugates properties. The single isomer 5 is mainly used for protein labeling, while single isomer 6 is mainly used for nucleotides labeling and sequencing of nucleic acids.

Advantages :

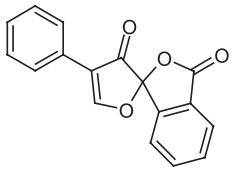
- Relatively high absorption.
- Excellent fluorescence quantum yield.
- Excitation maxima ~494 nm closely matches the 488 nm spectral line of the argon-ion laser (confocal laser scanning microscopy, flow cytometry).
- Extensively used for long times making of it a well characterized fluorophore with low cost.

Drawbacks :

- Many researchers are sometimes so accustomed with their fluorescein reagents that they are not aware of drawbacks, or accommodate with it! Poor sensitivity in some conditions or un-accurate detection can however ruin their efforts, even though simply trying a new dye might make a breakthrough!
To solve these issues, have a look at our alternative FluoProbes®488 dye (page B52).



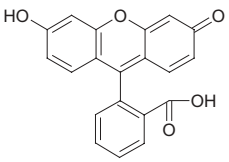
Related products :
Fluorescein labeling kits with a long spacer Fluorescein (FP-AX1350) and with the superior FluoProbes488 FP-BE3750) (page B55).



Fluorescein derivatives

Fluorescein is the basic and standard green dye ($\lambda_{abs.}/\lambda_{em.}$ 494 /519 nm) used for labeling biomolecules (see introduction to fluorescein based dyes). Its isothiocyanate derivatives, and then the more amine specific reactive Succinimidyl esters, are popular for labeling.

Fluorescein



MW : 332.52

Fluorescein is widely used as basic building block for fluorescein labeling in synthesis. For industrial applications, please ask bulk pricing, or the Fluorescein Na salt FP-40500 in 500 g package !

$\lambda_{abs}/\lambda_{em}$ (MeOH) : 492/520 nm

$\lambda_{abs}/\lambda_{em}$ (pH>7.0) : 494 /519 nm

EC : 88 000 M^{-1} cm^{-1}

QY : 0.8 ; tem: 3.5 ns (water)

Description	Cat.#	Qty
Fluorescein	**FP-19365A**	1 g

Fluorescein diacetate (FDA)

MW : 419.39

Non-fluorescent until Acetate group is hydrolyzed ; used mainly for cell applications

Description	Cat.#	Qty
Fluorescein diacetate (FDA)	**FP-29403A**	1 g

FITC

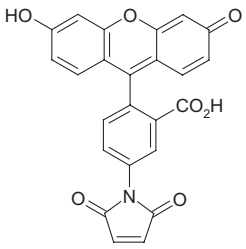
Fluorescein-IsoThioCyanate

MW : 389.39

General purpose protein labeling (isomer 5), or for specification (isomer 6)

Description	Cat.#	Qty
5-FITC (FITC I)	**FP-01739K**	100 mg
	FP-01739L	1 g
6-FITC (FITC II)	**FP-47555A**	1 g

Maleimide-C5-fluorescein



Fluorescein-5-maleimide

MW : 427.34

Soluble in DMF

$\lambda_{abs}/\lambda_{em}$ (coupled) : 492/515 nm

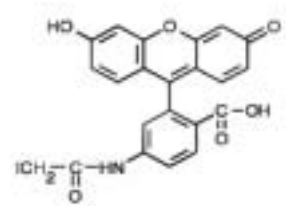
EC : 83 000 $M^{-1}cm^{-1}$

The maleimide derivative of fluorescein with an extended spacer, for improved fluorescence features and SH reactivity.

References : [1] Stephens AN, et al. (2003) ; J Biol Chem [2] van der Sluis EO, et al. (2002) ; FEBS Lett 527, 159-65 [3] Polyakov V, et al. (2000) ; Bioconjug Chem 11, 762-71 [4] Meuller J and Rydstrom J (1999) ; J Biol Chem 274, 19072-80 [5] Bigelow DJ and Inesi G (1991) ; Biochemistry 30, 2113-25.

Description	Cat.#	Qty
Maleimide-C5-fluorescein	**FP-47551A**	25 mg

AF



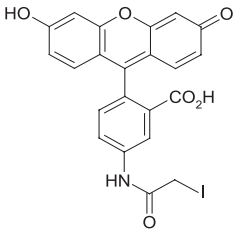
Iodoacetamidofluorescein

MW : 515.3

Soluble in DMF or aqueous buffers above pH 6.

$\lambda_{abs.}/\lambda_{em.}$ (coupled) : 493/515 nm

The iodoacetamide derivative of Fluorescein for SH reactivity. In addition, methionines can sometimes react with iodoacetamide reagents. These make more selective maleimide derivatives preferable to this very popular reagent. It remains very popular for applications including structure [2] and function [1] studies.



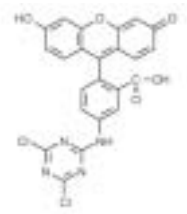
References :

[1] Baty JW, et al. (2002). Proteomics 2, 1261-6 ; [2] Moens PD, et al. (1994). Biochemitry 33, 13102-8.

Description	Cat.#	Qty
6-IAF	**FP-11368A**	25 mg
5-IAF	**FP-11339A**	25 mg

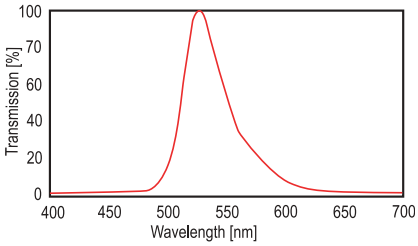
DTAF

(4,6-dichlorotriazinyl)aminofluorescein
MW : 495.28
Soluble in DMF at pH>6
$\lambda_{abs}/\lambda_{em}$: 492/517 nm



The isomer 5 is for standard applications, isomer 6 for specific applications

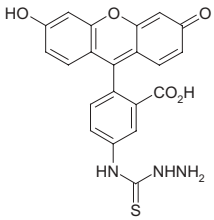
Description	Cat.#	Qty
5-DTAF	**FP-46732A**	25 mg
6-DTAF	**FP-46733A**	25 mg



FTSC

Fluorescein-5-thiosemicarbazide
MW : 421.43
Soluble in DMF or DMSO
$\lambda_{abs}/\lambda_{em}$ (pH>7.0) : 492/516 nm

FTSC hydrazine derivatives react with ketones to yield relatively stable hydrazones and with aldehydes to yield hydrazones that are somewhat less stable, though they may be formed faster. These hydrazones are generally reduced with sodium borohydride ($NaBH_4$) to further increase the linkage stability. The FITC hydrazine derivative has been extensively used to modify reduced sugars for analysis in gels[2] and sequencing. Additionally, hydrazine derivatives can also be coupled to carboxy groups in drugs, peptides and proteins[1]. FTSC has been used for structure and function studies with a wide variety of biomolecules such as L-aspartase decarboxylase, enzyme-oxydized live plant protoplasts, immunoglobulins, thrombin and antithrombin[3].



References :
[1] Hase, S. (1992) ; J Biochem (Tokyo) ; 112, 266-8.
[2] Ahn, B, et al. (1987) ; Anal Biochem, 161, 245-57.
[3] Atha, DH, et al. (1984) ; Biochim Biophys Acta 785, 1-6.

Description	Cat.#	Qty
5-FTSC	**FP-47552A**	25 mg

Fluorescein cadaverine

5-((5-Aminopentyl)thioureidyl)fluorescein dihydrobromide
MW : 653.4
$\lambda_{abs}/\lambda_{em}$ (pH>7.0) : 492/516 nm

Description	Cat.#	Qty
Fluorescein cadaverine	**FP-46576A**	5 mg

See page E.115 Enzyme probes.

Carboxyfluorescein (FAM)

FAM reagents are used for labeling (nucleic acids, proteins), with a prominent role in genomics, and also as pH indicators.

FAM (carboxyfluorescein), with primary or secondary aliphatic amines, gives carboxamides that are more resistant to hydrolysis than the widely used FITC. It requires less stringent reaction conditions, gives better conjugation yields, and the resulting conjugates have superior stability. Now, several FAM derivatives (i.e. long spacer versions, more reactive groups (i.e. Succinimidyl Esters) that are more easy to use than standard FAM. Pleaser refer to section 'cross-linking for more informations on chemical reactivities. Mixed isomers are generally used, but one or the other single isomers serves specific applications where it might significantly affect some biological properties or the underlying conjugated. I.e. 6-FAM SE is mainly used for nucleic acids sequencing of and nucleotides labeling rather than for peptides or proteins, at the opposite of the 5-FAM isomer. Various other derivatives are also available, including the diacetate form, CFDA-SE (FP-52493A) that is very popular for long term cell tracing.

References: 1. Hahn, M, et al. (2001) ; Electrophoresis 22, 2691-700[2] Hung, SC, et al. (1996) ; 2. Anal Biochem 243, 15-27[3] Banks, PR and Paquette DM. (1995) ; 3. Bioconjug Chem 6, 447-458.

For more information about reactivities (SE, Maleimide... See page B60).

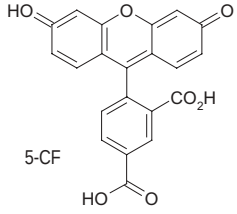
See page E69 for application of FAM to pH indicators.

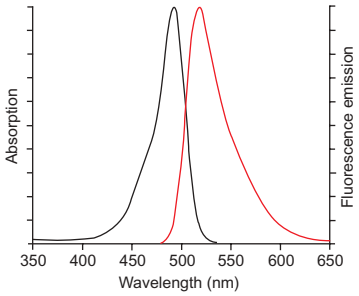
See FluoProbes 488 for a great alternative page B52

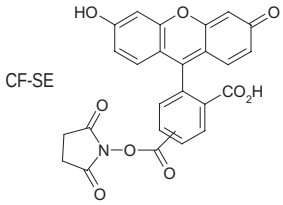
CF (FAM)

$\lambda_{abs}/\lambda_{em}$: 492/518 nm ; Soluble in DMF (pH>6)

Description	Cat.#	Qty
CF (FAM) (5,6-Carboxyfluorescein)	**FP-46641A**	100 mg
5-CF (FAM) the single 5-isomer for FAM-SE (FP-46641)	**FP-34426A**	100 mg
6-CF (FAM) the single 6-isomer for FAM-SE (FP-46641)	**FP-84858A**	100 mg







CF-SE (FAM-SE)

5(6)-carboxyfluorescein succinimidyl ester
MW : 472.39
The SE derivative of FAM (FP-46641A), reactive to amines, and the standard green fluorescein labeling agent to replace FITC.

Description	Cat.#	Qty
F-SE (FAM-SE)	**FP-48189A**	100 mg
5-CF-SE (5-FAM-SE) the single 5-isomer for FAM-SE (FP-48189)	**FP-24977A**	10 mg
6-CF-SE (6-FAM-SE) the single 6-isomer for FAM-SE (FP-48189)	**FP-M1324A**	10 mg

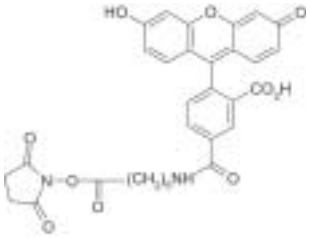
CFX (SFX)

Fluorescein-5(6)-carboxamidohexanoic acid
MW : 586.6
$\lambda_{abs}/\lambda_{em}$ (pH9) : 494/520 nm
EC : 74 000 $M^{-1}cm^{-1}$
EC and QY decrease markedly at pH <7
The longer spacer (7C) version of FAM (FP-46641A). Recommended when the fluorescence quenching after conjugation is a serious problem.

Description	Cat.#	Qty
SFX	**FP-45831A**	25 mg

CFX-SE (SFX-SE)

Fluorescein-5(6)-carboxamidocaproic acid succinimidyl ester
MW : 586.6
The SE derivative of SFX (FP-45831), reactive to amines.
The longer spacer (7C) version of FAM (FP-46641A). Recommended when the fluorescence quenching after conjugation is a serious problem.

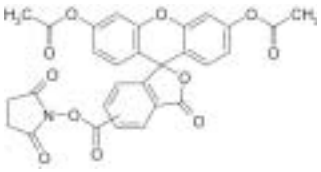


References : 1. Czapski, GA, et al. (2001); Med Sci Monit 7, 606-9[2] Cooper, WC, et al. (2000) ; Biophys J 78, 1449-57[3] Johansson, AG, et al. (1996) ; Hepatology 24, 169-75.

Description	Cat.#	Qty
SFX-SE	**FP-40295A**	25 mg
6 CF-X-SE (6-FAM-X-SE)	**FP-M1299A**	5 mg

CFDA-SE (CFSE, Green Cell Tracker)

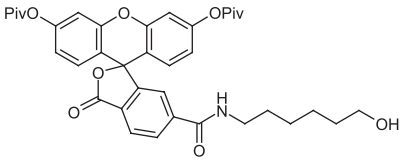
carboxyfluorescein Diacetate Succinimidyl ester
MW : 557.47
Non fluorescent until Acetate groups are hydrolyzed to give CFDA (FP-33953A); used mainly for cell applications (long-term tracing).



Description	Cat.#	Qty
CFDA-SE (CFSE, Green Cell Tracker)	**FP-52493A**	25 mg
5-CFDA-SE the single 5-isomer for CFDA-SE (FP-52493)	**FP-BA8461**	10 mg
6-CFDA-SE the single 6-isomer for CFDA-SE (FP-52493)	**FP-AM497A**	10 mg

CFDP-X

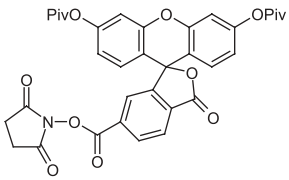
N-(6-Hydroxyhexyl)-6-carboxamidofluorescein dipivalate
MW : 643.73
A special derivative of fluorescein.



Description	Cat.#	Qty
CFDP-X	**FP-BA844A**	10 mg

6-CFDP-SE

carboxyfluorescein dipivalate hydroxysuccinimide
MW : 641.64
NH_2 reactive through NHS group



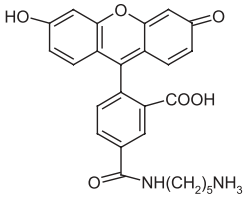
Description	Cat.#	Qty
6-CFDP-SE	**FP-BA844A**	10 mg
5-CFDP-SE 5-carboxyfluorescein dipivalate hydroxysuccinimide	**FP-BA8455**	10 mg NHS

FAM-PA

6'-Fluorescein Phosphoramidite
For 6'-terminus DNA labeling. See page B96.

Description	Cat.#	Qty
5-FAM-PA	**FP-FI9661**	50 µmol

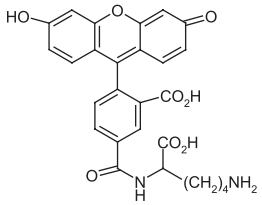
FAM cadaverine



Fluorescein-5-carboxamide cadaverine
MW : 460.5
Soluble in DMF or DMSO
$\lambda_{abs}/\lambda_{em}$ (pH>7.0) : 494/521 nm
An excellent building block to prepare fluorescent ligands for receptor binding assays.

Description	Cat.#	Qty
5-FAM cadaverine	**FP-AM846A**	10 mg

5-FAM lysine



Fluorescein-5-carboxamide lysine
MW : 504.5
Soluble in DMF or DMSO
$\lambda_{abs}/\lambda_{em}$ (pH>7.0) : 494/521 nm

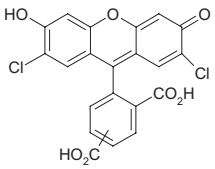
An excellent building block to prepare fluorescent peptides.
It has been shown to be a good transglutaminase substrate for site-specific protein labeling like FITC cadaverine.

Description	Cat.#	Qty
5-FAM lysine	**FP-AM847A**	10 mg

Carboxy DiChloroFluorescein (CDCF)

Dichlorosubstitution of fluorescein heterocycle lowers the pKa to 4.8. Hence, CDCF is useful as acidic pH and hydrophobic probe (i.e. fluid phase markers for endocytosis). See page E68. It does readily enter cells.
CDCF is available as several derivatives, that are mainly used for cell applications (morphology, dynamics or acidic organelle), including diacetate for better loading/cell retention, and succinimidyl for amine conjugation.

CDCF



5(6)Carboxy 2,7'-DichloroFluorescein
MW : 445.21
$\lambda_{abs}/\lambda_{em}$ (pH4) : 495/529 nm
EC : 38 000 $M^{-1}cm^{-1}$
$\lambda_{abs}/\lambda_{em}$ (pH8) : 504/529 nm
EC : 107 000 $M^{-1}cm^{-1}$

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

CDCFDA (Carboxy-DCFDA)

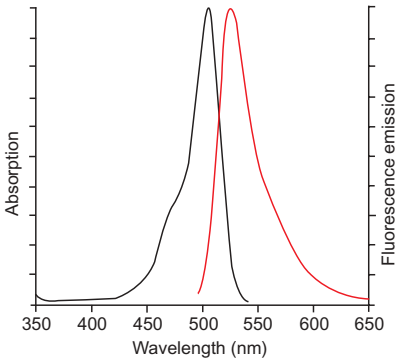
5(6)-carboxy-2',7'-DiChloroFluorescein DiAcetate
MW : 529.29
Acetate group hydrolysis gives CDCF product (FP-46629)

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

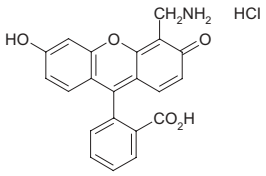
6-CDCFDA-SE

5(and 6)-Carboxy-2',7'-DiChloroFluorescein DiAcetate Succinimidyl Ester
MW : 626.36
SE group reacts on amines. Acetate group hydrolysis gives CDCF product (FP-46629)

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



Absorption and fluorescence emission spectra in pH 9.0 buffer.



Aminomethyl derivatives of fluorescein

AMF

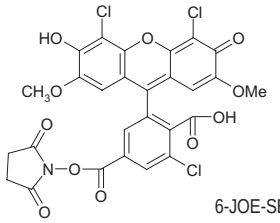
4'-(Aminomethyl)fluorescein, hydrochloride
MW : 397.8
Soluble in DMF or DMSO
$\lambda_{abs}/\lambda_{em}$: 493/516 nm (pH 8-9)
An amino-containing fluorescein used to label small drug molecules and other biological substances. It is also widely used for FRET and DNA sequencing [1].

References : [1] Lee LG, et al. (1997); Nucleic Acids Res 25,2816-22 ; [2] Shipchandler MT, et al. (1987); Anal Biochem 162, 89

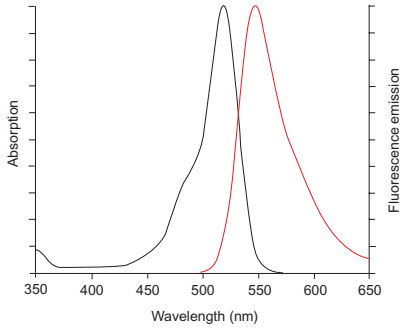
Description	Cat.#	Qty
AMF	**FP-M1161A**	25 mg

Chlorinated derivatives of fluorescein

JOE, TET and HEX are three dyes derived from FAM, with increased red-shifted fluorescence. They are important for genetic analysis, especially for automated DNA sequencing applications. They are notably used as fluorescent donors with rhodamine dyes ROX and TAMRA. They also can serve as FRET acceptors for DABCYL and other quenchers.



6-JOE-SE



6-CDCDMF-SE (JOE-SE)

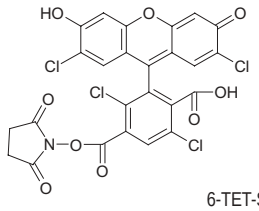
6-Carboxy-4',5'-DiChloro-2',7'-DiMethoxyFluorescein, Succinimidyl ester
MW : 602.34
Soluble in DMF or DMSO
$\lambda_{abs}/\lambda_{em}$ (pH>9.0) : 520/548 nm

Description	Cat.#	Qty
6-CDCDMF-SE (JOE-SE)	**FP-M1326A**	5 mg

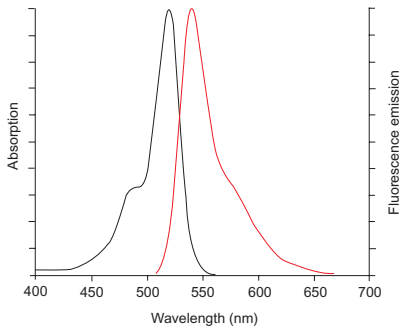
TCCF-SE(TET-SE)

Carboxy-4,7,2',7'-TetraChloro-Fluorescein, Succinimidyl Ester
MW : 611.2
$\lambda_{exc}/\lambda_{em}$: 524/538 nm
EC : 99 000 M^{-1} cm^{-1}
TET is a red-shifted version of JOE

Description	Cat.#	Qty
TCCF-SE(TET-SE)	**FP-AM575A**	10 mg
5-TCCF-SE (5-TET-SE)	**FP-AM576A**	5 mg
The single 5-isomer for TET-SE (FP-AM575), mostly used for genomic analysis.		
6- TCCF-SE (6-TET-SE)	**FP-T8214A**	5 mg
The single 6-isomer for TET-SE (FP-AM575)		



6-TET-SE



5-TCCF-PA (5-TET-PA)

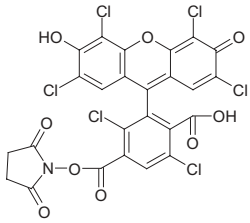
5'-Tetrachloro-Fluorescein PhosphorAmidite
MW : 981.74
For 5'-terminus DNA labeling. See section Synthesis reagents (B97)

Description	Cat.#	Qty
5-TCCF-PA (5-TET-PA)	**FP-FI9681**	50 µmol

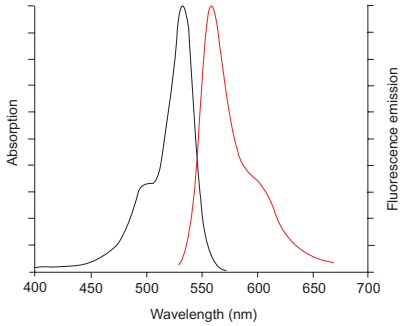
HCF-SE (HEX-SE)

Carboxy-2',4,4',5,7,7'-HexaChloroFluorescein Succinimidyl Ester
MW : 680.07
$\lambda_{exc}/\lambda_{em}$: 524/538 nm
Addition of 4 chlores gives to this fluorescein derivative the longest wavelength.

Description	Cat.#	Qty
HCF-SE (HEX-SE)	**FP-AM574A**	10 mg
5-HCF-SE (5-HEX-SE) The single isomer 5 of HEX-SE (FP-AM574A)	**FP-AM573A**	5 mg
6-HCF-SE (6-HEX-SE) The single isomer 6 of HEX-SE (FP-AM574A)	**FP-T8123A**	5 mg



6-HEX-SE



5-HCF-PA (5-HEX-PA)

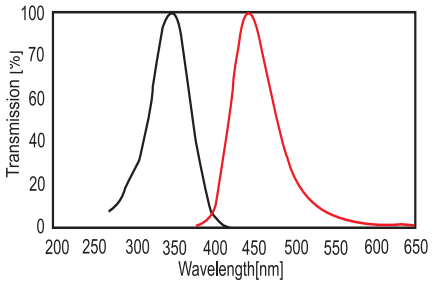
5'-Hexachloro-Fluorescein Phosphoramidite
For 5'-terminus DNA labeling. See section Synthesis reagents (page B97).

Description	Cat.#	Qty
5-HCF-SE (5-HEX-PA)	**FP-FI9671**	50 µmol

Coumarins fluorochromes

AMCA(Amino Methyl Coumarin)

AMCA (Amino Methyl Coumarin Acetic acid) is an excellent blue dye. Its main use is for multiple color analysis by FCM and IHF.
AMCA emits in the blue region (440-460 nm) when activated with UV light (350 nm). It suits well to mercury lamps, and can be excited by argon laser (FCM). The Stokes shift of 100 nm compared to 30 nm for FITC allows easy filter discrimination of exciting and emitting radiation. The bright blue fluorescence is easy to visualize and photograph. It allows photographic exposure time of fluorescent labeled sections to be reduced to a quarter of that required for a corresponding FITC conjugate. In general, AMCA-immunoglobulin conjugates are not or minimally susceptible to photobleaching and have a storage life at -20°C of more than two years. All that make of it a popular dye for double or triple detections with a green and red dye, but not preferred for single labeling because of lower brightness than green, orange or red dyes.



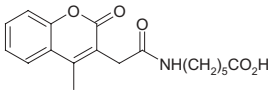
AMCA is available as several derivatives of the base structure, with extended spacer (AMCA-X), convenient reactivity toward amines (AMCA-SE) or thiols (AMCA-Maleimide, -MTS). Modified versions of the coumarin base are also available (see page B.52).

FluoProbes®390 is a potential alternative label (see page B.52).

References :
Eis PS and Lakowicz JR (1993) ; Biochemistry 32, 7981-93
Nederlof PM, et al. (1989) Cytometry 10, 20-7
Khalfan H, et al. (1986) ; Histochem J 18, 497-9.
Malicka J, et al. (2003) ; Anal Biochem 315, 160-9
Aubry JP, et al. (1990) ; J Immunol Methods 128

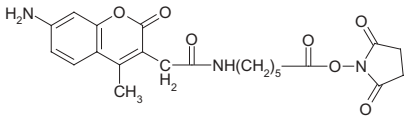
AMCA-X

6-((7-Amino-4-methylcoumarin-3-acetyl)amino)hexanoic acid
MW : 346.4
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 353/442 nm
EC : 19 000 $M^{-1}cm^{-1}$
The acid is readily activated to form N-hydroxysuccinimide ester that reacts with lysine residues under mild conditions to form photostable amide links. Contains a 7C spacer between the fluorophore and the reactive group. This increases sensitivity, potentially reduces the quenching that typically occurs upon conjugation, and renders the dye more available for recognition by secondary detection reagents.



Description	Cat.#	Qty
AMCA-X	**FP-AZ393A**	25 mg

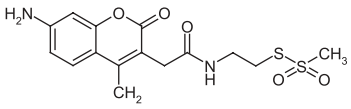
Protein Labeling



AMCA-X-SE

6-((7-Amino-4-methylcoumarin-3-acetyl)amino)hexanoic acid, succinimidyl ester
MW : 443.46
A popular derivate of AMCA-X for convenient protein labelings.
SE group reacts readily with amines.

Description	Cat.#	Qty
AMCA-X-SE	**FP-84695A**	10 mg

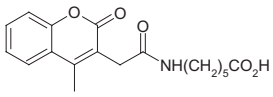


MTS-AMCA

Methanethiosulfonate 7-amino-4-methylcoumarin-3-acetic acid
MW : 370.45
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 353/442 nm.
The MTS derivative of AMCA, for SH-reactivity

Description	Cat.#	Qty
MTS-AMCA	**FP-AM364A**	5 mg

Methoxy coumarin dyes



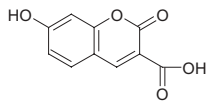
MCA

7-methoxycoumarin-4-acetic acid
An acceptor/donor for developing FRET-based fluorescent probes with DNP.

Description	Cat.#	Qty
MCA	**FP-46925A**	500 mg

Hydroxy coumarin dyes

Hydroxycoumarin is a blue-fluorescent polar tracer ($\lambda_{exc}/\lambda_{em.}$ ~388/445 nm) useful to complete green-fluorescent dyes. It is mainly use for cell applications as polar tracers (free acid) or for cell tracing (SE ester), but also increasingly used to label peptides, nucleotides and carbohydrates.



HCC

7-Hydroxycoumarin-3-carboxylic acid
MW : 206.24
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 387/448 nm

References : [1] Higai K, et al. (1999); Biol Pharm Bull 22, 333-8 ; [2] Li H, et al. (199-); J Cell Biol 134, 1019-30.

Description	Cat.#	Qty
HCC	**FP46857A**	100 mg

HCC-SE

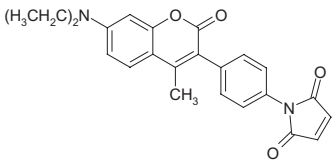
7-Hydroxycoumarin-3-carboxylic acid, succinimidyl ester
MW : 303.2
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 420/447 nm

References: [1] Chakrabarti S, et al. (1999); Int J Radiat Biol 75, 1055-65; [2] Li H, et al. (1996); J Cell Biol 134, 1019-30.

Description	Cat.#	Qty
HCC-SE	**FP-M1151A**	25 mg

For reactivities (SE, MTS,...see information page B60)

7-diethylamino-4-methyl coumarin

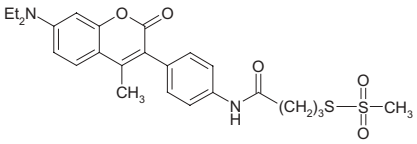


DEAMCA (CPM)

7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin
MW : 402.5
$\lambda_{exc}/\lambda_{em}$ (coupled) : 384/470 nm

Quite non fluorescent until it reacts with thiol-reactive of maleimide group at pH 7-9. Used for thiol detection without a separation step. Also a good acceptor from tryptophan and a good donor to fluorescein.

Description	Cat.#	Qty
DEAMCA (CPM)	**FP-46714A**	25 mg



MTS-DEAMCA (CMPTS)

7-diethylamino-4-methyl-3-(4-(4(methanethiosulfonato)butanoyl)amino)phenylcoumarin
MW : 502.66
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 384/470 nm

The MTS derivative of CPM (FP-46714). A thiol reactive fluorescent dye with quite identical fluorescence spectrum than CPM (FP-46714), but with higher selectivity and reactivity for sulfhydryls.

Description	Cat.#	Qty
MTS-DEAMCA (CMPTS)	**FP-AM360A**	5 mg

Rhodamines based fluorophores

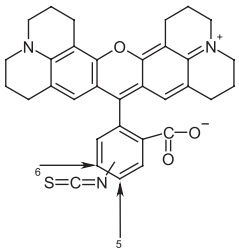
Rhodamines have supplemented fluorescein-based fluorophores, as they offer longer wavelengths emission maxima. They thus opened opportunities for multicolor labeling in applications such as DNA sequencing, microarrays and FISH.
Rhodamine based dyes fill in following families :

- CarboxyRhodamine110 CR110 (page B70)
- CarboxyRhodamine6G CR6G (page B72)
- TetraMethylRhodamine (TMR) / CarboxyTetraMethylRhodamine (TAMRA) (B73)
- CarboxyRhodamine ROX (page B76)
- SulfoRhodamine B (page B77)
- SulfoRhodamine 101 (page B79).

See also Rhodamine labeling kit and FluoProbes®547 labeling kit # FP-BC0900

Rhodamine (base)

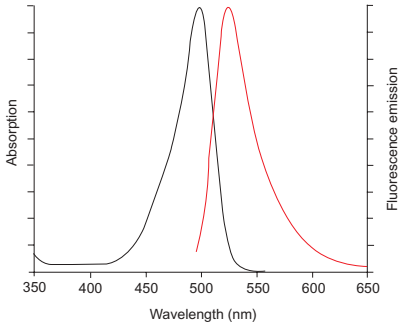
Rhodamine basic compound is mainly use as a polar tracer for cell applications. Fluorescence spectra a similar to CarboxyRhodamine.



XRITC

Rhodamine-X-5(6)-Isothiocyanate
MW : 547.68
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MetOH) : 572/596 nm

Description	Cat.#	Qty
XRITC	**FP-M1135A**	10 mg

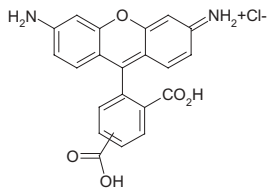


Absorption and emission spectra of CR110

CR110 (Carboxyrhodamine 110)

CR110 is a green dye $\lambda_{exc}/\lambda_{em}$: 502/524 superior to Fluoresceins (including FAM) in many applications, because it is not sensitive to pH between 4 and 9, with high extinction coefficient and it is much more photostable than fluoresceins. It is compatible with standard fluorescein filter sets.
CR110 is available with an extended spacer for improved fluorescent features (CR110-lc, FP-AM393), and derived with standard reactivities (SE, MTS, Maleimide; see page B60 for reactivities information).

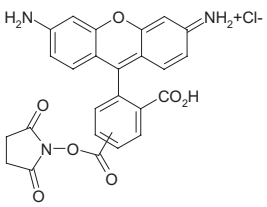
See also FluoProbes®488, that is even more photostable, and brighter than CR110.



CR110, HCl

5(6)-CarboxyRhodamine 110 HydroChloride
MW : 426.86
Soluble in Water (pH>6)/DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 502/524 nm

Description	Cat.#	Qty
CR110, HCl	**FP-AM383A**	10 mg

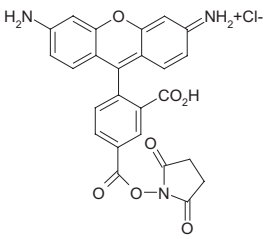


CR110-SE, HCl

CarboxyRhodamine110 succinimidyl ester, hydrochloride
MW : 507.85
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 502/524 nm
EC : 85 000 $M^{-1}cm^{-1}$

The SE ester of CR110, for selective amine reactivity. Single isomers 5 and 6 are available, and used for specific applications where it might significantly affect some biological properties of the underlying conjugates.

Description	Cat.#	Qty
CR110-SE, HCl	**FP-84372A**	5 mg
5-CR110-SE, HCl	**FP-AM385A**	5 mg
5-CarboxyRhodamine 110, Succinimidyl Ester hydrochloride, single isomer		
6-CR110-SE, HCl	**FP-AM386A**	5 mg
6-CarboxyRhodamine 110, Succinimidyl Ester hydrochloride, single isomer		



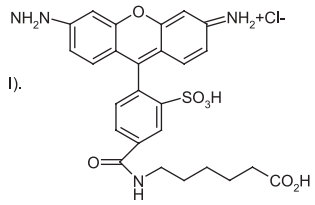
CR110 TFA, SE

CarboxyRhodamine110 carboxylic acid, trifluoroacetamide, succinimidyl ester
MW : 663.4

Description	Cat.#	Qty
CR110 TFA, SE	**FP-M13010**	5 mg

CR110-Ic, HCl

CarboxyRhodamine110-5(6)Hexanoic acid, mixed isomers (5,6)
MW : 528.01
Soluble in Water : DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 502/524 nm

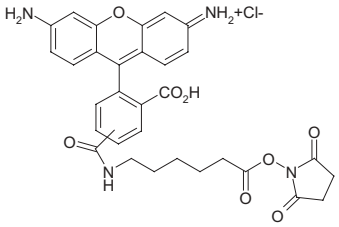


A derivative of CR110 with an extended spacer improving the fluorescence (lower interaction between the dye and protein).

Description	Cat.#	Qty
CR110-Ic, HCl	**FP-AM393A**	5 mg

CR110-Ic-SE, HCl

5,6-CarboxyRhodamine110-amidohexanoatesuccinimidyl ester hydrochloride
MW : 621.05
Soluble in DMF or DMSO

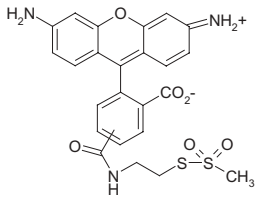


A long spacer and SE, amino reactive, derivative of CR110-Ic (FP-AM393), for improved fluorescence features and selective amine reactivity.

Description	Cat.#	Qty
CR110-Ic-SE, HCl	**FP-AM394A**	5 mg

MTS-CR110

Methanethiosulfonate CarboxyRhodamine 110
MW : 511.58
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 502/524 nm.

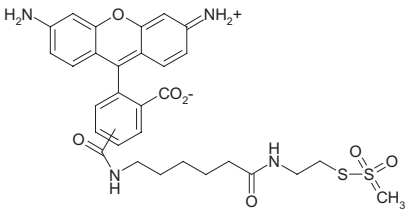


A MTS derivative of CR110, for highly selective thiol reactivity.

Description	Cat.#	Qty
MTS-CR110	**FP-AM367A**	5 mg

MTS-Ic-CR110

MethaneThioSulfonate-Ic-CarboxyRhodamine 110
MW : 624.74
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 502/524 nm

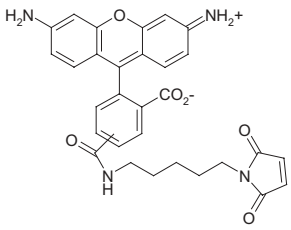


A long spacer and MTS derivative of CR110, for improved fluorescence features and highly selective thiol reactivity.

Description	Cat.#	Qty
MTS-Ic-CR110	**FP-AY800A**	5 mg

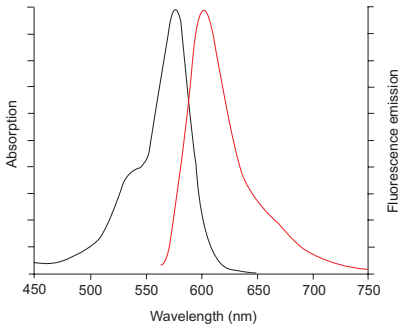
Maleimide-C5-CR110

Maleimido-CarboxyRhodamine110
MW : 522.61
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 502/524 nm



The maleimide derivative of CR110 with an extended spacer, for improved fluorescence features and SH reactivity.

Description	Cat.#	Qty
Maleimide-C5-CR110	**FP-AM380A**	5 mg



Absorption and fluorescence emission spectra in pH 7.0 buffer.

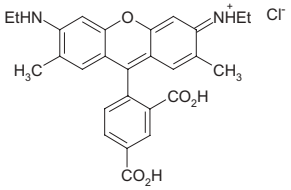
CR6G (Carboxyrhodamine 6G)

Rhodamine 6G elicits excitation and emission wavelengths ($\lambda_{exc}/\lambda_{em}$: 520/546 nm; EC : 102 000 $M^{-1}cm^{-1}$) between those of fluorescein and tetramethylrhodamine derivatives, making it useful for the multicolor fluorescence imaging applications. Additionally the maximal absorption of CR6G conjugate matches well to the 514 nm spectral line of the argon-ion laser.

CR6G is widely used for the FRET labeling of nucleic acids.
The pure separate isomers (FP-M1300A and FP-26412A) are preferred when reproducibility is more critical than material cost since the minor positional difference between 5-CR6G and 6-CR6G might significantly affect some biological properties of the underlying conjugates.

References:
Hung, SC, et al. (1997) ; Anal Biochem 252, 78-88; Hung, SC, et al. (1996) ; Anal Biochem 238, 165-70.
Arezi B, et al. (2002) ; J Mol Biol 322, 719-29; Hung, SC, et al. (1997) ; Anal Biochem 252, 78-88.

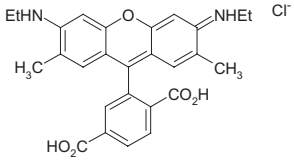
See also the alternative dye FluoProbes®520



CR6G, HCl

Carboxyrhodamine 6G, hydrochloride
MW : 494.98
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 520/546 nm

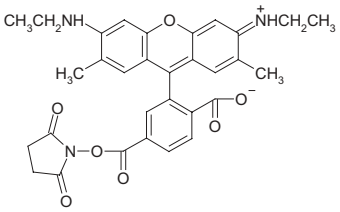
Used to modify amino and hydroxy groups using EDC-mediated coupling chemistry when there are difficulties in using 5-(and 6)-CR6G, SE.



Description	Cat.#	Qty
CR6G, HCl Mixed isomers	**FP-AM843A**	25 mg
5-CR6G, HCl 5-CarboxyRhodamine6G HydroChloride	**FP-M1300A**	5 mg
6-CR6G, HCl 6-CarboxyRhodamine6G HydroChloride The single 6-CR6G isomer is predominantly used for nucleotides labeling.	**FP-26412A**	5 mg

CR6G-SE

CarboxyRhodamine 6G, Succinimidyl Ester
MW : 555.59
$\lambda_{exc}/\lambda_{em}$ (MetOH) : 520/546 nm
EC : 102 000 $M^{-1}cm^{-1}$(MeOH)
The SE ester (amine reactive) of CR6G, with similar fluorescent properties.



References : [1] Hung, SC, et al. (1997) ; Anal Biochem 252, 78-88 ; [2] Hung, SC, et al. (1996) ; Anal Biochem 238, 165-70.

Description	Cat.#	Qty
CR6G-SE Mixed isomers	**FP-AM844A**	10 mg
5-CR6G-SE	**FP-M1308A**	5 mg
6-CR6G-SE	**FP-M1309A**	5 mg

CR6G-MTS

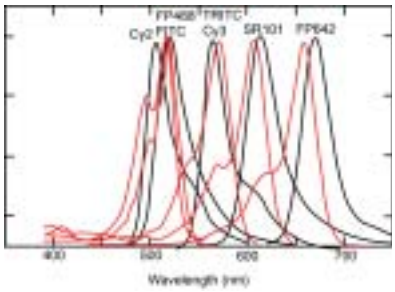
MethaneThioSulfonate-CarboxyRhodamine 6G
MW : 595.74
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 502/546 nm
A MTS derivative of CR6G for highly selective thiol reactivity.

Description	Cat.#	Qty
CR6G-MTS	**FP-AM376A**	5 mg

TMR (TetraMethylRhodamine)

TetraMethylRhodamine (TMR) has been largely used for protein labeling in immunochemistry, for sensitive aminoacid derivatization in CE. Its carboxilic acid form (TAMRA, see below) remains prominent for oligonucleotide labeling (DNA sequencing).The absorption and emission in pH 8 buffer are red-shifted approximately 8 nm compared to MeOH, with EC ~10% lower. $\lambda_{abs.}/\lambda_{em.}$: 543/571nm

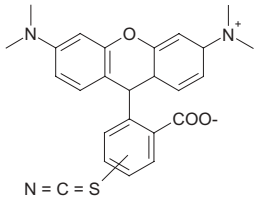
The absorption spectrum of TMR/TRITC-labeled proteins is frequently dependent on the labeling location and on the degree of substitution, and may even show splitting into two absorption peaks at about 520 and 550 nm. Such limitation can be adressed using alternative dyes: When the fluorescence quenching by protein of the labeling dye is a serious problem, try an extended spacer version TAMRA-X-SE, or our excellent FluoProbes®547 (#FP-AK773 for NHS ester) this has brighter and more stable fluorescence.



Antibody (Gt) – FITC fluorescence spectrum (may be subject to considerable variations depending or coupled protein and ratio)

TRITC

TetramethylRhodamine isothiocyanate
MW : 443.53
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 543/572 nm
EC : 99 000 $M^{-1}cm^{-1}$

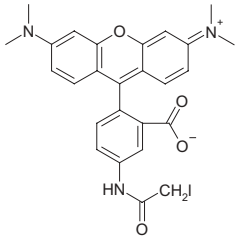


The standard red fluorescent labeling agent. Single isomers are available separately for most demanding applications.

Description	Cat.#	Qty
TRITC	**FP-47004A**	10 mg
6-TRITC (6-TetramethylRhodamine Isothiocyanate)	**FP-06276A**	5 mg
5-TRITC (5-TetramethylRhodamine Isothiocyanate)	**FP-17503A**	5 mg

TMRIA

5-Tetramethylrhodamine-5-iodoacetamide dihydroiodide
MW : 825.2
The iodoacetamide derivative of TMR, for thiol reactivity.
TMRIA was widely used to label proteins via the cystein residues for protein structural studies [5], protein-protein [2,5] and protein-DNA interactions.

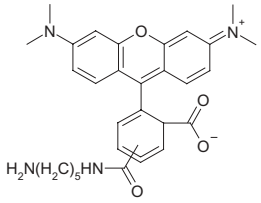


References :
[1] Martyn, DA, et al. (2001); Biophys J 80, 360- 70
[2] Hopkins, SC, et al. (1998); Biophys J 74, 3093-110
[3] Ajtai, K and TP Burghardt (1995) ; Biochemistry 34, 15943-52
[4] Wang, YL (1991) ; Methods Enzymol 196, 497-505
[5] Tait, JF and C Frieden (1982); Arch Biochem Biophys 216, 133-41.

Description	Cat.#	Qty
5-TMRIA	**FP-96468A**	5 mg

TMR cadaverine

5-(6)-((N-(5Aminopentyl)amino)carbonyl)tetramethylrhodamine; tetramethylrhodamine 5-(and 6)-carboxamide cadaverine
MW : 414.6
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 544/570 nm



Used for fluorescent labeling of carboxy groups via EDAC-mediated reactions. It's also a good glutamate transglutaminase substrates [5] (section "Enzymes Probes")
FluoProbes® provides also single pure isomers 5 (FPAM872) and 6 (FP-AM874) that may be preferred for some applications where reproducibility is more critical than material cost since the minor positional difference between 5-isomer and 6-isomer might significantly affect some biological properties of the resultant products.

References :
[1] Nurminskaya, MV, et al. (2002); Dev. Dyn. 223,24-32
[2] Patricelli, MP, et al. (2001); Proteomics 1, 1067
[3] Hileman, RE, et al. (1994); Bioconjug Chem 5, 436-444
[4] Kasprzak, AA, et al. (1988); Biochemistry 27, 4512-4522.

Description	Cat.#	Qty
5-(6)-TMR cadaverine	**FP-60053A**	10 mg

TMR Lysine

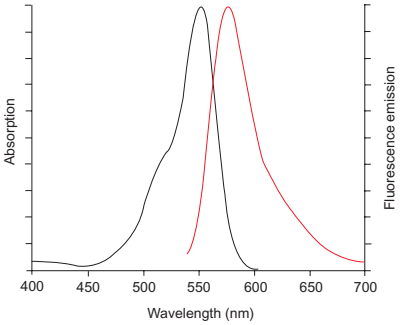
5-((N-(5-Amino-5-carboxypentyl)amino)carbonyl)tetramethylrhodamine, tetramethylrhodamine-5-carboxamide lysine
MW : 558.64
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 545/577 nm
This product is a very useful building block for fluorescent peptides preparation. It's also a good transglutaminase substrate (section "Enzymes Probes").

References :
[1] Nurminskaya, MV, et al. (2002) ; Dev. Dyn. 223,24-32
[2] Olorundare OE, et al. (2001) ; Blood 98, 117-24
[3] Chowdhury ZA, et al. (1997) ; Exp Cell Res 231, 38-49.

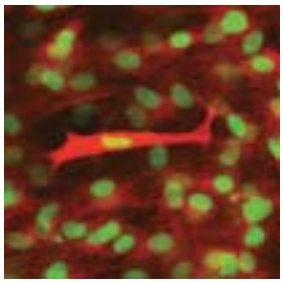
Description	Cat.#	Qty
5-TMR Lysine	**FP-AM873A**	5 mg

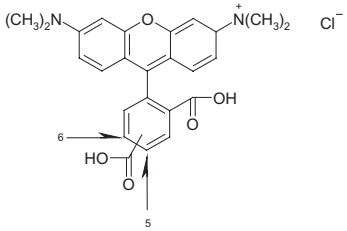
TAMRA (CarboxyTetramethyl Rhodamine)

Carboxy tetramethylrhodamine (TAMRA) is one of the most popular yellow-orange fluorophore used in various bioconjugations for immunochemistry, notably with nucleic acids as well as peptides and proteins. It is an excellent fluorescence acceptor for fluorescein derivatives in FRET-based assays.
The absorption spectrum of TRITC-labeled proteins is frequently dependent on the labeling location and on the degree of substitution, and may even show splitting into two absorption peaks at about 520 and 550 nm. Such limitation can be addressed using alternative dyes: When the fluorescence quenching by protein of the labeling dye is a serious problem, try an extended spacer version TAMRA-X-SE, or our excellent FluoProbes®547 (#FP-AK773 for the ester) this has brighter and more stable fluorescence.



Absorption and fluorescence emission spectra of TAMRA in pH 7.0 buffer.





TAMRA

CarboxyTetraMethylRhodamine
MW : 466.92
Soluble in DMF or DMSO or MeOH, or H2O.
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 540/565 nm
In pH 8 buffer compared to MeOH, absorption and emission are red-shifted ~8 nm with EC lowered by ~10%.

Used to modify amino and hydroxy groups using EDAC-mediated couplings when there are difficulties in using TAMRA-SE. Pure isomers are available, they are preferred for specific biological applications where reproducibility is more critical than material cost since the minor positional difference between 5-TAMRA #FP-05867 and 6-TAMRA # FP-M1306 might significantly affect some biological properties of the underlying conjugates.

References :
[1] Evans, NA, et al. (2001) ; J Neurochem 77, 476-85
[2] Kask, P, et al. (2000) ; Biophys J 78, 1703-13
[3] Hess, KL,et al. (1997) ; Cytometry 27, 145-5
[4] Yoo, H and RL Juliano (2000), Nucleic Acids Res.28, 4225-31
[5] Gelsthorpe, AR, et al. (1999), Tissue Antigens 54,603-14
[7] Brunner, A, et al. (1998)
[8] Hsu, TM, et al. (2001), Clin Chem 47, 1373-7
[9] Schutz, E, et al. (2000), Clin Chem 46, 1728-37

Description	Cat.#	Qty
TAMRA	**FP-46644A**	100 mg
5-TAMRA (5-CarboxyTetraMethylRhodamine)	**FP-05867A**	10 mg
Single 5-isomer of TAMRA (FP-46644), predominantly used for protein labeling.		
6-TAMRA (6-CarboxyTetraMethylRhodamine)	**FP-M1306A**	10 mg
Single 6-isomer of TAMRA (FP-46644), predominantly used for nucleotide labeling.		

TAMRA-SE

carboxytetramethylrhodamine N-succinimidyl ester
MW : 527.54
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 546/575 nm ; EC : 95 000 M-1cm-1

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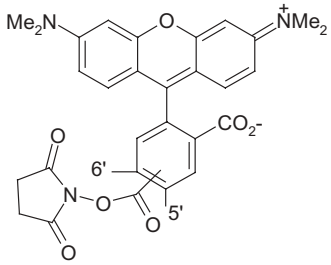
The succinimidyl ester (amino reactive) derivative of TAMRA, and a standard labeling reagent.

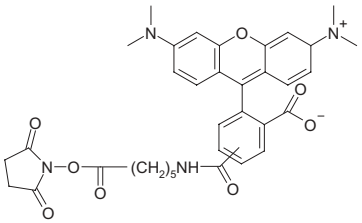


References : Hsu, TM, et al. (2001) ; Clin Chem 47, 1373-7 ; Jordan, JA, et al. (2001) ; J Clin Microbiol 39, 3819-22 ; Sanders Sevall, J (2000); Mol Cell Probes 14, 249-53 ; Yoo, H and RL ; Juliano (2000) ; Nucleic Acids Res 28, 4225-31
Evans, NA, et al. (2001) ; J Neurochem 77, 476-85 ; Lyttle MH, et al. (2000) ; J Org Chem 65, 9033-8 ; Nasarabadi S, et al. (1999); Biotechniques 27, 1116-8 ; Brunner.A, et al. (1998) ; Eur J Pharm Biopharm 45, 265-73.
Hsu, TM, et al. (2001) ; Clin Chem 47, 1373-7 ; Sanders Sevall, J (2000) ; Mol Cell Probes 14, 249-53 ; Schutz,E, et al. (2000) Clin Chem 46, 1728-37.

Description	Cat.#	Qty
TAMRA-SE	**FP-52498A**	25 mg
5-TAMRA-SE	**FP-67480A**	5 mg
5-carboxytetramethylrhodamine N-succinimidyl ester, HCl The single 5-isomer is predominently used for peptides and proteins labeling		
6-TAMRA-SE	**FP-84634A**	5 mg
6-carboxytetramethylrhodamine N-succinimidyl ester, HCl The single 6-isomer of TAMRA-SE (FP-52498), predominantly used for nucleotides labeling in DNA sequencing.		

TAMRA-X-SE

6-(Tetramethylrhodamine-5-(and-6)-carboxyamido)hexanoic acid, succinimidyl ester
MW : 640.7
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 544/572 nm



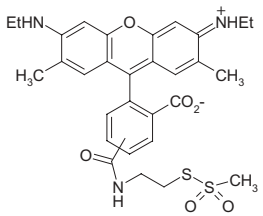
Extended spacer version of TAMRA-SE. Similar features as TAMRA-SE (FP-52498A). Contains a 7C spacer between the TAMRA fluorophore and the succinimidyl ester. It potentially reduces the quenching that typically occurs upon conjugation, improving detection sensitivity.

References : [1] Biophys J 80, 360-70 ; [2] Wazawa, T, et al.(2000); Biophys J 78, 1561-1569 ; [3] Moore, KJ, et al. (1999); J Biomol Screen 4, 335-354 ; [4] Allen, TS, et al. (1996); Biophys J 70, 1847-62 ; [5] Andreev, OA, et al. (1993); Biophys J 65, 1027-38.

Description	Cat.#	Qty
TAMRA-X-SE	**FP-33406A**	10 mg

MTS-TAMRA

MethaneThioSulfonate-CarboxyTetraMethylRhodamine
MW : 567.69
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 540/565 nm

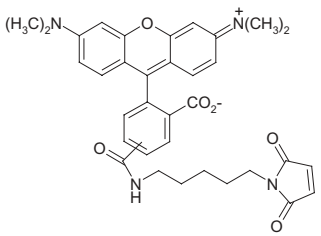


MTS derivative of TAMRA, for highly selective thiol reactivity.

Description	Cat.#	Qty
MTS-TAMRA	**FP-60222A**	5 mg

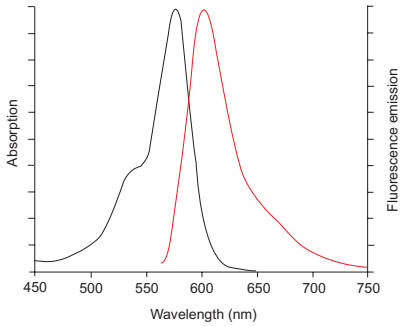
Maleimide-C5-TAMRA

TAMRA : carboxytetrarhodamine
MW : 594.67
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 540/565 nm



The maleimide and extended spacer version of TAMRA, for improved fluorescence features and SH selective reactivity.

Description	Cat.#	Qty
Maleimide-C5-TAMRA	**FP-AM381A**	5 mg



Absorption and fluorescence emission spectra of 6-ROX in pH 7.0 buffer.

ROX (Carboxy-X-Rhodamine)

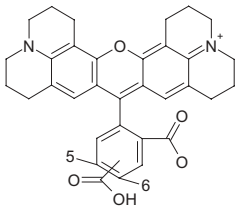
$\lambda_{exc}/\lambda_{em}$: 570/590 ; 568/593

RhodamineX (ROX) generally refers to the rhodamine dyes that are derived from julolidines, ROX dye has longer excitation and emission wavelengths ($\lambda_{exc}/\lambda_{em}$: ca 566/600 nm) than other "conventional" rhodamines.

ROX, NH3 salt

5,6-Carboxy-X-Rhodamine triethylamonium salt, mixed isomers (5 and 6)
MW : 635.81
Soluble in DMSO, DMF, MeOH, or H_2O (pH>6).
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 568/593 nm
EC(MeOH) : 113 000 $M^{-1}cm^{-1}$

In pH 8 buffer compared to MeOH, absorption and emission are red-shifted ~8 nm with EC lower by ~10%.



Used to modify amino and hydroxy groups (peptides, proteins, and other biological ligands) using EDC-mediated couplings when there are difficulties in using ROX-SE. Pure isomers are available, that are preferred for specific biological applications when reproducibility is more critical than material cost since the minor positional difference between 5-ROX and 6-ROX might significantly affect some biological properties of the underlying conjugates.
Applications include nucleic FRET probes [2], automated DNA sequencing applications, and nucleic acid chromatography analysis [4].

References : [1] Hahn, M, et al. (2001), Electrophoresis 22, 2691-700 ; [2] Li, Y and AN Glazer (1999), Bioconjug Chem 10, 241-5 ; [3] Yoshikawa, Y, et al. (1998) ; Anal Biochem 256, 82-91 ; [4] Oefner, PJ, et al. (1994) ; Anal Biochem 223, 39-46.
References : [6] Slateva K, et al. (2001); Tissue Antigens 58, 250-4 ; [5] Hung, SC, et al. (1998); Anal Biochem 255, 32-8.
[8] Lu, H, et al. (1994); J Chromatogr A 680, 497-501 ; [9] Carson, S, et al. (1993); Anal Chem 65, 3219-26.

Description	Cat.#	Qty
ROX, NH_3 salt	**FP-AM395A**	25 mg
5-ROX, NH_3 salt (5-Carboxy-X-Rhodamine triethylammonium)	**FP-M1307A**	10 mg
The single 5-isomer of ROX useful for specific applications notably FRET analysis [6], chromatography and CE analysis [5].		
6-ROX, NH_3 salt (6-Carboxy-X-Rhodamine triethylammonium)	**FP-AM396A**	10 mg
The single isomer (6), useful for specific applications in DNA sequencing[7], chromatography and CE analysis[8,9].		

6-ROX

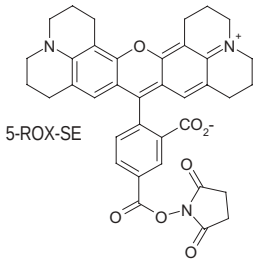
6-Carboxy-X-Rhodamine free acid
MW : 534.62

The single 6-isomer of ROX for specific applications

Description	Cat.#	Qty
6-ROX	**FP-M1319A**	10 mg

ROX-SE

5,6-Carboxy-X-Rhodamine succinimidyl ester, mixed isomers (5 and 6)
MW : 631.69
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 568/595 nm

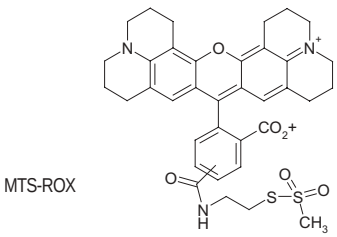


The SE ester derivative, for standard amine targetted labeling.

Description	Cat.#	Qty
ROX-SE	**FP-96292A**	25 mg
5-ROX-SE (5-Carboxy-X-Rhodamine succinimidyl ester)	**FP-68336A**	5 mg
The single 5-isomer of ROX-SE (FP-96292), predominantely used for nucleotides labeling and nucleic acids sequencing.		
6-ROX-SE (6-Carboxy-X-Rhodamine succinimidyl ester)	**FP-47253A**	5 mg
The single 6-isomer of ROX-SE (FP-96292), predominantely used for nucleotides labeling and nucleic acids sequencing.		

MTS-ROX

MethaneThioSulfonate-5(6)carboxy-X-rhodamine
MW : 671.84
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 568/595 nm



The MTS derivative of ROX, for highly selective thiol reactivity.

Description	Cat.#	Qty
MTS-ROX	**FP-58296A**	5 mg

Maleimide-C5-ROX

Maleimido-carboxy-X-rhodamine
MW : 698.83
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 568/595 nm

The maleimide and extended spacer derivate of ROX, for improved fluorescence features and selective thiol reactivity.

Description	Cat.#	Qty
Maleimide-C5-ROX	**FP-AK306A**	5 mg

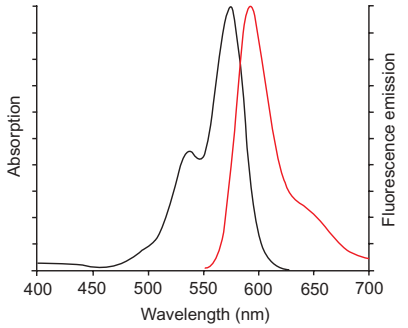
SRB (sulfoRhodamine B)

$\lambda_{exc}/\lambda_{em}$: 560/580 nm

SRB

SulfoRhodamineSRB is mainly used as cell tracer for in vitro cell-based screening of anticancer drugs, where it is believed to bind basic aminoacids of cellular proteins.

Description	Cat.#	Qty
SRB	**FP-700710**	5g



Spectra of SRB-coupled protein.

SRB-lc-SE

SulfoRhodamine B-propionic-succinimidyl ester
MW : 768.9
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 560/580 nm
EC : 129 000 $M^{-1}cm^{-1}$

The SE derivative of SRB. FluoProbes® recommends the superior alternative SRB-EOP-SE (FP-AM408).

Description	Cat.#	Qty
SRB-lc-SE	**FP-M1321A**	5 mg

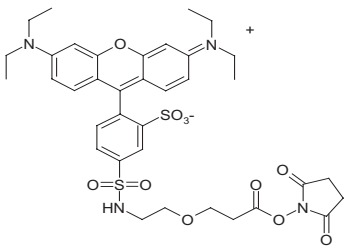
SRB-PE0SE

Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 560/580 nm

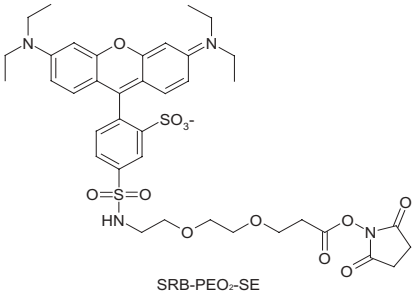
A superior alternative to the standard SRB-SE as well as SR101-SE reagents. It has similar fluorescence, and also reacts readily with amines, but with following advantages:

- Elicits much slower hydrolysis rate, making it easier to store and use.
- Contains a PEO spacer that enhances the fluorescence, minimizes interactions between the dye and the biomolecule to be labeled (better hydrophilicity than SRB-lc-SE, resulting in reduced dye aggregation and self quenching).
  It is available with different spacer lengths, for even improved fluorescent properties.

Description	Cat.#	Qty
SRB-EO-P-SE	**FP-AM408A**	5 mg
SulfoRhodamineB-EO-Propionic acid succinimidyl ester - MW : 770.88		
SRB-PEO2-P-SE	**FP-AM412A**	5 mg
SulfoRhodamineB-PEO_2-Propionic acid succinimidyl ester - MW : 814.94		
SRB-PEO8-P-SE	**FP-BW739A**	5 mg
SulfoRhodamineB-PEO_8-Propionic acid succinimidyl ester - MW : 1079.26		
SRB-PEO12-P-SE	**FP-BW740A**	5 mg
SulfoRhodamineB-$PE0_{12}$-Propionic acid succinimidyl ester - MW : 1255.47		



SRB-EO-P-SE



SRB-PEO_2-SE

SRB-SC

SulfoRhodamine B Sulfonyl Chloride (mixed isomers), also known as LRB-SC
Sulfonyl Chloride ; MW : 577.12
Soluble in DMF (DO NOT use DMSO)
$\lambda_{exc}/\lambda_{em}$ (dichloromethane) : 568/584 nm

The sulfonyl choride derivative or SRB (FP-70071), with strong but not selective reactivity. SRB-SC was popularized originally and is relatively inexpensive. However, it is quite labile in aqueous basic solutions [2], making it somewhat difficult to achieve reproducible conjugations. Reaction should be carried out at low temperature (over ice or 4°C) usually at pH 8[5] so we recommend using the superior alternative SRB-EOP-SE (FP-AM408).

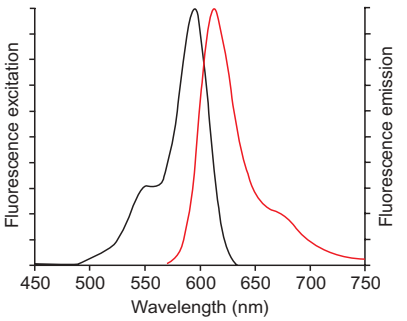
Applications : Membranes heterogeneity study [1,4], DNA labeling [3].

References :
[1] Lour, LM, et al. (2001) ; Biophys J 80, 776-88 , [2] Smith SN and Steer RP (2001) ; J Photochem Photobiol, A139, 151; [3] Neves C, et al. (2000) ; Bioconjug Chem 11, 51-55 ; [4] Pedersen S, et al. (1996) ; Biophys J 71, 554-60 ; [5] Wessendorf MW and TC Brelje (1992) ; Histochemistry 98, 81-85

Description	Cat.#	Qty
SRB-SC	**FP-18798A**	100 mg

SR101 (SulfoRhodamine 101)

SulfoRhodamine101 (SR101) is an excellent red fluorophore ($\lambda_{exc}/\lambda_{em}$: 583/603 nm), especially for microscopy, with strong and stable fluorescence (brighter than LRB). It has been used extensively as its sulfonylchloride derivative. It is used alone, or combined to TRITC, or more often paired to green labels (i.e. Fluoresceins), and IR labels. Triple labeling, along with FITC/FP488 and G5.5/FP682 or equivalent, is possible using a confocal laser scanning microscope. SR101 is also available coupled to our secondary antibodies (page A324) and several cell biology probes.



Absorption and emission spectra of SR101-protein conjugates

SR101

SR101 basic compound is mainly used for cell biology applications, as polar tracer. See section Cell Tracing.

Description	Cat.#	Qty
SR101	**FP-46999A**	25 mg

SR101-lc-SE

SulfoRhodamine101-lc-SE
MW: 816.9
Soluble in DMF, DMSO
$\lambda_{exc}/\lambda_{em}$(MeOH) : 583/603 nm
EC : 94 000 $M^{-1}cm^{-1}$

Description	Cat.#	Qty
SR101-lc-SE (Single isomer) SulfoRhodamine succinimidyl ester	**FP-R1404A**	2 mg
SR101-lc-SE (mixed isomers) 5(6)-SulfoRhodamine 101-succinimidyl ester	**FP-47243A**	5 mg

SR101-PEO$_x$-SE

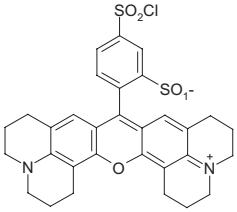
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 583/603 nm
EC : 94 000 $M^{-1}cm^{-1}$

A superior alternative to the popular SulfoRhodamine101 sulfonyl Chloride, as well as SR101-lc-SE. It is available in 4 versions, with a single and a double spacer lenght, for even improved properties.
It has similar fluorescence, and also reacts readily with amines, but with following advantages :

- Elicits much slower hydrolysis rate, making it more easier to store and use.
- Contains a PEO spacer that enhances the fluorescence, minimizes interactions between the dye and the biomolecule to be labeled (better hydrophilicity than SR101-lc-SE resulting in reduced dye aggregation and self quenching).

Description	Cat.#	Qty
SR101-PEO-SE SulfoRhodamine101-EO-Propionic acid Succinimidyl Ester - MW : 818.93	**FP-AM402A**	5 mg
SR101-PEO$_2$-SE SulfoRhodamine101-PEO$_2$-Propionic acid succinimidyl ester - MW : 862.98	**FP-AM409A**	5 mg
SR101-PEO$_8$-SE SulfoRhodamine101-PEO$_8$-Propionic acid succinimidyl ester - MW : 1127.28 - Spacer 32 Å	**FP-BV107A**	1 mg
SR101-PEO$_{12}$-SE SulfoRhodamine101-PEO$_{12}$-Propionic acid succinimidyl ester - MW : 1303.49	**FP-BV108A**	1 mg

FluoProbes® suggest trying our alternatives FluoProbes 590A or 594A (see page B56).



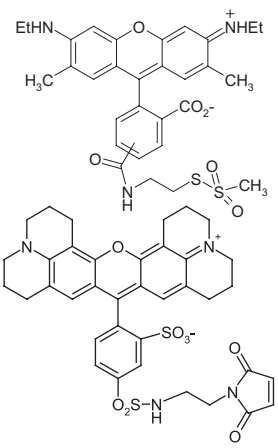
SR101-SC

SulfoRhodamine 101 Sulfonyl Chloride
MW : 625.17
Soluble in DMF (DO NOT use DMSO)
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 588/603 nm
EC : 84 000 M^{-1} cm^{-1} ; QY : 0.85
$\lambda_{exc}/\lambda_{em}$ (water) : 580/602 nm ; QY 0.95

A popular dye, that is long wavelength and amine reactive (reacts with amino acid, peptides, and proteins). It gives bright red fluorescent conjugates that are extremely stable, and resistant to protease-catalyzed hydrolysis. Reaction should be carried out at low temperature (over ice or 4°C) usually at pH8 [5] As it is quite unstable in water, especially at the higher pH required for reaction with aliphatic amines, we recommend the superior alternative SR101-PEO-SE (FP-AM402).

References :
1. Larramendy ML, et al. (1998) ; Cytometry 31, 174-9. - 2. Brismar H, et al. (1995) ; J Histochem Cytochem 43, 699-707.
3. Schneider H (1989) ; J Neurosci Methods 30, 107-15. - 5. Titus JA, et al. (1982) ; J Immunol Methods 50, 193-204.

Description	Cat.#	Qty
SR101-SC	**FP-47006**	10 mg



SR101-MTS

MethaneThioSulfonate-SulfoRhodamine101
MW : 743.94
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (coupled) : 583/603 nm

The MTS derivative of SR101, for selective thiol reactivity.

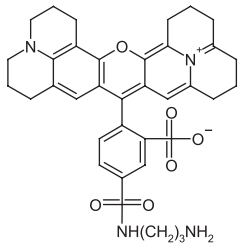
Description	Cat.#	Qty
SR101-MTS	**FP-AM379A**	5 mg

SR101-Maleimide

SulfoRhodamine101-Maleimide, RRX-Maleimide
MW : 728.8
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 588/601 nm
EC :112 000 M^{-1} cm^{-1}

The maleimide derivative or SR101, for selective thiol reactivity.

Description	Cat.#	Qty
SR101-Maleimide	**FP-37796A**	5 mg

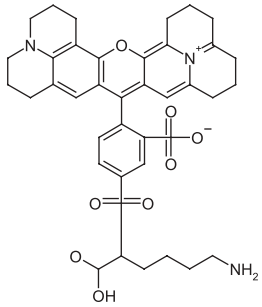


SR101 SA Cadaverine

SulfoRhodamine 101 Sulfonamide Cadaverine
MW : 690.89
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 588/601 nm

Used as building block for preparing red fluorescent biomolecules. It has also been proven to be a good transglutaminase substrate.

Description	Cat.#	Qty
SR101 SA Cadaverine	**FP-M1206A**	5 mg



SR101 SA Lysine

SulfoRhodamine101 Sulfonamide Lysine
MW : 734.9
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (MeOH) : 583/600 nm

Used as building block for preparing red fluorescent peptides through attachment to COOH. It has also been proven to be a good transglutaminase substrate

Description	Cat.#	Qty
SR101 SA Lysine	**FP-AM871A**	5 mg

Other fluorophores

It is impossible to list here exhaustively available fluorochromes. FluoProbes® aims to index any old and new fluorochromes and source them for you, or provide alternatives. Below are presented by alphabetic order some dyes non-classified above, and links to other sections for other dyes that are available in kits. If you dont find the dye you want, please inquire at interbiotech@interchim.com, we may have it in our "A to Z biodirectory of fluorescence".

Benzofuran based fluorochromes (ABD, SBF)

Benzofurazan moiety readily reacts with thiol compounds to generate highly fluorescent products, which make them useful as derivatization agents and for cell structure studies. The ABD/thiol adduct has absorption maximum at ca 386 nm and fluorescence maximum at 514 nm. It is available as the original SBF reagent, and the more reactive ABD reagent.

ABD-F

4-Fluoro-7-Aminosulfonylbenzofurazan ; 4-Aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole
MW : 232.21
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (free) : 315nm/none
$\lambda_{exc}/\lambda_{em}$ (coupled) : 389/513 nm

Readily reacts with thiol compounds. The reaction rate is 30 times faster than that of SBD-F, it completes within 5 minutes in aqueous solutions at 50 °C, pH 8, and ABD-F generates a highly fluorescent compound. Like SBD-F, ABD-F also reacts with amine compounds. It is widely used for TLC and HPLC derivatizations of thiol compounds (superior sensitivity and selectivity than OPA). The detection limits of cystein, glutathione, N-acetylcystein, and cysteamine are 0.6, 0.4, 1.9 and 0.5 pmol/injections respectively with pre-labeled ABD-thiol compounds.

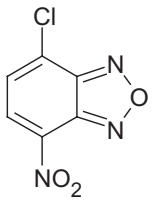
References :
[1] Uchiyama S, et al. (2001); Biomed Chromatog 15, 295-318 ; [2] Treuheit, MJ and TL Kirley (1993); Anal Biochem 212, 138-42

Description	Cat.#	Qty
ABD-F	**FP-57564A**	10 mg

NBD-Cl

4-Chloro-7-NitroBenzoFurazan
MW : 199.55
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (free) : 337 nm/none
$\lambda_{exc}/\lambda_{em}$ (NH_2 bound) : 464/512 nm

NBD reacts with amino groups such as aliphatic amines, amino acids, peptides, and proteins to form highly fluorescent compounds. The fluorescence spectra of NBD/amine adducts are highly environment-sensitive, the fluorescence intensity decreases significantly in aqueous solutions. NBD-Cl also reacts with thiol groups to form fluorescent adducts. Widely used to label peptides, proteins, drugs and other biomolecules, for localization [4], structural studies [5], function and transport [2] ; NBD is also a popular derivatization reagent for HPLC analysis.



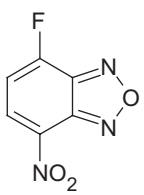
References :
[1] Babia, T, et al. (2001) ; Traffic 2, 395-405 ; [2] Schramm, U, et al. (1993); J Lipid Res 34, 741-57 ; [3] Schramm U. et al. (1991); J Lipid Res. 32, 1769-79 ; [4] Detmers, PA, et al.(1985); Cell Motil 5, 415-30.

Description	Cat.#	Qty
NBD-Cl	**FP-T3226A**	1 g

NBD-F

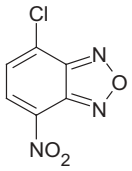
4-Fluoro-7-nitrobenzofurazan
MW : 183.1
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (free) : 337 nm/none
$\lambda_{exc}/\lambda_{em}$ (NH_2 bound) : 464/512 nm

NBD-F has similar properties and applications to NBD-Cl. Compared with NBD-Cl, it is more reactive, and should be more carefully stored.



References :
[1] Suzuki S, et al. (2001) ; Electrophoresis 22, 4023-31 ; [2] Tani M, et al. (1998) ; Anal Biochem 263, 183-8 ; [3] Chejanovsky N, et al. (1986) ; Biochemistry 25, 4810-7

Description	Cat.#	Qty
NBD-F	**FP-U0573A**	25 mg



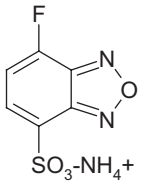
SBF-Cl

4-Chloro-7-sulfoBenzoFurazan, ammonium salt
MW : 251.65
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (free) : 380 nm/none
$\lambda_{exc}/\lambda_{em}$ (coupled) : 385/515 nm

SBF-Cl is a water-soluble fluorescent-labeling reagent for thiol compounds, and is not cytotoxic or mutagenic. It selectively reacts with thiol groups to generate a highly fluorescent product. In aqueous solutions, SBF-Cl does not readily react with amines. However, it may react with amine compounds in DMSO solutions. It has been used for derivatization in chromatography [2], for enzyme substrates design [3].

References :
[1] Ozkan Y, et al. (2002) ; Int J Cardiol 82, 269-77. ; [2] Chen, XP, et al. (1998); J Chromatogr B Biomed Sci Appl 709,19-25
[3] Bolton, RM, et al. (1994) ; Anal Biochem 216, 418-23. ; [4] Andrews JL, et al. (1982); Arch Biochem Biophys 214, 386-96.

Description	Cat.#	Qty
SBF-Cl	**FP-AM858A**	5 mg



SBF-F

4-Fluoro-7-sulfobenzofurazan, ammonium salt
MW : 235.2
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$ (free) : 385 nm/none
$\lambda_{exc}/\lambda_{em}$ (coupled) : 385/515 nm

SBF-F has the same features than SBF-Cl. It is widely used for HPLC derivatizations of thiol compounds[1]. The HPLC detection limit of thiol compounds such as glutathione, cystein, N-acetylcystein, CoA, and BSA is in the range of 100-500 pmol/injection.

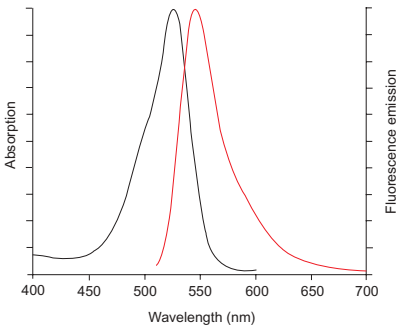
References:
[1] Uchiyama S, et al. (2001) ; Biomed Chromatogr15, 295-318 ; [2] Fermo, I and R Paroni (2000) ; Methods Mol Biol 159, 237-44
[3] Imai, K and T Toyo'oka (1987) ; Methods Enzymol 143, 67-75

Description	Cat.#	Qty
SBF-F	**FP-AM859A**	10 mg

Cyanines

Cyanine based dyes were popularized with the Cy™2, Cy™3, Cy™5 series. FluoProbes® provides labeling kits (page D.134 in Genomics section) because Cy3 and Cy5 have become standards in microarray applications, and also coupled to our secondary antibodies (pages A324-A345). We recommend alternatively, respectively, our FluoProbes®546/547 (FP546 labeled antibodies, FP547 reactive agents and labeling kits) and our FluoProbes®642/647 labels (FP642 labeled antibodies, FP647 reactive agents and labeling kits).

IndoCyanines dyes have long wavelengths and intense fluorescence which excitation/emission maximal depend of the number of methine carbons in their structure. ask our FluoProbes®550D and 640D dyes.



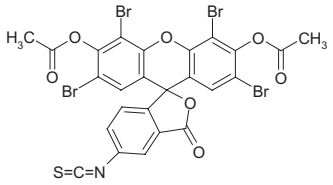
Absorption and emission spectra of Eosin-Isothiocyanate

Eosin

Eosin is used primarily as a phosphorescent probe or as photosensitizer.
Fluorescence $\lambda_{exc}/\lambda_{em}$: 521/544 nm is far lower than fluorescein.

Applications :

- Effective photoxidizer of DAB for electron microscopy (high quantum yield (~0.57) for singlet oxygen generation).
- Measuring the rotational properties in solution and in membranes, FRET.
- Also a potent reversible inhibitor of the erythrocyte calcium pump (IC_{50} : <0.2 µM).



Eosin-5-isothiocyanate

MW : 704.89
Soluble in DMF at pH >6
$\lambda_{exc}/\lambda_{em}$ (MetOH) : 521/544 nm
EC : 95 000 $M^{-1}cm^{-1}$

Description	Cat.#	Qty
Eosin-5-isothiocyanate	**FP-47527A**	100 mg

Eosin-5-isothiocyanate diacetate

MW : 789.04
$\lambda_{exc}/\lambda_{em}$: 520/545 nm

Acetate groups facilitate loading and retention in cell (hydrolysis by intracellular esterases).

Description	Cat.#	Qty
Eosin-5-isothiocyanate diacetate	**FP-AM519A**	5 mg

Naphtalene based fluorophores

ANTS

8-Aminonaphtalene-1,3,6-trisulfonic acid
MW : 427.34
Soluble in DMF or DMSO
$\lambda_{exc}/\lambda_{em}$: 353/520 nm
EC : 7 200 $M^{-1}cm^{-1}$

A green fluorescent and anionic dye for labeling glycoproteins or sugars in general. Reaction of ANTS amine with the aldehyde or ketone of the sugar involves a reductive amination forming a Schiff's base. Reduction forms a stable C-N bond. It has been widely used for oligosaccharides and glycoproteins sequencing (Yamazaki 1990), and for electrophoresis analysis of degradation products from carbohydrate polymers. It is also used in conjunction with the quencher DPX.

Description	Cat.#	Qty
ANTS	**FP-46574A**	500 mg

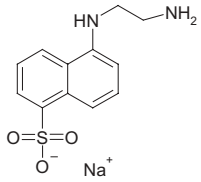
EDANS

5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid, sodium salt ; MW : 288.3
Soluble in DMSO
$\lambda_{exc}/\lambda_{em}$: 335/493 nm

EDANS is one of the most popular donors for developing FRET-based nucleic acid probes and protease substrates, often paired with DABCYL or DABSYL. It shows an environment-sensitive fluorescence.

References :
[1] Becker BF, et al. (2002) ; Biol Chem 383, 1821-6 ; [2] Cottaz S, et al. (2000) ; Eur J Biochem 267, 5593-600 ; [3] Beekman B, et al. (1996) ; FEBS Lett 390, 221-5. 4. Wang GT, et al. (1993) ; Anal Biochem 210, 351-9

Description	Cat.#	Qty
EDANS	**FP-46479A**	1 g



See also EDANS building blocks (t-BOC, Fmoc derivatives) in section Synthesis page B96.

INA (5-iodonaphthyl-1-azide)

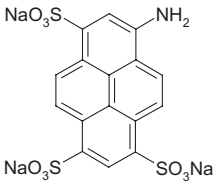
5-iodonaphthyl-1-azide
MW : 295.08

INA is a lipophilic photoreactive probe, which has been used to selectively label membrane-embedded cysteine residues of proteins (Proc. Natl. Acad. Sci. USA 100, 886(2003).)

Description	Cat.#	Qty
INA	**FP-BT9531**	10 mg

Pyrene based fluorophores

The fluorescence of pyrene based dyes may vary considerable depending on environment factors.



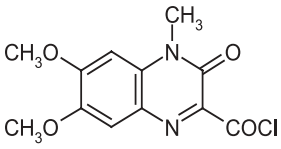
APTS

8-AminoPyrene-1,3,6-Trisulfonic acid, trisodium salt
MW : 523.4
Soluble in water
$\lambda_{exc}/\lambda_{em}$ (free) : 424/505 nm

A green fluorescent and multi-anionic dye ($\lambda_{exc}/\lambda_{em}$ (free) : 424/505 nm) for glycoproteins or sugars labeling in general. Reaction of APTS amine with the aldehyde or ketone of the sugar involves a reductive amination forming a Schiff's base. Reduction forms a stable C-N bond. It suits ideally high-resolution capillary electrophoresis of carbohydrates.

Description	Cat.#	Qty
APTS	**FP-33972A**	10 mg

Quinoxaline based fluorophores



DMEQ-COCl

3-Chlorocarbonyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone
MW : 282.69
$\lambda_{exc}/\lambda_{em}$: 400/500 nm
Solubility : 0.85 mg/ml benzene

DMEQ-COCl is a labeling reagent for primary and secondary alcohols. In organic solvents such as benzene and acetonitrile, this reagent readily reacts with alcohols to yield highly fluorescent derivatives. The HPLC detection limits of benzyl alcohol, n-hexanol, and cyclohexanol are 2-3 femtomoles per injection. Steroids that have primary and secondary alcohols can be detected as fluorescent DMEQ derivatives. However, *tert*-alcohols, hydroxycarboxylic acids, and phenols do not react under the same labeling conditions. DMEQ-COCl also reacts with amines ; as little as 0.3 pmol/ml of b-phenylethylamine has been detected in human serum. The excitation and emission wavelengths of the labeled materials are 400 nm and 500 nm, respectively.

Description	Cat.#	Qty
DMEQ-COCl	**FP-69129A**	10 mg

DPX

Pyridinium, 1,1'-(1,4-phenylenebis(methylene))bis-, dibromide
MW : 422.18
Soluble in Water
$\lambda_{exc}/\lambda_{em}$: 259 nm/none
EC : 8 800 $M^{-1}cm^{-1}$

A positively charged quencher that is often used as polar tracer with ANTS to study membrane fusion or permeability including complement-mediated immune lysis.

Description	Cat.#	Qty
DPX	**FP-47017A**	2 x 500 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

Fluorescence Reference Standards

Fluorescence Reference Standards [Comments]	$\lambda_{exc}/\lambda_{em}$ (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 * [Structural base of Magic Red™]	601/632	**BS5990**	100 mg
Oxazine 1 [Redox –sensitive fluorescence]	646/670	**BS6410**	25 mg
Pyrromethene 546 [Excellent dye for dyeing latex]	494/519	**BS6420**	25 mg
Rhodamine 700 [Unstable in strong base (pH>12)]	643/nd	**BS6430**	25 mg
Rhodamine 800 [Unstable in strong base (pH>12)]	682/nd	**BS6460**	25 mg
Rose bengal [pH-dependent fluorescence; Singlet oxygen generator.]	556/577	**BS6440**	100 mg
Sulfofluorescein [Water-soluble; Fluorescence is similar to that of fluorescein]	495/520	**BS6450**	100 mg

* Coumarin Fluorescence Reference Standards are excellent dyes for dyeing latex, liposomes, membrane and films.

Quenchers

This section presents conventional as well unique black quenchers. Most associated fluorophores, that are quenched when paired, are described with fluorescent dyes in pages B51-B81. Great applications include the study of molecular interactions and the development of sensitive assays :

♦ Genomics
Real-time, Quantitative PCR
SNP Discovery, Detection & Scoring
Allelic Discrimination
Spectral Genotyping
in situ Hybridization
Single-Tube Multiplexing

♦ Molecular Biology
Proteolysis
Receptor/ligand interactions
Distribution and transport of lipids
Membrane potential sensing
Cyclic AMP detection

Interchim provides conventional pairs of quencher/fluorophore as **DABCYL**/EDANS and **DNP**/MCA that were popularized. Limitations of use come from their short absorption wavelength and low extinction coefficient. To address these limitations, FluoProbes developed FRET acceptors that are optimized dark quenchers covering all the Fluorophores commonly used, such as fluoresceins and rhodamines: **DABCYL Plus** (B88), **FluoQuench™ FRET detectors** (B86), and **BHQ (Black Hole Quenchers)** (B87).

Technical tip

Conventionnal quenchers
The following table displays the popular FRET dyes pairs, to help you selecting the best one depending on your light excitation source and light detector, as well expected FRET efficiency (Ro value [£]). Table1: Some Typical Ro Values of D/A pairs*

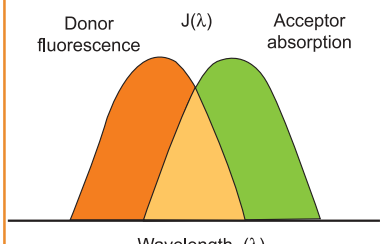
Donor	Acceptor	R_o (Å) [£]
Fluorescein	Tetramethylrhodamine	49-56
Fluorescein	Fluorescein	44
IAEDANS **	FITC	49
IAEDANS	5-(Iodoacetamido)	49
EDANS	Dabcyl	33
Tryptophan	IAEDANS	22
Tryptophan	Dansyl	21-24
Tryptophan	Pyrene	28
Dansyl	Fluorescein	33-41
Naphthalene	Dansyl	22
Pyrene	Coumarin	39
B-Phycoerythrin	Cy5	79

[£] R_o (Å) is the Förscher radius (distance at which 50% of energy is transferred).
[*] The value may change under different conditions.

Technical tip

Quenching
Fluorophores molecules in close contact may inhibit reciprocally their fluorescence properties (self-quenching). This is of big importance for labeling experiments (degree of conjugation should be optimized), as well for any detection where a high concentration of dye is reached (high density of antigen on membranes, or concentrated in organelles for example). This is a common limitation with PhycoErythrin dye for example. Quenching however is taken to good account in some techniques as quenched molecular probes and FRET probes (see below).

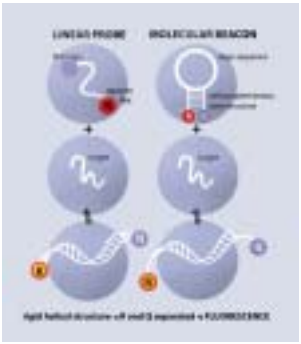
FRET / quenching principle
Fluorescence Resonance Energy Transfer (FRET) occurs with some fluorophores when absorption spectrum from an Acceptor molecules (A) overlaps the emission spectrum from a Donor (D), and molecules are in vicinity, typically at 30 to 60 Angstroms. In this situation, a fluorophore 'acceptor' is excited, not by light, but by direct transfer of the excited state energy from an initially excited donor. Return to the ground energy state releases photons = fluorescence. For non-fluorescent acceptors, FRET results in a decrease of donor fluorescence quenching.



Overlapping spectra of Donor emission and Acceptor absorption.

Technical tip

A **FRET pair (or tandem)** is a donor fluorophore and acceptor fluorophore that generate fluorescence with a long Stocke's shift when they are in vicinity.
When the acceptor is a quencher, it abolishes the fluorescent when paired, and the pair should rather be called Quenching tandem. As an example, **Molecular Beacons** are oligonucleotides "hairpin" (two "arms" of 5-7 nucleotides self-complementary to each other), conjugated to a fluorescent dye (donor) and a quencher. Interestingly, this structure is very stable, by design, and should only open in the presence of target. In the absence of target, the fluorescent reporter and quencher molecules are brought close together in the probe's self-complementary stem structure, and the fluorescent signal is suppressed. When the molecular beacon hybridizes to its target, the fluorescent reporter and the quencher are separated, and the reporter dye emits at its characteristic wavelength. So, hybridization event turns on fluorescence.

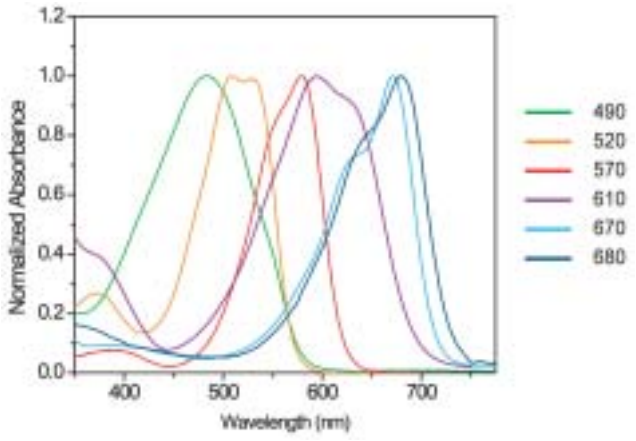


Molecular beacons are widely used to detect DNA hybridization [1-4], i.e. virus replication in HIV, rifampicin resistant Mycobacterium. These properties make them superior in hybridization-based investigations of single nucleotide polymorphisms (SNPs)[5,6]. They also detect nucleases [8], i.e. the 5' exonuclease activity of Taq polymerase. Finally, quenchers have been used to perform multiplex assays (One dark quencher + severa fluorophores).

[1] Heyduk T., Heyduk E. (2002) Molecular beacons for detecting DNA binding proteins. Nat Biotechnol, 20, 171-6.
[2] Kuhn H., et al. (2001) PNA beacons for duplex DNA. Antisense Nucleic Acid Drug Dev, 11, 265-70.
[3] Marras S. A., et al. (1999) Multiplex detection of single-nucleotide variations using molecular beacons. Genet Anal, 14, 151-6.
[4] Li J. J., et al. (2000) Using molecular beacons as a sensitive fluorescence assay for enzymatic cleavage of single-stranded DNA. Nucleic Acids Res, 28, E52.
[5] Shi M. M. (2002) Technologies for individual genotyping: detection of genetic polymorphisms in drug targets and disease genes. Am J Pharmacogenomics 2, 197-205.
[6] Shi M. M. (2001) Enabling large-scale pharmacogenetic studies by high-throughput mutation detection and genotyping technologies. Clin Chem, 47, 164-72.
[7] Yamamoto R., et al. (2000) Molecular beacon aptamer fluoresces in the presence of Tat protein of HIV-1. Genes Cells, 5, 389-96.
[8] Tapp I., et al. (2000) Homogeneous scoring of single-nucleotide polymorphisms: comparison of the 5'-nuclease TaqMan assay and Molecular Beacon probes.Biotechniques, 28, 732-8.

FluoQuench™ FRET Detectors

FluoQuench™ FRET detectors are dark quenchers superior to most conventional quenchers. This products family provides a valuable set of tools to build FRET probes covering the full visible spectrum with unusually high efficiency. Relatively large absorption spectra make them perfect fluoresceins and rhodamines. High extinction coefficients ensure total quenching. Finally their good water solubility facilitates FRET probes preparation and improves the results of FRET probes in many applications.
FluoQuench™ FRET detectors are available under a variety of derivatives allowing biomolecules labeling through their amines, sulfhydryls or carboxyl groups. You can thus design your own FRET probes for some special applications.



FluoQuench™490	FluoQuench™520	FluoQuench™570	FluoQuench™610	FluoQuench™670	FluoQuench™680
l abs. max (nm) 488	508, 530	538, 577	594, 628	668	679
Recommended FRET donor EDANS, AMCA, FP390-425	FAM, FITC,CR6G, FP488	Cy3, TAMRA, ROX, FP547	ROX, SR101	Cy5, FP647	Cy5, F647
MW (free acid) 377.42	553.43	597.77	590.65	623.81	705.93
acid **FP-BC9410**, 100mg	**FP-BC9460**	**FP-BC9510**, 25mg	**FP-BC9330**, 100mg	**FP-BU1850**, 10mg	**FP-BU1800**, 10mg
Amine **FP-BC9430**,10mg	**FP-BC9480**, 10mg	**FP-BC9530**, 5mg	**FP-BC9390**, 25mg	**FP-BU1870**, 5mg	**FP-BU1820**, 1mg
NHS **FP-BC9420**, 25mg	**FP-BC9470**, 25mg	**FP-BC9520**, 10mg	**FP-BC9380**, 25mg	**FP-BU1860**, 5mg	**FP-BU1810**, 5mg
Maleimide **FP-BC9450**, 10mg	**FP-BC9500**, 10mg	**FP-BC9540**, 5mg	***FP-BC9340**, 10mg	**FP-BU1880**, 5mg	**FP-BU1830**, 1mg
Hydrazide **FP-B1890**, 5mg	**FP- BU1760**, 5mg	**FP-BU1770**, 5mg	**FP-BU1780**, 10mg	**FP-BU1790**, 5mg	**FP-BU1840**, 1mg

*FluoQuench610 FP-BC9340 is derivatized by vinylsulfone group (not maleimide), also a thiol reactive group.

All FluoQuench Detectors are soluble in DMF or DMSO, but QXL520 acid, amine, Maleimide, Hydrazine and QXL610 acid, amine, Vinylsulfone, Hydrazine are soluble also in water.

MW is given for free acid forms.

Please inquire for additional information (FRET spectral overlap with a dye, bulk price,...).

BHQ FRET Quenchers

BHQ dyes function has efficient dark quenchers over the entire visible spectrum and into the near-IR, re-emitting their energy as heat rather than light. Probes made with BHQ dyes exhibit extremely low background fluorescence, enabling enhanced detection sensitivity :

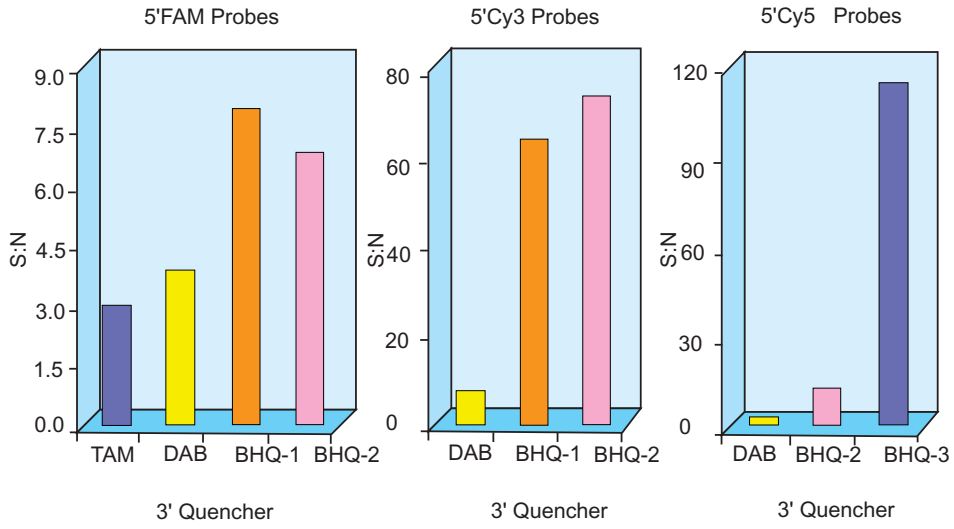
- Work into almost any probe formulation ; from linear DNA probes to molecular beacons
- Efficiently FRET quenching of most common fluorochromes
- Ability to multiplex detections (minimal or no cross talk between reporters)
- True dark quenchers NO native fluorescence
- Superior to conventional quenchers (DABCYL, TAMRA...)

BHQ Dyes have absorption and Quenching Range large enough to quench efficienctly by FRET following dyes.

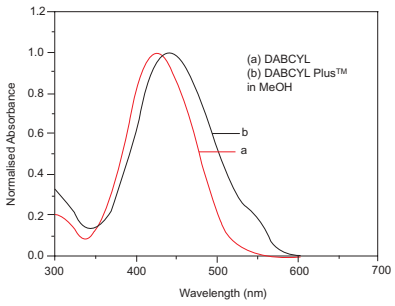
Quenchers	Abs max	Quenching Range (nm) Suggested Fluorophores	Carboxylic acid	Amine	NHS	Resin
BHQ-1	534 nm	480 - 580 nm FAM, TET, JOE, Oregon Green®	**FP-BC8540** 5 mg	**FP-BC8490** 5 mg	**FP-BC8510** 5 mg	**FP-BC8520** 100 mg
BHQ-2	579 nm	550 - 650 HEX, TAMRA, ROX, Cy™3, Cy™3.5,Texas Red™, Red 640	**FP-BC8500** 5 mg	**FP-BC8530** 5 mg	**FP-BC8550** 5 mg	**FP-BC8560** 100 mg
BHQ-3	672 nm	620 - 730 nm Cy™5, Cy™5.5, LC Red640	**FP-BC8580** 5 mg	**FP-BC8570** 5 mg	**FP-BC8590** 5 mg	

Application

DABCYL has an inadequate absorption footprint that overlaps very poorly with fluorophores emitting above 480 nm. TAMRA is not a dark quencher and contributes to an overall increase in background because of its own native fluorescence.
In comparison BHQ dye probes have much larger signal-to-noise ratios when compared to the corresponding DABCYL and TAMRA probes. This is a critical advantage for the development of new equipments that achieve ultimate sensitivities.



Signal-to-noise (S:N) ratios were calculated by dividing the fluorescence signal of a 25-mer in the presence of a five-fold excess of an exactly complementary target sequence by the fluorescence intensity of the probe alone. Each probe was formulated with a 5' reporter group (FAM, Cy™3, Cy™5) and a quencher (TAMRA, DABCYL, BHQ-1, BHQ-2 or BHQ-3).
The 3 BHQ quenchers are available derivatized by Carboxylic acid, Amine, Succinimidyl ester. Glycolate CPG resins are also availabe used to add the non-fluorescent quencher BHQ-1 to the 3' end of an oligonucleotide. The glycolate linker allows rapid cleavage of oligonucleotides and is labile enough for base sensitive oligonucleotides. Synthesis columns are available on inquire.



See DABCYL/EDANS probes for metalloproteases and transglutaminases substrates page E.112.
see also DABSYL-Amino-acids
see also DABSYL-Building blocks for synthesis (Fmoc, tBoc derivatives) (page B.97)

DABCYL Quenchers

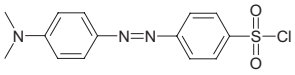
DABCYL has been routinely used as a general-purpose dark quencher for FAM, TET, and JOE. Also, DABCYL/EDANS pair has been intensively used to develop FRET-based nucleic acid probes and protease substrates. However, the extreme hydrophobicity and resultant poor water solubility of DABCYL have limited its use for sensitive fluorogenic FRET probe, because affinity for enzyme may be reduced.
DABCYL Plus™ has been designed to address this limitation : it elicits spectral properties similar to those of DABCYL, enabling researchers to keep all assay settings similar to DABCYL's probes to which they are accustomed. In addition, DABCYL Plus™ has much greater water solubility than DABCYL. Also, the slightly red-shifted absorption spectra overlaps well better with the EDANS emission spectrum. Lastly, the absorption spectrum of DABCYL Plus™ is environment-sensitive as in the case of DABCYL dyes. For example, in water, the spectrum of DABCYL Plus™ is red-shifted ~40 nm compared to that in methanol.

Quencher	$\lambda_{abs.\ max}$	EC	MWx	acid (free acid)	NHS	Maleimide
DABCYL	425nm	32 000 $M^{-1}cm^{-1}$	269.3	**FP-AY7630** 1 g	**FP-AY7640** 100 mg	**FP-AY7650** 25 mg
DABCYL Plus	437nm		377.4	**FP-AY7660** 100 mg	**FP-AY7680** 25 mg	**FP-AY7690** 10 mg

λabs max, EC and MW are given for the free acid from.

DABSYL chloride

[4-dimethylaminoazobenzene-4'-sulfonyl chloride] "high purity"
MW : 323.8
Soluble in DMSO or DMF
$\lambda_{exc}/\lambda_{em}$: 466 nm/none



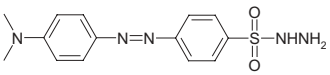
DABSYL is misused as "DABCYL" in some literature . DABSYL is used as an acceptor for developing FRET-based nucleic acid probes and protease substrates (often paired with EDANS). It is also an important derivatization reagent.

References :
[1] Sando S and Kool ET (2002) ; J. Am Chem Soc 124, 2096-7
[2] Sato E, et al. (1991) ; J Pharmacobiodyn 14, 599-604
[3] Hendrickson HS, et al. (1990) ; Anal Biochem 185, 80-3

Description	Cat.#	Qty
DABSYL chloride	**FP-19195B**	1 g

DABSYL hydrazine

MW : 319.39
Soluble in DMSO or DMF

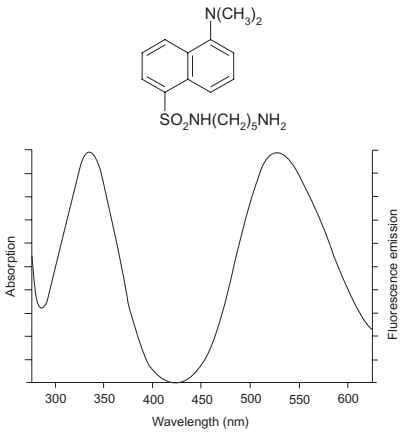


DABSYL hydrazine is a useful building block that can be attached to an aldehyde (for carbohydrates or glycoproteins) or carboxy group (for peptides or proteins).

Description	Cat.#	Qty
DABSYL hydrazine	**FP-AY7720**	100 mg

Dansyl cadaverine

MW : 335[5]1
$\lambda_{exc}/\lambda_{em}$: 333/518 nm



Has been used to prepare other small fluorescent biomolecules via amidation or reductive amination of carboxyls. Also used as transglutaminase probe.

*Description	Cat.#	Qty
Dansyl cadaverine	**FP-75581A**	100 mg

Other Quenchers (DNP)

DNP (2,4-dinitroaniline) was widely paired with **MCA** (7-methoxycoumarin-4-acetic acid) for developing FRET-based probes. Compared with the pair of DABCYL/EDANS, they usually have shorter and weaker wavelengths. However, they often demonstrate better affinity or turnover rate due to their smaller size. DNP is also a good FRET acceptor paired with Tryptophan or 2-aminobenzoic acid (Abz) or Abz derivatives such as Abz(N-Me).
Pyrene is excited by and emits in UV spectrum ($\lambda_{exc}/\lambda_{em}$: 340/376 nm ; EC : 43000 $cm^{-1}M^{-1}$) and has an extensive lifetime. Excited-state dimers (excimers) can form, with emission shifted to longer wavelengths than that of the monomer.

Enzyme Labeling

Interchim provides the popular enzymes used in biotechnologies, peroxidase (so called POD, or HRP for the popular HorseRodish Peroxidase), and the alkaline phosphatase (AP), both in ready to use labeling kits and reagent formats.

Peroxidase Labeling kits – Spin format

Interchim provides peroxidase labeling kits in convenient spin formats, with 2 coupling strategies: conjugation though amines, and through sulfhydryls.
The labeling process of NH_2 type is very simple, and the sensitivity of the peroxidase conjugate is sufficient for most of the detections or assays. Ca 1-3 peroxidase per IgG are coupled.
The labeling process of SH type is also simple but requires a reducing reaction to create SH group(s) prior to label with SH-reactive peroxidase. The sensitivity of the prepared peroxidase-conjugated IgG is usually higher than the one prepared by NH2 type kit because of the site specific labeling reaction. Ca 1-2 peroxidase per IgG are coupled.

- Quick : only 3 hours (/NH_2) to get conjugates
- Easy : all processes in a single filtration tube
- Reliable : high recovery of conjugates
- Efficient : applicable for 50-200 µg IgG*

Both kits are dedicated to IgG labeling, but can be applied easily for other proteins, peptides or oligonucleotides provided their MW is greater than 50 000 or less than 5 000 and they have reactive primary or secondary amino groups (or SH groups for SH labeling kit).

Compared to other coupling strategies, these kits offer rapidity and efficiency :

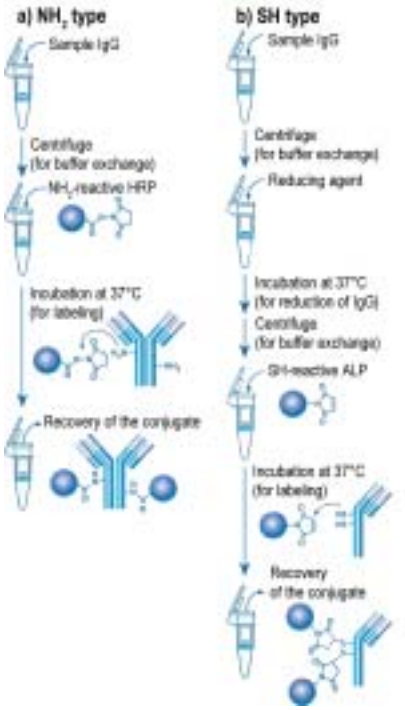
	Kit #BT3771	Glutaraldehyde method	Periodate method
Reactivity	High	Low	High
S/N ratio	High	Low	Low
Polymerization	No	No	Yes
Blocking step	No	Yes	Yes
Required time	3 days	2 days	2 days

Description	Cat.#	Qty
Peroxidase labeling kit – NH2	**BT3771**	3 rxn*
Peroxidase labeling kit – SH	**BG7691**	3 rxn*

Kit #BT3771 contains :
NH_2-reactive peroxidase : 100 µg x 3
Washing buffer : 4 ml x 1
Reaction buffer : 200 µl x 1
Storage buffer : 4 ml x 1
Filtration tube : 3 tubes
*This kit works for 50-200 µg IgG ; it is also available in greater sizes : kit #BT3772 to make 1 labeling of 1mg IgG, and kit #BT3773 make 1 labeling of 10mg or 2 labelings of 5mgs

Kit #BG7691 contains :
SH-reactive peroxidase : 100 µg x 3
Reducing agent : 3 tubes
Solution A : 4 ml x 1
Solution B : 1 ml x 1
Reaction buffer : 200 µl x 1
Storage buffer : 4 ml x 1
Filtration tube : 3 tubes
*This kit works for 50-200 µg IgG ; it kit is also available in greater sizes : kit #BG7692 to make 1 labeling of 1mg IgG

The reaction mechanism of NH2 type and SH type as well as the required steps are illustrated :



Peroxidase labeling kit

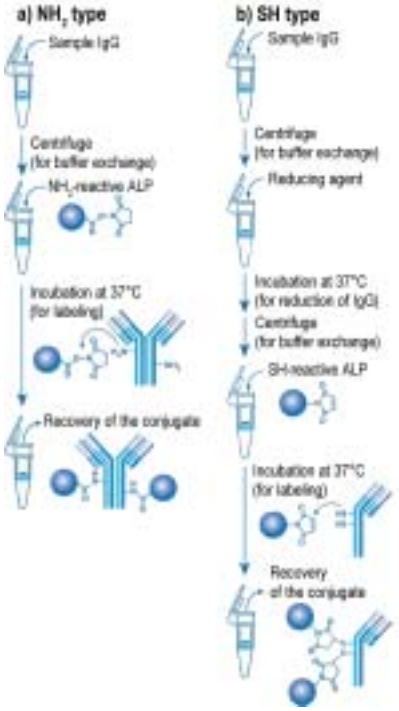
This kit is recommended for economic labeling, and larger quantities than 200 µg per labeling. All reagents are provided to label easily ca 2 mg of proteins (i.e. antibodies). It uses preactivated HRP that is stable for up to 1 year.

Description	Cat.#	Qty
Peroxidase labeling kit	**U27381**	1kit

Contains : 2 mg activated HRP, conjugation buffer, blocking buffer, conjugate stabilizer, dialysis bag

Below 200 µg sample quantity, kit #BT3771 may be more convenient

The reaction mechanism of NH2 type and SH type as well as the required steps are illustrated:



Alkaline Phosphatase (AP) labeling kits – spin format

Interchim provides Alkaline Phosphatase labeling kits in convenient spin formats, with 2 coupling strategies : conjugation though amines, and through sulfhydryls.
The labeling process of NH_2 type is very simple, and the sensitivity of the AP conjugate is sufficient for most of the detections or assays. Ca 1-3 AP per IgG are coupled. No oligomers forms. The labeling process of SH type is also simple but requires a reducing reaction to create SH group(s) prior to label with SH-reactive AP. The sensitivity of the prepared AP-conjugated IgG is usually higher than the one prepared by NH_2 type kit because of the site specific labeling reaction. Ca 1-2 AP per IgG are coupled.

- Quick : only 3 hours to get conjugates
- Easy : all processes in a single filtration tube
- Reliable : high recovery of conjugate
- Efficient : applicable for 50-200 ug IgG

Both kits are dedicated to IgG labeling, but can be applied easily for other proteins, peptides or oligonucleotides provided their MW is greater than 50 000 or less than 5 000 and it has reactive primary or secondary amino groups (or SH groups for SH labeling kit).

Description	Cat.#	Qty
Alkaline Phosphatase (AP) labeling kits – NH2	**BG7700**	3rxn*

Contains :
NH_2-reactive ALP : 100 µg x 3
Washing buffer : 4 ml x 1
Reaction buffer : 200 µl x 1
Storage buffer : 4 ml x 1
Filtration tube : 3 tubes
*This kit works for 50-200µg IgG. A greater size exists : kit #GB7702 for 1 labeling of 1mg.

Description	Cat.#	Qty
Alkaline Phosphatase (AP) labeling kits – SH	**BG7710**	3rxn*

Contains :
SH-reactive ALP : 100 µg x 3
Reducing agent : 3 tubes
Solution A : 4 ml x 1
Solution B : 1 ml x 1
Reaction buffer : 200 µl x 1
Storage buffer : 4 ml x 1
Filtration tube : 3 tubes
*This kit works for 50-200µg IgG. A greater size exists : kit #GB7712 for 1 labeling of 1mg.

Alkaline Phosphatase (reagent)

EC 3.1.3.1 from bovine calf intestinal mucosa
MW: ~140 000Da
Our AP is purified from bovine calf intestinal mucosa (>90% pure by FPLC), and supplied in 50 solution containing % glycerol, 5mM Tris/HCl, 5mM MgCl2 and 0.1mM ZnCl2, pH7.0. Our catalog item is of high activity (>2000U/mg), selected for immunolabeling.
Several other qualities are available on inquire (specific activity from 700 to 2500 U/mg; TEA buffer (UP16657), freeze-dried powder for use as serum controls). We also have a grade tested for absence of DNase, RNase, and DNA nicking activity (#71689), for molecular biology applications (for the removal of terminal phosphate groups of DNA and RNA, preventing the re-annealing of cohesive ends after digestion).

Description	Cat.#	Qty
Alkaline Phosphatase	**UP852857**	10 000 Units
	UP852858	100 000 Units

Peroxidase (reagent)

Hydrogen-peroxide oxidoreductase
EC1.11.1.7
MW : ~44 000Da

Our peroxidase is prepared from Horseradish, purified chromatographically and supplied as freeze-dried powder. I suits especially immunolabeling.
Activity : > 250 U/mg material

Description	Cat.#	Qty
Peroxidase	**UP146500**	250 mg
	UP146501	1 g

Unit definition :
1 unit = amount of enzyme causing the hydrolysis of one micromole of p-nitrophenyl phosphate per minute at pH 9.6 and 25°C (glycine buffer). This unit corresponds to approximately three DEA units at pH 9.8 and 37°C.

See also :
Crosslinkers see section B11, including SMCC (UP), sulfoSMCC (UP), EDC (UP52005),...
Desalting see section B101
Biotinylation see section B41
DNA labeling see section D134

A purer grade (#UP189160, with highest isoenzyme C content) is available for most demanding applications. Please inquire for other preparations dedicated for using as indicator enzymes for reaction in which hydrogen peroxide is produced (i.e. glucose oxidase assays for determination of glucose, lipases assays...): #857280, >60U/mg, RZ>0.6 691.HRP2, and #882990 (mainly acidic isoenzymes) >80 PgP U/mg, RZ:ca3.5.