# **Cell Staining and probing**

### Introduction

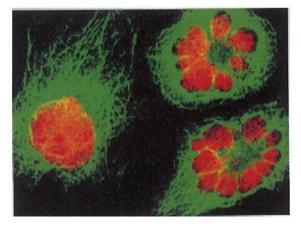
Cell staining and probing is performed for different purposes (cell vizualisation, tracking, structures study,...), targetting various cell components or activities, using chromogenes or fluorescent probes (great for quantitative and multiplex assays).

The most commonly stained cell structures are cell membranes/proteins (i.e. with CFSE), cytosol (i.e. with Calcein and BCECF, notably promoted

by esterases activity – using their AM derivatives), <u>mitochondria</u> (I.e. Rh123, MitoRed), and <u>nucleic materials</u> (DNA nucleus i.e. with DAPI). Staining of cells and tissues is also performed to study the morphology of cells (using i.e. dextrans; especially for specific cells like neurones) or the becoming of cells (tracking), and various intracellular events (ion signaling, phagocytosis, adhesion, apoptosis,... See cell biology assay section).

Cells staining applies for microscopy observation, colony forming assays, cytometry,...They are widely used as more or less **specific stains in anapathology** (tissue staining), and as **counterstains** (when other detections are performed) in IHC/IF and IF-Cytometry techniques (fixed cells hence dead cells in frozen cells or tissues, or paraffin embeded tissue sections). See '<u>Conterstaining section</u>.

Yet, some stains are designed for **staining living cells** and used in IF microscopy or for other purpose/techniques (cell tracing), such as with CFSE stain (see '<u>Cell tracers' section</u>), and RedDot stain (see 'Nucleus staining'). **Vital stains** stain cells that are kept alive (intravital stains), while



supravital stains stain dead cells and are excluded form living cells (differential staining). See 'Vital stains' section.

FluoroQuest<sup>™</sup> Antifading Kit I ; FluoroQuest<sup>™</sup> Antifading Kit II )

## 1) Cell general staining if main structures (nucleus, cytoplasm, mitochondria)

## Cell staining

## **CellStain and BacStain solutions**

Following are convenient staining solutions for cytology, histology and other microscopy observations (IC/IF,...). They are chromogenic or fluorescent, stains more or less specifically certains tissues, cell structures, .... See use protocols technical sheete, and more stains in sections corresponding to each application (mitochondria, nuclear staining,...).

-Cellstain- DAPI solution	BE8260, 1ml	
4',6-Diamidino-2-phenylindole, dihydrochloride, aqueous solution; CAS: 2871		
2.9 mM DAPI buffer solution (1 mg DAPI/1 ml) (slightly yellow to yellow liquid		n audam A296 40, 4mm (L)
-Cellstain- AO solution	<b>Ri6430-, 1ml</b>	powder: A386-10, 1mg (L)
3,6-Bis(dimethylamino)acridine, hydrochloride, aqueous solution; CAS: 494-3 Orange or yellow solution 3.3 mM AO (1 mg AO/1 ml water)	30-2 ; 10100: 301.01	
-Cellstain- EB solution	T31440, 1ml	
Ethidium Bromide (M)	See solution 0.625mg/ml #32790A	
-Cellstain- Pl solution	367740. 1ml	powder: A346-10, 1mg (L)
3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium d	,	
Orange to red solution 1.5 mM PI (1 mg/1ml water (M)		10 4(1), 1111. 000.00
-Cellstain- Calcein-AM solution	855425, 1ml	
3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein tel		n CAS: 148504-34-1 (Calcein-AM); MW: 994.86
Colorless liquid 1 mM Calcein-AM in DMSO (1.0 mg/1ml DMSO) (J)		
-Cellstain- CytoRed solution	T30820, 1ml	
7-Isobutyloxycarbonyloxy-3H-phenoxazin-3-one; MW: 313.31 (J)		
1 mM CytoRed DMSO solution (yellowish-orange)		
-Cellstain- Hoechst33258 solution	BD6061, 1ml	
Bisbenzimide, 2'-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-ber 1 mg/ml aqueous solution (yellow solution)	nzimidazole, trihydrochloride, solution; (	CAS: 23491-45-4; MW: 533.88 (L)
-Cellstain- Hoechst33342 solution	BE8270. 1ml	
Bisbenzimide, 2'-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benz	,	AS: 23401 52 3 (free base): MINI: 561 03 (1)
1 mg/ml aqueous solution (Yellow liquid)		A3. 23431-32-3 (liee base), MW. 301.33 (L)
-Cellstain- MitoRed solution	T32840, 50µqx8vials	
9-[2-(4'-Methylcoumarin-7'-oxycarbonyl)phenyl]-3,6-bis(diethylamino)xanthyli	· • • •	
Red purple to purplish-brown solid		
-Cellstain-Double Staining Kit (Calcein & PI) / Live & Dea	ad cells	486301. Kit
To simultaneously stain and observe live and dead cells by microscopy. λex.		



-BacStain- CFDA solution	FO8001, 100 tests
-BacStain- DAPI solution	BE8261, 100 tests
-BacStain- AO solution	RI6431, 100 tests
-BacStain- EB solution	T31441, 100 tests
-BacStain- PI solution	367741, 100 tests
-Bacstain- CTC Rapid Staining Kit (for Flow cytometry) I	FO8010, 100test
-Bacstain- CTC Rapid Staining Kit (for Microscopy)	FO8011, 100tests

Also available:		
-Cellstain- CFSE	-	powder: 294038-A375
5- or 6-(N-Succinimidyloxycarbonyl)-fluorescein 3',6' diacetate; C/	AS:150347-59-4; MW: 557.46 (M)	
-Cellstain- FDA	-	powder: T31520-F209
Fluorescein diacetate; CAS: 596-09-8; MW: 416.38 (M)		
-Cellstain- Rh123	-	powder: 47372-R233
Rhodamine 123, 2-(6-Amino-3-imino-3H-xanthen-9-yl)benzoic aci	id methyl ester, hydrochloride; CAS:62	2669-70-9; CAS: 62669-70-9; MW: 380.82(L)
Red to reddish-brown powder or solid		
-Cellstain- Trypan Blue		powder: T33190-H342
3,3'-{[3,3'-Dimethyl-(1,1'-biphenyl)-4,4'-diyl]bis(azo)}-bis(5-amino-	4-hydroxy-2,7-naphthalenedisulfonic a	acid), tetrasodium salt; CAS:72-57-1; MW: 960.81 (Z)
Blackish brown crystalline powder		
-Cellstain- BCECF	-	powder: 45440Z-B262
3'-O-Acetyl-2',7'-bis(carboxyethyl)-4 or 5-carboxyfluorescein, diac orange or orange-brown crystals, >90%(HPLC)	etoxymethyl ester; CAS:117464-70-7;	; MW: 688.59 (M)

## **Misceaneous Cell staining**

Trypan Blue MW: 960.82; CAS: 72-57-1 (Z)	T33190, 5g		
Trypan Blue Solution 0.4% solution	BX1370, 100ml		
Eosin Y	12504C, 1g		
Eosin Y counter stain	FP-QX7825, 15 ml ; FP-QX7826, 100 ml ; FP-QX7827, 250 ml ; FP-QX7828,500 ml		
fluorescent red dye used to stain cytoplasm, collagen and muscle fiber. Sliv Red Counterstain C	Q69260. 50 ml	can only be mounted with organic mounting mediums.	
provides an alternative for a red counterstain	<b>400-00</b> , <b>00</b>		
Red Aqueous Counterstain	WU1520, 15ml ; WU1521, 5	i0ml	
formulated for staining of protein when NBT/BCIP is used in IHC or <i>in Situ</i> hybridization.			
Stain-All Stain - Protein, DNA, & RNA	JQ6530, 25mg	Q70481, 100mg	

See also Cell Stains for DNA materials (section countertains such a PI/, DAPI, Hoechst, Pyronins, Acridins & Methyl Green, NuclearFastRed, DMAO, Hematoxylin, AAD7,...), as well as for cytosol (Trypan, Eosin,...) and other cell structures (Mitochrondria with NAO,...; Cytoskeletton with Phalloidin,...) or <u>cell tracking</u> (CFDA, Calcein,...).

## Specific Cell stains for anapathology / diseases

Stains RNA iln Purple, DNA in Blue, and Proteins in Red on PAGE Gels

### HistoStain Kits

A.F.B. Stain Kit (T. bacilllus)	BP2890, 100 Test
Alcian Blue PH 1.0 Stain Kit (Mucosubstances)	BP2891, 100 Tests
Alcian Blue PH 2.5 Stain Kit (Mucosubstances)	BP2892, 100 Tests
Alcian Blue-P.A.S Stain Kit	BP3945, 100 Tests
Bielschowskys Modified Stain Kit	BP3947, 100 Tests
BILE Stain Kit	BP3952, 100 Tests
Bodian Stain Kit	BP3948, 100 Tests
Brown & Brenn Modified Stain Kit	BP3946, 100 Tests
C E M Stain Kit (Ecsinophil)	BP3946, 100 Tests
Brown & Brenn Modified Stain Kit	BP3946 , 100 Tests
C.E.M. Stain Kit (Eosinophil)	BP2893, 100 Tests
Colloidal Iron Stain Kit (Mucosubstances)	BP2894, 100 Tests

### • Alzheimer"s Disease Research

### **FBS Solution**

CG2370, 100µl 1-Fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene, 1% w/v DMSO solution; MW: 420.39, C24H17FO6 (L) Pale yellow to yellowish brown liquid; Absorbance: 0.6-0.85 (around 370nm). Technical sheet High affinity with  $\beta\mbox{-sheet}$  structure for high detection sensitivity of Amyloidosis



## Living cell staining: Cell tracers: Cell cytosol markers

Labeling a specific cell population in vitro is a common approach to trace cells in tissues and organisms for survival or redistribution studies. A long term staining is expected, such as with CFSE, or CMTMR. Short term cell follow up can be achieved with the very popular Calcein, or many other dyes. All these are cell cytoplasme or membrane stains described in this section. However some other dyes may be used as cell marker, see next sections 'Staining of cells membranes' and 'Staining of cells nuclei'.

Staining of cells cytoplasm is achieved using many colored or fluorescent stains that can be loaded passively- or not - in cells. Most interesting are stains that cross membranes of viable cells only, but dead cell stains (see vital stains section) are also useful and used in combination. Viable cell stains use mostly groups such as DiAcetate (DA) and AcetoxyMethyl esters (AM) that neutralize electric charges of the dve. prompting membrane permeability, and also decrease the fluorescence of reagent in cell medium. Once internatized in cytoplasm, these groups are clived by intracellular esterases of viable cells, releasing a highly fluorescent compound. The fluorescence can be sensitive to the pH (of cytoplasme, like with the popular BCECF-AM probe). The retention by cells is favored by polyanions such s found in Calcein. Other cell stains can react with amines (CFSE) for a lasting cell staining (use for 'cell tracing'), like with SE esterd (i.e. CFSE) or aminated probes that can be fixed by glutaraldehyde. The CMTMR cell stain conjugate with thiols. Green fluorescent stains are very popular, but stains are available with other colors: orange (CMTMR), far red (DDAO), blue (CMHC)

Staining of cells membranes is achieved using amine reactives dyes, such as Fluoresceins (FITC, FDA-SE) or superior alternatives (FluoProbes488-NHS). More specific markers and polar probes that can be useful to stain cells are described elsewhere. .Staining of cells nuclei is achieved using:

Diacetate derivatives are quite non fluorescent and passively diffuse into cells, then become brightly green fluorescent once they are hydrolyzed by intracellular esterases.

Polyanionic derivates, with calcein as leading dye, are far less sensitive to pH than other fluorescein derivates. This favored their wide usage. especially for short term labelings in studies of cell structure, function, and many other applications.

**D** Chemical fixation of the tracer is achieved, either on amino-bearing dyes (with glutaraldehyde), or using amino reactive derivatives as CFSE. This leads to a long-term cell labeling, that is stable to formaldehyde or glutaraldehyde fixations in IHF techniques.

Several alternatives were proposed to reduce fluorescence quenching of fluorescein based tracers (CFDA), including acidification of the extracellular medium, addition of tryptan blue or an anti-fluorescein antibody.

### **CFDA/CFSE.** Green Cell Tracker

CFDA-SE (also known as CFSE) is the most popularized long term cell tracer in tissues and organisms for survival or redistribution purposes, i.e. during division / embryogenesis studies, migration or moving / cancerogenesis, cell transplantation... This colorless dye passively diffuses into cells where its acetate group is cleaved by intracellulare esterases, and binds to amine groups as a highly fluorescent intracellular label (same spectra that CDCF #FP-46629). It is compatible with subsequent fixation with formaldehyde or glutaraldehyde. CFSE is a better alternative to reduce fluorescence quenching observed with Fluorescein-based tracers, including acidification of the extracellular medium, addition of tryptan blue or an anti-fluorescein antibody. Its amide linkage is also more stable than the thiourea linkage formed by isothiacyanate fluorescent dyes. CFDA-SE is available as the 2 pure isomers for specific applications. Technical sheet

#### CFDA-SE (CFSE, Green Cell Tracker)

FP-52493A 25 mg 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester "mixed isomers"; MW : 557.47; Soluble in DMF, DMSO; (J) λexc./λem. (after hydrolysis, pH 7.0) : 495/519 nm; pKa : 6.4

5-CFDA-SE (5-CFSE) FP-AM496A 10 mg 5-carboxyfluorescein Diacetate Succinimidyl ester . The pure isomer 5 of CFDA-SE FP-52493; (J) 6-CFDA-SE (6- CFSE) FP-AM497A 10 mg 6-carboxyfluorescein Diacetate Succinimidyl ester. The pure isomer 6 of CFDA-SE FP-52493A.; (J) CAS:[150347-59-4]; MW : 557.47; Soluble in DMF, DMSO; (J) λexc./λem. (after hydrolysis, pH 7.0) : 495/519 nm pKa : 6.4

Main use is labeling cells in-vitro, allowing further tracing of cell becoming (during division / embryogenesis studies, during migration or moving / cancerogenesis, cell transplantation...). It is also used for labeling amine containing probes (antibodies, aminoallyl nucleic acids...). The amide linkage formed by the coupling reaction of CFDA-SE is much more stable than the thiourea linkage formed by the coupling of an amine with a isothiocvanate.

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

### **CDCFDA-SE**

Dichloro substitution of CFDA lowers pKa below CFDA's one, thus making it more useful to follow probes in acidic organelles (vacuoles, endosomes...). It gives after hydrolysis the same fluorescence than CFSE #FP52493A and CDCF #FP-46629.

#### **CDCFDA-SE**

FP-52495A, 25 mg

5-(and-6)-carboxy-2',7'-dichlolorofluorescein diacetate succinimidyl ester; MW : 626 .36; Soluble in DMF, MeCN; (J) λexc./λem. (free) : <300 nm/none λexc./λem. (after hydrolysis, pH 7.0) : 495/519 nm; pKa : 6.4 Technical sheet



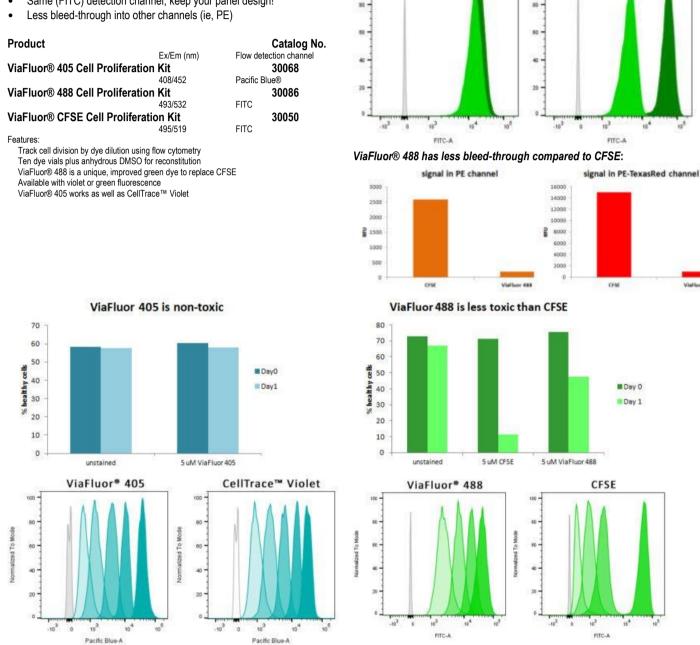
## ViaFluor® SE Cell Proliferation Dyes

## ViaFluor® SE superior alternative to CFSE for Cell Proliferation Assays

CFSE continue to be a popular dye to monitor cell proliferation by flow cytometry. It however has several drawbacks including leakage from the cell, cell toxicity, and bleed-through into the PE and PE-TexasRed® channels.

Reasons to switch from CFSE to ViaFluor® 488

- Less toxic, doesn't inhibit T cell activation
- Better proliferation peaks
- More fixable, less dye leakage
- Same (FITC) detection channel, keep your panel design!



## ViaFluor® 488 improved fixability: does not lose signal after fixation or incubati

CFSE

ViaFluor® 488



### CMTMR/CMFDA/CMAC Cell Tracking dves

These fluorescent dyes freely enters in living cells, where they react mildly with thiol-containing intracellular components and becomes highly fluorescent in cytoplasm at all physiological pH levels (low pKa).

In peculiar, the popular CMTMR localizes essentially in cytoplasm. Fluorescent cells remain viable for at least 24 hours after loading, and it has been shown to be present in several cell generations up to two weeks. It also can be fixed in situ with glutaraldehyde. CMTMR is widely used for long term cell tracing by cytometry, but also in 2 colors imaging microscopy analysis thanks to its stable fluorescence. Technical sheet



## CMAC. Blue Cell Tracking dye

FP-63323A,, 5 mg

7-amino-4-chloromethylcoumarin, Celltracker Blue, CellHunt Blue; CAS: 147963-22-2; MW : 209.63 (M) Soluble in DMSO, DMF, Methanol (M) λexc./λem. (MeOH) : 353 / 466 nm ; EC : 16 000 Mol<sup>-1</sup>cm<sup>-1</sup>.

CMFDA, Green Cell Tracking dye FP-38855A, 1 mg

5-chloromethyllfluorescein diacetate; Celltracker Green, CellHunt Green;CAS: 136832-63-8; MW: 464.86 (M) Aexc./Aem. (hydrolized): 492 / 517 nm; EC: 29 000 Mol<sup>-1</sup>cm<sup>-1</sup>; Soluble in DMSO, DMF, Acetonitrile and Chloroform

See also Sensor dyes for Acidic pH FT-44201A (LysoSensor dyes; 500-ratiom. Green , 550-Green, 600-Red)

### CytoRed, Red Cell Tracer

CytoRed is cell membrane permeable and accumulates inside of viable cells as resorufin. Incubation with esterase at pH 8.0 results in a 80-90 nm shift of emission maxima. CytoRed has a much wider spectrum than BCECF or Calcein, so filters for fluorescein and rhodamine can also be used, whil being 2 times more sensitive in viability assay.

#### CvtoRed solution

T30820. 1ml

7-Isobutyloxycarbonyloxy-3H-phenoxazin-3-one; CAS: 251292-24-7; MW: 313.31 (J) λex./λem.: 560/590nm. Technical sheet

### VFSE, Red Cell Tracking dye

This new tracer is an alternative to CFSE (CFDA-SE) with red emission. It is non-fluorescent until it enters easily (only viable) cells where it is clived and binds to amines (proteins). It excitation/emission wavelenght (398/545nm) are compatible with double labeling with green fluorochromes such as FITC of GFP transfected cells.

ZE7750 inquire

**FP-AK196A** Inquire

**VESE 550** 

MW: 451.86; Soluble in DMSO; (M) λexc./λem. (MeOH) : 398/545 nm . Technical sheet

## DDAO-SE, Far Red Cell Tracking dye

DDAO has pH-dependent red fluorescence with excitation close the He-Ne red laser 633nm

DDAO-SE

MW: 505.34; Soluble in DMSO; (M) λexc./λem. (MeOH) : 398/545 nm **DDAO** (reference standard)

FP-M1367A, 10mg 7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one);MW : 308.17 ; Soluble in DMSO; (M) λexc./λem. (MeOH) : 398/545 nm . Technical sheet

## Calcein, short term Green Cell Tracer

Calcein dye is a polyanionic derivate of fluorescein that exhibits fluorescence that is essentially independent of pH between 6.5 and 12. It is well retained in cells. These features have made it a popular and versatile dye for various applications, including cell volume changes in neurons and other cells, endocytosis, Gap junctional communication, membrane integrity and permeability, angiography, liposomes... It is worthy to notice that calcein is strongly quenched by several ions, including Fe3+, Co2+, Cu2+ and Mn2+ at physiological pH (not by Ca2+ or Mg2+ ions). Ions levels should thus be monitored. Technical sheet

#### Calcein

### FP-466251 100 mg

FP-895514 1 mg

FP-895515 20 x 50 µg

MW : 622.54 ; Soluble in DMSO, DMF or pH >6 water. Membrane impermeant. λexc./λem. (pH 8) : 494/517 nm ; EC : 75 000 M-1cm-1 Can be introduced into cells by microinjection.

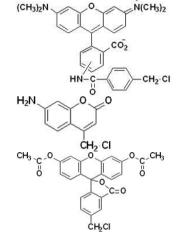
#### Calcein-AM MW · 994 88 (M)

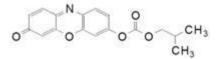
Soluble in DMSO. Enters readily cell membranes. Intracellular esterases converts it into calcein. Aexc./Aem. (hydrolyzed) : see Calcein FP-46625

Contact your local distributor

interbiotech@interchim.com

## InterBioTech, powered by Dinterchim 213 Avenue J.F. Kennedy - BP 1140 03103 Montlucon Cedex - France 161,0470 0388 55 - Fax 04 70 03 82 60





Calcein-AM. 1 mg/ml solution Calcein, AM, 1 mg/ml in DMSO Calcein, AM, 4 mM in DMSO

FP-855422 1 ml @ 1mg/ml This solution in dry DMSO is more convenient (time saving : no solubilization) and increase reproducibility of screening assays. FP-855422, 1 ml FP-FI9820, 100 µl

Calcein Orange -AM MW: 880 (M); λexc./λem.: 525 / 550nm

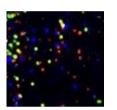
See also Viability kit #876981 (uses Calcein-AM), and Cytotoxicity assay #BF4710 (uses Calcein-AM combined with EthD-III)

FP-ZE7840, 1 mg

## CellVue® irreversible labeling of plasma membranes of live cells

Fluorescent probes for irreversible labeling of the lipid regions of plasma membranes, providing stable labeling of live cells.

- Versatile use with any cell type or bioparticle with a membrane
- Provides stable labeling with minimal transfer from cell to cell
- Provides rapid. uniform membrane labeling
- Combine with fluorescent antibodies or markers of cell function
- Suitable for cell tracking and proliferation studies
- Several colors (UV to NIR) for multi-parameter studies (use with existing fluorochromes for more colors)
- Greater signal to noise with Far-Red and NIR versions (reduced background autof
- · Compatible with flow cytometers, confocal and in vivo imaging equipment
- Convenient, easy-to-use kit format



The CellVue® cell linker kits use proprietary membrane labeling technology to stably incorporate a fluorescent dye with long aliphatic tails into the lipid regions of the cell membrane, see Figure 1. The labeling vehicle provided with the kit (Diluent C) is an iso-osmotic aqueous solution which contains no physiologic salts or buffers, detergents, or organic solvents and is designed to maintain cell viability while maximizing dye solubility and staining efficiency. The pattern of staining is dependent upon the cell type being labeled and the membranes of the cells. Video on how to use CellVue.

Lipid

CELLVUE - BURGUNDY KIT FOR MEMBRANE LABELING KIT (683/707nm) CELLVUE - CLARET KIT FOR MEMBRANE LABELING KIT (655/675nm) CELLVUE - LAVENDER KIT FOR MEMBRANE LABELING KIT (424/461nm) CELLVUE - MAROON KIT FOR MEMBRANE LABELING KIT (647/667nm) CELLVUE - PLUM KIT FOR MEMBRANE LABELING KIT (652/671nm) CELLVUE NIR780 KIT FOR MEMBRANE LABELING KIT (745/776nm) CELLVUE NIR815 KIT FOR MEMBRANE LABELING KIT (786/814nm)

MIDI C-1004-MIDI, 200 µL Kit
C-1005-MIDI, 1 Kit C-1001-MIDI, 1 Kit C-1003-MIDI, 1 Kit C-1006-MIDI, Kit

Cell Tracking with Lipophilic

Membrane Intercalating Dyes

ipophilic tails

Non-covalent labeling

mediated by hydrophobic

interactions between dve

Fluorescent head group

Cytoplasm

MINI C-1004, 100 µL Kit **CLARET-MINI, 1 Kit** C-1005-MINI, 1 Kit C-1001-MINI. 1 Kit C-1003, 1 Kit C-1006, 1 Kit C-1007, 1 Kit

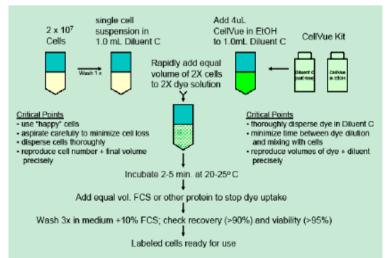
Green Fluorescent Cell Linker Kit for General Cell Membrane Labeling, CellVue Jade C-1009, 100 µL Kit Green Fluorescent Cell Linker Kit for General Cell Membrane Labeling, CellVue Jade C-1009, 2 x 100 µL Kit Green Fluorescent Cell Linker Kit for General Cell Membrane Labeling, CellVue Jade C-1009, 500 µL Kit

Mini Kit, Midi Kits/Maxi Kits contain 0.1ml / 0.2ml / 0.5ml of plus 10/60/60ml diluant #C-1008 The CellVue Fluorescent Cell Linker Kit contains a 1mM dye stock solution and cell labeling diluent.

CellVue® is a trademark of PTI Research

Far Red and Near Infrared Fluorescence benefits:

- · Reduced autofluorescence background
- · Greater signal to noise
- · Greater ability to multiplex with visible probes due to minimal spectral overlap
- · Excellent for use in combination with other



### Others

See also labeled Dextrans section, and cytoplasmic membrane labeling section.

Contact your local distributor



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## Dead cell staining:

## Cell Explorer Fixable Dead Cell Staining Kit *Red Fluorescence Optimized For Flow Cytometry* MycoLight Green JJ98, live/dead bacteria DNA stain ##	UVC610-22599, 200 Tests 24000, 100 ul
Live-or-Dye NucFix Red Staining Kit, red nuclei staining in dead cells (520/610nm) Live-or-Dye NucFix Red Staining Kit, red nuclei staining in dead cells (520/610nm)	32010 , 1 Ki 32010-T , 1 Ki
Yeast Live-or-Dye Fixable Live/Dead Staining Kit ##	31064 , 1 Ki
Live:Dead/Cytotoxicity Assay Kit, Allows Fast And Easy Measurement Of Both Living & Dead cells " Blue/Green Staining,	M1811 , 1 Ki
" Blue/Red Staining	M1810 , 1 Ki
" Green/Blue Staining Sophiagreen 421, Green Fluorescent Dna Stain For Dead Cells	M1812 , 1 Ki M1716, 200 ul
Sophiagreen 471, Green Fluorescent Dna Stain For Dead Cells, 1mm Solution In DMSO	M1714, 200 ul

 $^{+}$ 



## 2) Cell Structures staining:

below sections are: Cell Cytoplasmic membrane staining | Nucleus staining / DNA&RNA staining | Mitochondria staining | CytoSkeletton staining | Organelles staining | other special cell staining

## Cell Membrane staining

The labeling of cytoplasmic membranes can by labeled by Dil, DiO dyes and related carbocyanine dyes, or by cellbrite dyes.

### FluoProbes Dialkylcarbocyanine series dyes: DiO / DiI / DiD / DIR / DiA / DiB

Carbocyanine dyes have hydrophilic/hydrophobic pattern (amphiphilicity), with strongest fluorescence when in membranes. This makes them suitable for many membrane studies (structure, morphology, dynamic, ...), starting with the popular Dil (FP-46804) and DiO (FP-46805).

Several modifications are available, offering color detections from green (DiO) to IR (DiR), and several lengths or saturation degree of hydrophobic tails.

-Fluorescence, that is weak in water, is intense in lipidic environment thanks to high extinction coefficients, modest quantum yields, and short excited-state lifetimes (~1 nanosecond). -Short chain carbocyanines, i.e DiOC6 (available with our FluoCDTM technology) are mainly dedicated to potential measurement.

-Longer alkyl tails (>C12, i.e. DilC18(3)) are best for detection of particularly rigid gel phase. They are used for neuronal tracing long-term labeling of cells in culture and non-covalent labeling of lipoproteins.

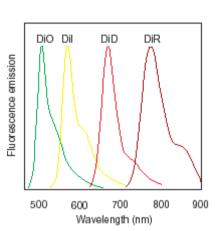
They are used in living and fixed tissues and cells. These dyes insert into the membrane, and diffuse rapidly, staining the entire cell surface. They allow the synaptic terminals tracing in a single motor unit. Dil and DiO are also efficient postmortem neuronal tracers and used in neuroanatomy and visual science (Lukas 1998) Technical sheet

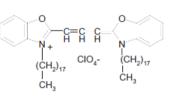
One of the most classic dyes. Can be used with standard fluorescein and rhodamine

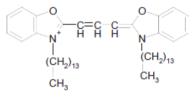
DiO analog with shorter alkyl tails, but more soluble in aqueous media. Staining is

accomplished by simple incubation of cells in the buffer containing the dve. Because the

staining is usually nontoxic and very stable, the dye is useful for long term cells tracing.







HOCH<sub>2</sub>CH<sub>2</sub>SO<sub>3</sub>

**SP-DiOC18(3)** CAS: ; MW:1115.55 (L) Aexc./Aem.: 498 / 514nm; EC: 175 000; Soluble in MetOH / EtOH / DMSO **5,5'-Ph2-DiOC18(3)** CAS: 217199-21-8; MW: 969.91(L) Aexc./Aem.: 496 / 512nm; Soluble in MetOH, DMF DMSO

3,3 -ditetradecyloxacarbocyanine, hydroxyethanesulfonate; CAS: 53213-82-4 C47H74N2O6S MW : 795.19; Store at 4°C Soluble in methanol, ethanol or DMSO

FP-46805A 50 ma

3,3° -dioctadecyloxacarbocyanine, perchlorate; CAS: 34215-57-1 C53H85CIN2O6 MW : 881.73; Store at 4°C

λexc./λem. : 484/501 nm ; EC : 152 000 M<sup>-1</sup>cm<sup>-1</sup>

λexc./λem.: 484 / 501nm; EC: 154 000; Soluble in DMSO

FP-40265A, 10 mg FP-M1610A, 10 mg

FP-46804A, 50 mg

FP-46764A, 100 mg

FP-AM329A 50 mg

DilC18(3) [Dil]

DiOC18(3) [DiO]

Soluble in DMSO or DMF

CAS: 53213-82-4 ; MW: 72.53 (L)

λexc./λem.: 490/515 nm

optical filters. DiOC6(3)

DiOC14(3)

1,1' -dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate C59H97CIN2O4 MW : 933.88; Do not freeze Soluble in DMSO or ethanol

λexc./λem.: 549/565 nm ; EC : 148 000 M-1cm-1

One of the most standard lipophilic dye for ER, Golgi studies. Can be used with standard fluorescein and rhodamine optical filters, and combined to DiO.

Applications : fate mapping and cell lineage studies(1) and for the determination of spatial organization and connectivity patterns of central nervous systems.

References : Afferent and efferent connections of the cerebellum of the chondrostean Acipenser baeri : a carbocyanine dye (Dil) tracing study. Huesa G, Anadon R, Yanez J. J Comp Neurol 460, 327-44 (2003). Honig, M.G. and Hume, R.I. Trends in Neurosci.9, 333(1989) ; McConnell, S.K., et al. Science 245, 978(1989). Ruiz, et al. in essential developmental biology : a practical approach Oxford : IRL Press at Oxford University Pres

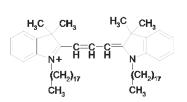
 Ruiz, et al. in essential developmental biology : a practical approach Oxford : IRL Press at Oxford University Press, 81-95 (1993)2) International Journal for Parasitology 28, 363 (1998).

 DilC18(3) [Dil] solution
 FP-AM328A, 0.5 ml

A convenient formulation of Dil (FP46804A) in oil, with uniform dissolution.

Contact your local distributor





P.8

interbiotech@interchim.com

### Catalogue BioSciences - Chap.Cell Biology

DilC1(3)	FP-46853A, 100 mg
CAS: 25470-94-4; MW:484.42 (L)	J
λexc./λem.: 541 / 540nm; Soluble in DMSO, CHCl3, DMF	
DilC1(5)	FP-20920A, 100 mg
CAS: 36536-22-8; MW: 432.25 (L)	
λexc./λem.: 638/ 658nm; Soluble in DMSO, DMF, EtOH	
DilC1(7)	FP-C86280, 100 mg
CAS: 16595-48-5; MW: 509.04 (L)	
λexc./λem.: 740 / 766; Soluble in DMSO, EtOH, CHCl3	ED DT5040 400 mm
<b>DilC5(3)</b> CAS: 53290-46-3; MW: 596.63 (L)	FP-BT5040, 100 mg
CAS: 55290-46-3, ΜW. 596.65 (L) λexc./λem.: 552 / 576nm; Soluble in DMSO, EtOH, DMF	
DilC12(3)	FP-46736A, 100 mg
CAS: 75664-01-6 : MW: 765.56	1 F-40750A, 100 mg
λexc./λem.: 550 / 566nm; EC: 144 000; Soluble in DMSO, DMF, EtOH, CHC	13
DilC16(3)	FP-46746A, 100 mg
CAS: 78566-75-3; MW: 877.77(L)	i i forfort, roo mg
λexc./λem.: 548 / 566nm; EC: 148 000; Soluble in DMSO, EtOH	
DilC18(3) [Dil] (*)	FP-46804A, 50 mg
CAS: 41085-99-8 ; MW: 933.88 (L)	
λexc./λem.: 551 / 566nm. EC: 148 000; Soluble in DMSO, EtOH, CH3CN	
DilC18(3) [Dil], crystalline	FP-162451, 25 mg
CAS: 41085-99-8 ; MW: 933.88 (L)	
λexc./λem.: 551 / 566nm; EC: 148 000; Soluble in DMSO, DMF, EtOH, CHC	
Dilinoleyl Dil Solid	FP-12792A, 5 mg
MW: 1017.97 (L)	
λexc./λem.: 549 / 564nm; EC: 134 600; Soluble in DMSO	
Delta9-Dil	FP-M1280A, 25 mg
MW: 925.49 (L)	
λexc./λem.: 550 / 565nm; Soluble in DMSO, CH3CN, EtOH, DMF	
6,6'-Ph2-DilC18(3)	FP-M1613A, 5 mg
CAS: 217199-28-5; MW: 1022.06 (L) λexc./λem.: 557 / 572; Soluble in DMSO, CHCl3, EtOH, DMF	
DilC18(5) [DiD] 4-chlorobenzenesulfonate salt	FP-22574A, 50 mg
MW: 1052.1 (M)	1 F-22314A, 30 mg
λexc./λem.: 644 / 663nm;EC: 193 000; Soluble in DMSO, EtOH, DMF	
DilC18(5) oil [DiD] perchlorate	FP-929099, 10 mg
CAS: 127274-91-3; MW: 959.9	· · · • • • • • • • • • • • • • • • • •
λexc./λem.: 644 / 665nm; EC: 270 000; Soluble in CHCl3, DMSO, MeOH, ac	etone
DilC18(5) oil [DiD] iodide	FP-DY3330, 25 mg
CAS: 127274-91-3; MW: 987.36	
λexc./λem.: 644 / 665nm; EC: 270 000; Soluble in DMSO	
DilC18(7) [DiR]	FP-69084A, 25 mg
CAS: 100068-60-; MW:1013.43 (M)	
λexc./λem.: 748 / 780(MetOH); EC: 270 000; Soluble in DMSO, EtOH	

#### Neuro-Dil

#### FP-AM330A 25 mg

C67H115IN2 MW : 1075.58; Store at 4°C Soluble in methanol, ethanol, DMF and DMSO. λexc./λem.: 550/565 nm ; EC : (MeOH) : 148 000 M-1cm-1 Diffuses faster than Dil in cells membranes and also may result in a more stable labeling.

#### DilC18(5) [DiD]

FP-22574A 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indodicarbocyanine, 4-chlorobenzenesulfonate salt C67H103CIN2O3S MW : 1052.1; Store at 4°C

Soluble in DMSO or ethanol λexc./λem. : 644/663 nm ; EC : 193 000 M-1cm-1 Similar to DiIC18(3), but excitable with longer wavelength than carbocyanines (He-Ne laser). It is useful when significant intrinsic fluorescence is observed with Dil or DiO.

### DilC18(7) [DiR]

## FP-69084A 25 mg

1,1'-dioctadecyltetramethyl indotricarbocyanine lodide C63H101IN2 MW : 1013.43; Store at 4°C Soluble in DMSO or ethanol λexc./λem. (MetOH) : 748/780 nm ; EC : 270 000 M-1cm-1

Lipophilic carbocyanine similar to Dil and DiO with near IR absorption and emission, allowing lowering the level of autofluorescence. Can be used in multicolor detection, combined to DiD (FP-22574A), Dil (FP-46804A) and Neuro-DiO (FP-AM330A).

### Neuro DiO

### FP-AM331A 25 mg

C67H105CIN2O5S MW : 1086.11; Store at -20°C and protect from light Soluble in ethanol, hexane, DMSO and oil. λexc./λem. : 484/501 nm

Similar features to DiO but with better solubility in membranes, less self quenching by aggregation, and higher diffusion rate in membranes.

NeuroDiO solution, for microinjections

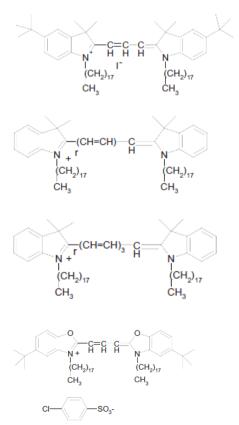
FP-BA641A 0.2 ml

Contact your local distributor

interbiotech@interchim.com

## InterBioTech, powered by





### Do not frezze.

A convenient formulation of NeuroDiO (FP-AM331A) in oil, with uniform dissolution. Applications: microinjection, combined to NeuroDil solution (FP-AM330A).

#### DiA

#### FP-66096A, 25 mg

4-(4-Dihexadecylaminostyryl)-N-methylpyridinium iodide; MW: 787.06 (L) Aexc./Aem.:491 / 613nm; EC: 52 000(MetOH); Soluble in DMSO and EtOH DiA is a green fluorescent membrane dye which diffuses much faster than DiO in cell membranes. DiA and DiI have been used together for two color membrane staining.

#### **DiB** MW: 1074 (L)

FP-YS2860, 10 mg

Aexc./Aem.: / nm; EC: 000(MetOH); Soluble in DMSO DiB is a lipophilic cationically charged blue fluorescent dye useful for staining cytoplasmic

membranes.  $\lambda Ex/\lambda Em$  (MeOH) = 353/442 nm.

### CellBrite cytoplasmic Membrane labeling

CellBrite<sup>™</sup> Blue Cytoplasmic Membrane Labeling Kit is designed to label cell cytoplasmic membranes with blue fluorescence. The labeling is stable and nontoxic, suitable for long-term tracking of cells. The kit includes Biotium's novel blue fluorescent membrane dye DiB (Abs/Em = 360/420 nm) pre-dissolved in an optimized staining solution. Similar to our lipophilic carbocyanine dyes, DiB has long hydrocarbon chains that insert into the lipid bilayer, resulting in stable labeling which is resistant to intercellular dye transfer. By combining multiple CellBrite<sup>™</sup> Cytoplasmic Membrane Stains, one can label multiple cell populations with different colors for studies of cell-cell interactions.

- Simple: Cell staining by direct addition of the supplied dye solution to normal culture media
- Stable Labeling: Highly lipophilic dye ensuring no dye transfer between cells

• Bright, Photostable and Nontoxic: Unique blue fluorescent membrane dye with high quantum yield, high photostability and relatively low toxicity

Cellbrite Cytoplasmic Membrane-Labeling Kits:	
CELLBRITE - BLUE (based on DiB, A/E:360/420nm)	JW7360, 250µl BTM.30024
Contains 250 uL DiB cell labeling solution, 250 uL DiB loading buffer	
CELLBRITE - GREEN (based on NeuroDiO, A/E:484/501nm)	JW7330, 1ml BTM.30021
Contains Neuro-DiO cell-labeling solution. TS/S. Add 5 uL of the cell labeling solution per 1 mL of cell suspension	: Incubate 1-20min 37°C.
CELLBRITE - ORANGE (based on Dil, A/E:549/565nm)	JW7340, 1ml BTM.30022
Contains Neuro-Dil cell-labeling solution. TS/S	
CELLBRITE - RED (based on DiD, A/E:644nm/665nm)	JW7350, 1ml BTM.30023
Contains Neuro-DiD cell-labeling solution. TS/S	

## Other membrane probes

• The **FP Membrane Marker** dyes, that are are cationic styryldyes, less lipophilic than above Carbocyanine dyes DiO/Dil/DiR but still amphilic. They become highly fluorescent once internalized in membranes. They are described in section "Neural Cell Study" as one important application is to follow synaptic activities. They are however very usefull as well for endocytosis of vesicules and vacuoles. See the <u>Technical sheet</u>. The most popular ones are:

Green FP Membrane Marker 1-43 A/E: 480/598 nm in membranes Green FP Membrane Marker 4-64 A/E: 480/598 nm in membranes FP-51254A, 1 mg FP-41109A, 1 mg

• The DialkylAminoStyryl dyes (Di-ASP) also insert in membranes. They are slightly less hydrophobic than above Carbocyanine dyes.

 4-Di-16-ASP [DiA]
 FP-66096A 25 mg

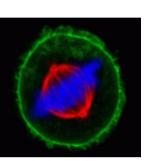
 4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide
 FP-66096A 25 mg

 C46H79IN2 MW : 787.06; Store at 4°C
 Soluble in DMSO or ethanol

 Xexc./Xem. : 491 / 613 nm ; EC : 52 000 M-1cm-1
 Commonly used for neuronal membrane tracing : diffuses faster than DiO.

 Has a very broad emission spectrum (can be detected with green, orange or even red filters), combined notably DilC18(3) for 2 colors staining.





 $H_3C - N^+$   $H_H^- C - C_H^- N[CH_2)_{15}CH_3]_2$ 

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## Lipophilic and Lipids staining

## Lipid stains:

Oil Red O Lipid stain (Sudan 5B)

N13002, 100 g

SUDAN BLACK B stains lipid granules NILE RED

EZE70-M1441, 10 mg

AR7910-BP2915, 100 Tests

a lipophilic dye that stains intracellular lipid droplets to produce a bright red fluorescence

LipidSpot<sup>™</sup> Lipid Droplet Stains: see below (70065 & 70069)

Lipophilic probes:		
ADIFAB, fatty acid indicator	FP-040791, 200µg	FP-040792, 1mg
ADIFAB # is a fluorescent dye that can detect fatty acids. See description i	0,1	
ADIFAB2, fatty acid indicator	FP-BB6681, 200µg	FP-BB6682, 1mg
high affinity version of the original adifab. <u>Technical sheet</u>	ED 400000 400	
1,6-DIPHENYL-1,3,5-HEXATRIENE (DPH) probe for viscosity, polarity and lipid order (350/420nm)	FP-123023, 100 mg	FP-12302B, 1g

FluoProbes 581/591 C11 (Lipid Peroxidation Sensor) FP-M12781, 1 mg

## Lipophilic charged probes:

FP-41391A / FP-EZE520

Tetramethylrhodamine, ethyl ester (TMRE) Positively charged lipophilic red-orange fluorophore; rapidly accumulates in mitochondria due to relative negative charge of active mitochondria with respect to cytosol

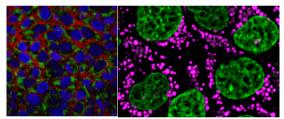
## LipidSpot Lipid Droplet Stains

rapidly stains lipid droplets in live cells or fixed cells, with no wash step required. Available with green or red/far-red fluorescence.

Intracellular lipid droplets are cytoplasmic organelles involved in the storage and regulation of triglycerides and cholesterol esters.

LipidSpot<sup>™</sup> dyes are fluorogenic neutral lipid stains that rapidly accumulate in lipid droplets, where they become brightly fluorescent. The dyes can be used to stain lipid droplets in both live and fixed cells, with no wash step required. Cells also can be fixed and permeabilized after staining. LipidSpot™ stains show minimal background staining of cellular membranes or other organelles, unlike traditional dyes like Nile Red.

LipidSpot™ 488 has excitation around 430 nm, and can be excited equally well at 405 nm or 488 nm. In cells, it stains lipid droplets with bright green fluorescence detectable in the FITC channel. LipidSpot™ 610 has



excitation/emission at ~592/638 nm in cells; it is optimally detected in the Texas Red® channel, but is also bright in the Cy®3 and far-red Cy®5 channels. Therefore, we don't recommend pairing LipidSpot™ 610 with other red or far-red probes.

### Features

- Rapidly and specifically stain lipid droplets •
- Stain live or fixed cells, or fix and permeabilize after staining
- Available with green or red/far-red fluorescence •
- Supplied as 1000X stock solutions in DMSO •

LipidSpot™ Lipid Stains	Abs/Em	Detection channel	Catalog no.	Size
LipidSpot™ 488 Lipid Droplet Stain,	427/585 nm	FITC, GFP	70065-T	20 uL
1000X in DMSO			70065	125 uL
LipidSpot™ 610 Lipid Droplet Stain,	610/663nm (in vegetable oil)	TR TexasRed® or Cy®5	70069-T	20 uL
1000X in DMSO	~592/638nm (in cells)		70069	125 uL

Cv Dve is a registered trademark of GE Healthcare

## Cytoplasm staining

For cytosol staining, see cell tracers section for dyes that accumulate in cytoplasm (CFDA, Calceins), and bind to solutes (CMTMR).



#

## Lipophilic and Lipids Assays

CELL NAVIGATOR FLUORIMETRIC LIPID DROPLET ASSAY KIT, Red Fluorescence AXEYF0-22735, 500 tests

CELL NAVIGATOR FLUORIMETRIC LIPID DROPLET ASSAY KIT, Green Fluorescence AXEMVA-22730, 200 tests

CELL METER PHOSPHATIDYLSERINE APOPTOSIS ASSAY KIT for Microplate Re	eader	
*Blue Fluorescence Optimized for Microplate Reader	AXEYK0-22790	
*Green Fluorescence Optimized for Microplate Reader	JQ8780-22791	
*Red Fluorescence Optimized for Microplate Reader	JQ8790-22792	
*Deep Red Fluorescence Optimized for Microplate Reader	ZE8030-22793	
*Orange Fluorescence Optimized for Microplate Reader	GCZ210-22794	
CELL METER PHOSPHATIDYLSERINE APOPTOSIS ASSAY KIT for Cytometry		
*Green Red Fluorescence Optimized for Cytometry	CJG080-22831	
*Deep Red Fluorescence Optimized for Cytometry	CJG080-22832	
*Red Fluorescence Optimized for Cytometry	CJG090-22831	
*Blue Fluorescence Optimized for Cytometry	CGZ350-22835	
CELL METER PHOSPHATIDYLSERINE APOPTOSIS ASSAY KIT Optimized for Cytometry*	CGZ360-22836	*Blue Fluorescence



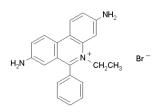
## Cell Nucleus staining / DNA&RNA probes

## Fluorescent DNA/RNA stains / counterstains

### EB, PI and other ethidium based dyes: stains for DNA

• Ethidium Bromide (EB, BEt) does not permeate viable cell membranes. However, it passes through the disrupted membranes of dead cells to stain nucleic DNA. The excitation and emission wavelengths of EB-DNA complex are 518 nm and 605 nm, respectively. Technical sheet

CAUTION: Because of EB and related dyes toxicity as potent mutagens, we offer and recommand to purchase the solutions -rather than more hazardous powdery form-, i.e. in convenient dropper (EB as #32790A), or to choose safer alternatives: see GelRed and EZ-Vision (non mutagenic).



Ethidium Bromide (EB, BET) stain
CAS:1239-45-8; MW: 394.32 (Z)
λexc./λem. (EB-DNA complex): 518/605nm. Technical sheet
Ethidium Bromide (EB, BET) stain, Solution
Ethidium Bromide (EB, BET) stain, Solution
Technical sheet

32790B, 5ml at 0.625mg/ml (dropper) UP89244B, 10ml at 10mg/ml/

FP-06022B, 5q

• Ethidium Homodimer, first developed by Dr. Le Pecq and his colleagues, is a high affinity fluorescent nucleic acid stain. It binds to both DNA and RNA in a sequence-independent manner and with a >30-fold fluorescence enhancement. The DNA binding of each Ethidium Homodimer covers four base pairs and is believed to occur by intercalation. Because the dye is highly positively charged, it can not cross cell membranes to stain living cells. However, it is very useful to detect nucleic acids in solution, or cells with disintegrated cell membranes. Our high purity grade Ethidium Homodimer while other suppliers often haves a high amount (as much as 20%) of inorganic salt that lowers the weight percent purity.

FP-06022A, 5x1g

Ethidium homodimer I (EthD-II. EtDi Ethidium homodimer I solution

FP-258105, 1mg FP-AT758A. 500µl at 1mM in DMSO

CAS:61926-22-5; MW:856.78 (L) λexc./λem. (with DNA) = ~528/617 nm; λexc.-H2O)=493nm; Red solid soluble in DMSO or MeOH. Technical sheet

• Ethidium Homodimer III is an alternative to Ethidium Homodimer I. It has absorption and emission spectra similar to those of Ethidium Homodimer I. However, the dye stains DNA 70% brighter than Ethidium Homodimer I. It is used for Cell Viability/Cytotoxicity Assay for detecting both live and dead cells in the same population of animal cells (see kit #BF4710) and of bacteria (see Kit #BU1040)

Ethidium homodimer III (EthD-III)

Ethidium homodimer III solution

FP-BP9340. 1ma MW: ~1000; λexc./λem. (with DNA) = ~530/~620 nm; Red solid soluble in DMSO, MeOH, or H2O . Technical sheet BP93441, 200µl at 1mM in DMSO

• Propidium Iodide (PI) does not permeate viable cell membranes, but it passes through disturbed cell membranes and stains the nuclei. Pl is often used in combination with green fluorescent compound, such as Calcein-AM or FDA or Annexin V-FluoProbes® 488, for simultaneous staining of viable and dead cells. The excitation and emission wavelengths of PI-DNA complex are 535 nm and 615 nm, respectively. Pl is carcinogenic.

Propidium Iodide (PI) stain Propidium Iodide (PI) stain, Fluopure grade Propidium lodide (PI) stain, Solution

FP-31238B, 100mg FP-31238C, 1g FP-R1345A, 25mg FP-R1345B, 5g FP-31238B, 1ml 10mM FP-36774A, 10ml at 1mg/ml

CH<sub>2</sub>CH<sub>3</sub> -CH<sub>3</sub> CH<sub>2</sub>CH<sub>3</sub>

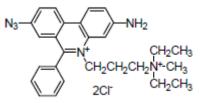
CAS: 25535-16-4; MW: 668.41 (L);  $\lambda_{exc}\lambda_{em}$  (no DNA, water) = 493 / 636 nm EC: 5900;  $\lambda_{exc}\lambda_{em}$  (DNA bound) = 535 / 617 nm EC: 5400. Technical sheet

• Other DNA stains (PMA, EMA, HI)

PMA<sup>™</sup> DNA modification agent Propidium Monoazide CAS: -; MW= 512.51 (M) Soluble in DMSO, DMF at least 5mg/ml λexc. (pH3) = 458 nm λexc./λem (free in water) = 462/625nm (weak) EC: 5 400 M-1 cm-1 λexc./λem (hydrolysed, DNA bound) = 504/600nm PMA<sup>™</sup> DNA modification agent, 20mM in H<sub>2</sub>O

BZ9340, 1 mg





PMA is a cell membrane-impermeable DNA modification dye that can be used to selectively covalently modify DNA from dead cells, rendering the modified DNA unamplifiable. Subsequently, DNA from viable cells can be quantified by qPCR. Thus, PMA can be used in selective detection of viable pathogens in the presence of dead pathogens by qPCR. Technical sheet

EMA, Ethidium Monoazide

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FP-48256A, 5 mg



#### Catalogue BioSciences - Chap.Cell Biology

CAS: 58880-05-0; MW= 420.32 (M) Soluble in DMSO, DMF, Methanol, EtOH and water at least 5mg/ml λexc. (pH3) = 458 nm λexc./λem (free in water) = 462/625nm (weak) EC: 5 400 M-1 cm-1  $\lambda$ exc./ $\lambda$ em (hydrolysed, DNA bound) = 504/600nm Similarly to PMA, EMA selectively and covalently label dead cells in the presence of live cells. Technical sheet

Hexidium lodide (HI) stain, FP-38915A, 5mg CAS:211566-66-4; MW: 497.42 (M) Soluble in DMDO & DMF; λexc./λem.(pH7) = 482/624nm HI can be used to stain dead cells with compromised membranes. Technical sheet

## DAPI (Phenyl Indol based dyes): stains for DNA

DAPI, di-HCI DAPI. di-HCI DAPI, FluoProbes Pure Grade powder fluorescent blue stain for nuclei; Technical sheet DAPI stain (dead cell staining) DAPI solution (diHCl) CAS:28718-90-23: MW:350.25 (M)

λexc./λem. (DAPI-DNA complex): 360/460nm Though DAPI is not permeable through viable cell membranes, it passes through disturbed cell membranes to stain the nucleus. DAPI is utilized for the detection of mitochondrial DNA in yeast, chloroplast DNA, virus DNA, mycoplasm DNA and chromosomal DNA. The excitation and emission wavelengths of DAPI-DNA complex are 360 nm and 460 nm, respectively. DAPI is carcinogenic. Technical sheet

See other vital stains (Trypan Blue, Neutral Red) and formazan dyes (MTT, WSTs.) in section 'Cell Viability'.

#### Hoechst dyes: stains for DNA

Hoechst are bisbenzimides dyes that bind to minor-grooves of DNA (multiple affinity types) with fluorescence enhancement. Fluorescence depends on pH (higher at pH5), and surfactants. Their use as nucleic acid stains has been popularized thanks to their relatively non toxicity (a substitute for DAPI), ability to be excited by most common light sources (i.e. argon/ion laser), and suitability for multicolor imaging (large Stocke's shift). Hoechst 33258 and Hoechst 33342 are popular cell-permeant nuclear counterstain. Both can be used in living cells because they can disrupt DNA replication during cell division. Care should be taken in their handling and disposal (potentially mutagenic and carcinogenic). Hoechst 333342 is relatively more specific for DNA.

37186D, 25mg

FP-371867, 10mg

FP-99963A, 10mg

AP4460, 50tests

BE8260, 1ml at 1mg/ml

Hoechst 33342 emits blue fluorescence when bound to dsDNA. This dye is often used to distinguish condensed pycnotic nuclei in apoptotic cells and for cell-cycle studies in combination with BrdU.

Hoechst 33258 emits blue fluorescence when bound to dsDNA. Its uses are similar to Hoechst 33342 for counterstaining, apoptosis and cell cycle studies, but Hoechst33258 is reportedly less cell-permeant. It also has been used to detect DNA in solution between 250ng/ml to 20µg/ml in solution.

Hoechst33342, TriHydrate	FP-71131A, 100 mg
Bisbenzimide, 2'-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-be	Inzimidazole, trihydrochloride, solution; CAS: 23491-45-4; MW: 533.88 (L) <u>Technical sheet</u>
Hoechst33342, FluoPure	FP-99964A, 100mg
Hoechst33342 solution, 10mg/ml	FP-59046A, 10mL
Hoechst33342 solution, 20mM in water	FP-BB1340, 5mL
Hoechst33258, PentaHydrate Bisbenzimide, 2-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-ben Hoechst33258, FluoPure Hoechst33258 solution, 10mg/ml Hoechst33258 solution, 20mM in water	FP-61248A, 100mg         FP-61248B, 1g           zimidazole, trihydrochloride, solution; CAS: 23491-52-3 (free base); MW: 561.93 (L) <u>Technical sheet</u> FP-P62759, 100mg           FP-41387A, 10mL           FP-BB1330, 5mL

See also CellStain 1mg/ml Hoechst solutions #BD60611 and #BE8270, and Hoechst Cell Proliferation Assays #Q70130 and Q0150

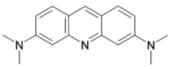
### Acridine based dyes: stains for DNA

A nucleic acid-selective fluorescent cationic dve useful for cell cvcle determination. Acridine Orange (AO) stains dsDNA in green and RNA or single stranded in DNA red. Membranepermeant. Used for cell-cycle studies. Has also been used for the detection of microorganisms in cerebrospinal fluid and other clinical specimens. Highly purified form while most of the other commercially available grades of AO are either in zinc chloride complex form or of low purity. Also

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interbiotech@interchim.com

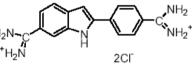


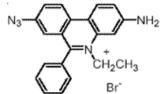


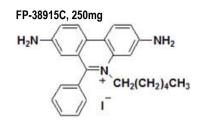
37186F, 500mg

371868, 25mg

AP4461, 2x625TS







Catalogue BioSciences – Chap.Cell Biology		
used for Electrophoresis. Technical sheet		
CAS:10127-02-3; MW : 438.1; Soluble in water, EtOH; (M) λabs./λem.(DNA bound) : 501/526 nm; EC : 53 000 M-1cm-1 λabs./λem.(RNA bound) : 460/650 nm		
Acridine Orange	FP-05920A, 1g FP-05920D 50 g	
Acridine Orange, for electrophoresis CAS:494-38-2; MW: 265.35; CAS:[10127-02-3]; C17H20CIN3 · HCI · 1/2ZnC Soluble 2mg/ml in Ethanol, 4 mg/mL in 2-methoxyethanol (EGME) Acridine Orange Solution, 10mg/ml in water 10mg/ml solution See also CellStain AO solution #RI6430.	05920E, 50g	05920F, 100g
Nile Blue Sulfate       Technica         CAS: 3625-57-8; MW:353.8; λexc\λem = 633 / 672nm (M)       Technica         Nile Red Sulfate       Technica         CAS: 73585-67-3; MW:318.37; λexc\λem = 450-500 / 528nm (M)       Technica         Nile blue sulfate,and Nile blue A, is a cationic dye that can be the gel buffer. At higher concentrations, Nile blue might chait       Technica	<b>FP-46875A, 100mg</b> I sheet be used to visualize DN	IA during electrophoresis. The dye is used both in the gel and in NA and inhibit resolution
Nonyl Acridine Orange (NAO) Acridine Orange 10-nonyl Bromide; CAS: 75168-11-5; MW: 472.52; (M) λabs./λem.(MeOH) : 495/522nm; EC : 63 000 M-1cm-1	FP-58566A, 50 mg	Me <sub>2</sub> N NMe <sub>2</sub> (CH <sub>2</sub> ) <sub>8</sub> Br
Used notably for mitochondria studies; also for phagosome-	lysosome fusion study	, and multidrug resistance. <u>Technical sheet</u>
Hematoxylin, MethylGreen, and Pyronin: stains fo	r DNA	
Pyronin Yellow, UltraPure CAS:92-32-0: MW: 302.81: λmax: 546-551 nm. Note: Pyronin Y is also called	FP-IT2831, 100mg	

CÁS:92-32-0; MW: 302.81; λmax: 546-551 nm. Note: Pyronin Y is also call <b>Pyronin B</b> CAS:[2150-48-3]; MW: 358.91	led Pyronin G, Pyronin J <b>N13070, 10g</b>		
Pyronin G	149794, 50	g	149795, 10g
• Methyl Green Pyronin (MGP) (stains RNA, DNA) Chromogenic RNA/DNA stain.	AP7340, 1	00tests	
Methyl Green, Zn Chloride MW: 653.24	25461A, 5	g	
Methyl Green stain (stains DNA)	P56540, 5	0ml	
•			
Hematoxylin, Immuno/Histo Aqueous stains nuclei in blue. Sides stained with this hematoxylin can be mounted w			441, 100 ml ; FP-WU1442, 250 ml ; FP-WU1443, 1L
Hematoxylin (Natural Black) Nucleic chromogenic stain used for general animal histology.	09192O, 2	5 g	09192P, 100 g
Mayer's Hematoxylin counter stain stains nuclei in blue	82342A, 5	00ml	

• Methylene Blue is a general non toxic but temporary stain for DNA and RNA (do not intercalate). For electrophoresis gels, it can only be used as a post-electrophoretical stain for DNA. It is useful especially for oligonucleotides. It also stains RNA on hybridization membranes in northern blotting to verify the amount of nucleic acid present. It also has many other uses for histological staining (intravital or supravital staining of nerve fibers; in different staining procedures such as Wright's stain and Jenner's stain to différentiate blod cells; indicator to show if bacteria or yeasts are alive or not) but also redox indicator, peroxide generator, MBAS assay for anionic surfactant in water.

Methylene Blue CAS:[61-73-4]MW: 319; Soluble in Water 50g/L, in ethanol at 10g/L; (x)	02284	$H_3C_N$ $CH_3$ $CI^ CH_3$
Methylene Blue, Chloride Trihydrate CAS:[7220-79-3]; MW: 373.90; Soluble in Water, DMSO; (x) Aabs.: 661nm	022843, 100mg	022846, 1kg
Methylene Blue, Staining solution	FP-WYM960, 15ml	FP-WYM960, 100ml





Contact your local distributor

Ask for Methylene Blue formulated products, i.e. Eosins Methylene Blue Agar #A2WQP0 for bacteria cultivation and differentiation. Ask for methylene derivatives, i.e. Benzoyl Leuco Methylene Blue #67647

Catalogue BioSciences - Chap.Cell Biology

Methyl Green counter stain, 1% solution Q69250, 50 ml stains nuclei in blue/gree. provides excellent contrast with DAB detection systems

## Other nucleic acid probes – specialities

AMCH-biotin CAS: 139585-03-8:MW : 331.4: (M)

An aldehyde reactive biotin. Application: detection of abasic sites of DNA (AP sites, depurinated/depyrimidated sites). Less than one abasic site in 104 nucleotides can be detected.

FP-L77845 10 mg

FP-R07565 10 ma

## **Psoralen-PEO-biotin**

MW: 688.8; (L) Labels nucleic acids in one step. Psoralen intercalates between thymine and other pyrimidine containing bases. Labeling occurs by photolysis at 350nm, 10-30min. PEO spacer confers excellent water solubility. DNA//RNA modification does not interfere with hydridization. Technical sheet

See also DNA stains for electrophoresis gels<sup>[PH</sup>, i.e. DNAzure Blue stain:

## Technical tip - DNA/RNA oligonucleotidess staining

[s]

The most effective method to visualize oligonucleotides is gel staining with methylene blue for both DNA and RNA oligos. oligonucleotides can be lectrophoresed in denaturing 7M urea 15% polyacrylamide gels for oligo resolution from 5 mer to over 150 mer, while 10 -12% polyacrylamide gels are recommended for longer oligos. Staining can be particularly useful if the gel is to be preserved or for photographic documentation.

As an analytical tool, staining has limited sensitivity; sequences present in low concentrations may not be visible. Nevertheless, staining is more sensitive than UV shadowing and is considered easier than radiolabeling.

The gel after electrophoresis and removal from the plates, may be soaked in a shallow pan with 0.02% methylene blue in water for approximately 10-15 min. Drain the pan and rinse the gel of all excess, unbound dye for several minutes. De staining with water should be continued till the back ground has almost no stain. Stained o ligonucleotides are visualized as blue bands and may be photographed under ambient light.

Ethidium bromide staining is a well -established technique for visualizing double -stranded DNA fragments. It is however not recommended, because of its toxicity, and because it is no effective or visualizing short, single -stranded DNA fragments such as synthetic oligonucleotides. (poorly intercalates in short intra-strand duplexes)

## Other nucleic acid probes - Fluorescent

AAD (7-aminoactinomycin D)

CAS:7240-37-1; MW : 1270.46; Soluble in DMF, DMSO; (M) λabs./λem. (DNA) : 546/647nm ; EC : 25 000 M-1cm-1

dsDNA intercalator with GC specificity. Weakly membrane permeant. Can penetrate cell membranes of dying or dead cells. The caused binding site distortion changes the pattern of other dyes or enzymes interacting with DNA.

Chromosome banding (polytene chromosomes and chromatin) multicolour fluorescence microscopy (argon-ion laser); Applications : Flow cytometry.

FP-132303, 1mg

Also available as non fluorescent form, Actinomycin D #FP-09086A (used in a similar way). Technical sheet

## ACMA

FP-155822, 25mg CAS: 3548-09-2; MW: 258.71; Soluble in DMSO; (K) λabs./λem.: 412/471nm A DNA intercaltor that selectively binds to AT sequences. Technical sheet

## AdyNA (DNA dyes)

## 2-Aminopurine, DiHydroChloride

CAS: 76124-64-6; MW: ; Soluble in DMSO; (K) λabs./λem.: 370/303nM A cell permeable adenine analog that is widely used as a versatile fluorescent probe to investigate RNA and

DNA secondary and tertiary structures and their conformation dynamics in response to local environment when interacting with other biomolecules with great sensitivity. Forms stable base pairs with nucleobase uracil found in RNA and thymine in DNA, and moderately stable base pairs with cytosine. Exhibits intensive fluorescence emission (IAbsmax = 303 nM, Iflmax = 370 nM, and fluorescence quantum yield in solution QFI = 0.68) and longer excitation wavelength compared with other nucleobases enabling its selective excitation with low background signals

#### DMAO MW: ; λabs/em.(dsDNA): 488/525nm

FP-CA8150, 1ml

2HCI H<sub>2</sub>N

NAB141, 5g

A probe of structural dynamics and charge transfer in DNA.

Inquire

NAB140, 500mg

InterBioTech, powered by Dinterchim 213 Avenue J.F. Kennedy - BP 1140 03103 Montlucon Cedex - France Tél. 04 70 03 88 55 - Fax 04 70 03 82 60 Applications: fluorescence green staining on live bacteria, when combined with dead red stain EthD-III. Technical sheet: see DMAO/EthD III Dead/Live Bacteria Viability Assay Kit <u>BU1040</u>.

### FionaGreen1 dssDNA stain, 1000X

; λabs/em.(dsDNA): xxx/520nm (M)

#### AW3DV0-M1900, 50 µl

FionaGreen<sup>™</sup>1 is a highly sensitive dsDNA binding dye. Upon binding to DNA the spectral properties of the dye are similar to other green intercalating dyes ensuring compatibility with a wide range of instrumentation. It is usefull for several molecular biology applications including qPCR, DNA melt curve analysis, and Isothermal Loop Mediated Amplification (LAMP)

References:

Chandler Y, Koelbl J, Puckett J, Moser MJ, Klingele AJ, Liles MR, Carrias A, Mead DA, Schoenfeld TW. (2014) " A novel thermostable polymerase for RNA and DNA loop-mediated isothermal amplification (LAMP)." Front. Microbiol., 01 August 2014 | doi: 10.3389/fmicb.2014.00395.

Moser MJ, Chander Y, Mead D, Klingele AJ, Schoenfeld T, Mielke C, Shrago A (2013)"One-step rapid molecular assay for the detection of Clostridium difficile" Presented at 29th Clinical Virology Symposium, Daytona Beach, FL

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. (2000)"Loop-mediated isothermal amplification of DNA." Nucleic Acids Res. 28(12):E63

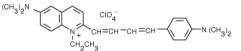
#### Hydroxystilbamidine methane sulfonate

#### FP-40766A 10 mg

Also known as Fluoro-Gold™; MW: 472.54; (M) λabs./λem. (DNA): 360 nm/ca450 nm, ca 625 nm; EC: 27 000 M-1cm-1 Exhibits AT-selective binding/II structure. Complex fluorescent properties allow DNA/RNA distinction. Technical sheet Also used as a neuronal tracer (initial use – see section Neurology).

#### LDS 751 CAS: 181885-68-7; MW: 471.99; Soluble in DMSO/DMF; (M)

FP-97384A 1g



Membrane permeant excited by the argon-ion laser at 488 nm. Stains DNA of live cells. High Stockes shift allows multicolour imaging. Application : analysis of intact nucleated or distinct cell types in mixed populations. <u>Technical sheet</u>

### Nuclear Blue™ DCS1

λabs./λem.(DNA): 543/712 nm λabs./λem. (RNA): 590/607 nm

.λabs/em.(dsDNA): 350/461nm; Soluble in DMSO(M) - TechSheet

#### SNX280-17548, 0.5 ml

Nuclear Blue<sup>™</sup> DCS1 is a fluorogenic, DNA-selective and cell-impermeant dye for analyzing DNA content in dead, fixed or apoptotic cells. The Nuclear Blue<sup>™</sup> DCS1 has its blue fluorescence significantly enhanced upon binding to DNA. It can be used in fluorescence imaging, microplate and flow cytometry applications. This DNA-binding dye might be used for multicolor analysis of dead, fixed or apoptotic cells with the DAPI filter sets. For example, Nuclear Blue<sup>™</sup> DCS1 can be used with GFP cell lines.

Thiazole Orange MW:476.61 (). Technical sheet	FP-24149B, 100mg
Thiazole Orange, FluoProbes Pure Grade	FP-241491, 100mg FP-Fl9531, 10ml
Thiazole Orange, 10mM Solution	FF-F19551, 10111

Stain-All Stain - Protein, DNA, & RNA .stains RNA iln Purple, DNA in Blue, and Proteins in Red on PAGE Gels JQ6531, 25mg

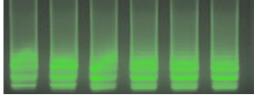
See also Cell Stains for DNA materials (section <u>countertains</u> such a PI/, DAPI, Hoechst, Pyronins, Acridins, NuclearFastRed...), as well as other cell structures.

### Others: see also

- DNA stains for electrophoresis gels<sup>[PH]</sup> i.e. GelRed&GelGreen stains for sensitive and non cancerigen DNA/RNA staining, RNA stain #FJ252.

InterBioTech, powered by

- Nucleic Acids Amplification PCR<sup>I</sup>, for DNA stains for PCRs i.e. Evagreen #BI1790 for RT-PCR).
- Stain-All Stain Protein, DNA, & RNA (JQ6530)
- Specific DNA detections ARP probes for abasic sites, damage,...
- ÷



## **Biochemicals for Nuclear Receptors**

* Nuclear receptors		
BADGE	S03610	25 g
Carbaprostacyclin	300370	1 mg
Ciglitazone	Q87580	1 mg
8(S)-HETE	127350	25 µg
(±)-lbuprofen	Q87360	500 mg
Indomethacin	27155A	1 g
Leukotriene B4	419051	25 µg
MCC-555	J74220	1 mg
Meclofenamate (sodium salt)	Q87420	1 g
MEDICA 16	Q88320	1 mg
Phenethyl Caffeiate	Q87560	10 mg
PPAR.alpha. Blocking Peptide	S00450	1 ea
PPAR.gamma. Blocking Peptide	Q88990	1 ea
Prostaglandin A1	244102	1 mg
Prostaglandin A2	401172	1 mg
Prostaglandin D1	Q84960	1 mg
Prostaglandin D2	366032	1 mg
Prostaglandin I2 (sodium salt)	401162	1 mg
Prostaglandin J2	300400	500 µg
15-deoxydelta.12,14-Prostaglandin J2	861300	1 mg
TO-901317	S03620	5 mg
Trichostatin A	Q88120	500 µg
Wy 14643	789395	5 mg

## **DNA/RNA differential staining**

DNA staining is widely used in fixed/dead cells and in living cells (i.e. with permeant DAPI or the impermeant PI), however used dye are not strictly selective of DNA and stain more or less RNA species. Many attempts have been made to stain distinctively DNA and RNA in cells, i.e. to distinguish G0 phase from G1 phase. Useful dyes include (see description above):

-Hoechst dyes, i.e. Hoechst 333342 #71131A: colorimetric, relatively specific for DNA

-Pyronine Y: colorimetric, relatively specific for RNA

In fixed cells, good DNA/RNA staining can be obtained after a few minutes incubation with 500 nm pyronin Y and 1 ug/ml Hoechst 33342 at room temperature. DNA versus RNA distinction can be precised by RNAse (or DNAse) treatment. However the staining is trickier in living cells, because of dye toxicity and mitochondria staining.

## Anti nucleic acid antibodies (probes)

+

See PrimAbs search engine- over 500 000 primary antibodies, including many specificities for the staining and research of cell nuclei (anti DNA damage, and transcription factors, histones,...).



## Mitochondria staining and probes

Stains and probes for mitochondria study are listed below. Most popular are classic ones as Rhodamine123, NAO, or new superior ones such as MitoRed and MitoVue (green). They probe typically the membrane potential (i.e. resazurin redox indicator), the mitochondrial-DNA material (i.e. NAO), or distribute preferentially in mitochondrial membrane (MitoRed, TMRE,Rh123). They are used in cell structure studies, for oxidative metabolism or apoptosis applications.

See also the sections Study of Mitochondry (kits), study of Oxidative Metabolism<sup>[BE100a]</sup>, and above membranes staining.

## Mitochondria stains

### MitoRed Viable Cell Stain

T32840, 8x50µg

9-[2-(4'-Methylcoumarin-7'-oxycarbonyl)phenyl]-3,6-bis(diethylamino)xanthylium chloride; MW: 637.18(L) Axec./em.(oxidized): 560 nm / 580 nm

MitoRed is a cell membrane permeable rhodamine-based dye. It localizes in mitochondria and emits red fluorescence. The interaction of MitoRed with mitochondria depends on the membrane potential of the mitochondria. Mitochondria can be stained with 20 to 200 nM MitoRed. The excitation and emission wavelengths of MitoRed are 560 nm and 580 nm, respectively. See <u>BE030d</u> for more info.

### Resazurine, sodium salt

Rezasuring sodium salt; MW: 251.18; CAS:[62758-13-8] Soluble in water

## FP-06224A, 10mg

A blue dye used mainly as an oxidation-reduction indicator for cell viability in bacteria and mammalian cells. <u>Technical sheet</u> Also available Resazurine (CAS:[550-82-3]; MW: 229.19) #08062, and in ready solution for cell viability assay (see UptiBlue #UP664162).

## Rhodamine B, hexyl ester perchlorate [R 6]

FP-89216A, 10mg

FP-17218A, 1mg

CAS: 877933-92-1; MW: 627.17; C34H43CIN2O7 (M) Nontoxic fluorescent cell permeant probe for mitochondria staining. Technical sheet

#### MitoVue Green

5,6-dichloro-2-(3-(3-methylbenzo[d]oxazol-2(3H)-ylidene)prop-1-en-1-yl)-1,3-bis(4-methylbenzyl)-1Hbenzo[d]imidazol-3-ium chloride (Z) [Known as Mitofluor® Green, TM of Mol ecular Pr obes,MitoHunt Green TM of SEH] Amax: 489 ± 3 nm in Methanol; EC: 125,000 ± 9,000 cm-1 M-1; λEm: 515 ± 4 nm in Methanol; Soluble in DMSO, DMF, Methanol; Red solid. Technical sheet

#### Nonyl Acridine Orange (NAO)

Acridine Orange 10-nonyl Bromide; CAS:[75168-11-5]; MW: 472.52; (M) Abs./Aem.(MeOH) : 495/522nm; EC : 63 000 M-1cm-1

A DNA stain used notably for mitochondria studies, being not dependent on mitochondrial membrane potential unlike JC-1 and Rhodamine 123; also for phagosome-lysosome fusion study, and multidrug resistance. <u>Technical sheet</u>

#### **Rhodamine 123**

FP-47372A, 50mg

FP-58566A, 50 mg

6-Amino-3-imino-3H-xanthen-9-yl)benzoic acid methyl ester, hydrochloride; CAS: 62669-70-9; MW: 380.82 (L) Red to reddish-brown powder or solid

Rhodamine 123 (Rh123) is cell-membrane permeable and localizes in mitochondria of viable cells to emit yellowish-green fluorescence. Rh123 is utilized for staining a wide variety of cells, including plant cells and bacteria. Since there is a correlation between the amount of ATP in a cell and the fluorescence intensity of Rh123, this compound is used for the detection of intracellular ATP. Rh123 is also used in cancer research. <u>Technical sheet</u>

See also: Dihydrorhodamine 123 (FP-83775A)

This reduced form of rhodamine 123 is useful for detecting reactive oxygen species: it readily enters most of the cells and is oxidized by oxidative species or by cellular redox systems to the fluorescent rhodamine 123 that accumulates in mitochondrial membrane. See description in section Oxidative metabolism / ROS probes or in the Technical sheet

FP-41391A / FP-EZE520

### Tetramethylrhodamine, ethyl ester (TMRE)

Positively charged lipophilic red-orange fluorophore; rapidly accumulates in mitochondria due to relative negative charge of active mitochondria with respect to cytosol

## MitoFlo Mitochondria Membrane Potential detection for Flow Cytometry

MitoFlow assay is a easy to use one color assay for Flow Cytometry to visualize mitochondrial membrane potential of cells. It utilizes the MitoFlo dye, a cell permeable cationic dye that has a strong fluorescent signal and exhibits low membrane potential independent (non specific) binding and toxicity

BENEFITS: Cell Permeable | Exc.488nm/Em.FL2 channel | Can be used with both suspension and monolayer adherent cell lines | Adaptable for High troughput format | Compatible with fluorescent protein expression vectors

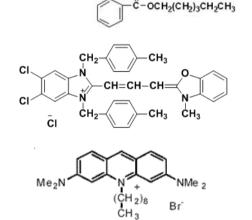
## •Other reagents for Mitochondria study

Other mitochondria probes (redox potential, pH, membrane,...) and assay kits on line[527, 530] or on inquire. +

Contact your local distributor

interbiotech@interchim.com





N(CH2CH3)2

CIO4

(CH3CH2)2N

FI 0200, 100tests

FL O200, 500tests

## CytoSkeletton probes

## Phalloidin

Phalloidin and ist derivatives with over 30 labels (biotinylated, fluorescent) are popular probe for F-actin detection, used in various analysis techniques (microscopy, FCM...). It allows to detect, identify, quantitate and stabilize F-actin in fixed and permeabilized tissue sections, cell cultures or cell free experiments. Phalloidin bind to a site at which few actin-binding proteins bind. So most of the F-actin in cells is available for phalloidin labeling. These properties make phalloidin more attractive than actin specific antibodies for fluorescence microscopy, giving high contracts staining. Technical sheet.

Phalloidin Bicyclic(Ala-DThr-Cys-cis-4-hydroxy-Pro-Ala-2-mercapto-Trp-4,5-dihydroxy- Phalloidin – FluoProbes® FITC	FP-547012, 1mg <sup>Leu)(S-3→6)</sup> FP-47548A 300 tests
FITC (494/518nm) with extended spacer Phalloidin-FluoProbes® 505 FP 505 is used alternatively to FITC, but with the stability of rhodamine *	FP-AZ0130, 300U
Phalloidin-FluoProbes® 547 FP547 (557/574nm) is used alternatively to Rhodamines, Cv3, AF546 *	FP-AZ0330, 300U
PP347 (557/574nm) is used alternatively to Rhodamines, Cy3, AF346 * Phalloidin-FluoProbes® 647 FP647 (652/673nm) is used alternatively to AF647 and Cy5 *	FP-BA0320, ,300U
Phalloidin-FluoProbes® 682 FP682 (679/702nm) is used alternatively to Cy7 *	FP- BG0480,300U
Phalloidin-SR101 SulfoRhodamine101 label*	FP-033991, 300U
Phalloidin- Rhodamine	FP-475741, 300U
over 30 available labels – Phalloidin conjugates Please inquire for other dyes	see in the <u>Technical sheet</u>

See more information on dyes in section of FluoProbes or conventional labeling agents..

## Tubulin probes (AnnexinV)

AnnexinV detects apoptotic cells in the early step, recognizes the cell surface-exposed phosphatidylserine (PS) that is translocated from plasma membrane surface. Annexin V and binds with high affinity to it, before the dying cell changes morphology and cleaves its DNA (Vermes 1995). Apoptotic cells are stained with a fluorescent conjugate of Annexin-V by a simple and quick one-step staining procedure. No fixation of the cells and no washing procedures are necessary. The stained cells can be measured by flow cytometry, microscopy or other fluorescent based instruments. AnnexinV is available labeld with various conventionnal fluorochromes, and biotin. <u>Technical sheet</u>

Annexin V-FluoProbes® 488, for FCM , for Confocal Microscopy	FP-BH9390 100 tests FP-BH4140 500 µl
FP488 (494/519nm) is a superior alternative to FITC and other gren dyes * <b>Annexin V-FITC</b> FITC(494/518nm) *	FP-M19651 500 µl
Annexin V-RPE R-PhycoErythrin (496.546.565/578nm) *	FP-AH191A 50 tests
Annexin V-APC APC (650/660nm)	FP-AK194A 100 tests
Tubulin-Tetramethylrhodamine Tubulin from bovine brain conjugated to TetraMethyl Rhodamine.	FP-966921 5x20 µg
Annexin V-Biotin Biotin is detected wit (strept)avidin conjugates	FP-FX8470 100 µl

See more information on dyes in section of FluoProbes or conventional labeling agents...

Related products:AnnexinV 10x binding bufferAnti-Annexin V-FITCR51380, 100 tests



## 3/Special probes for cell bioloby

## ExtraCellular Matrix (ECM)

+

See <u>Cell Adhesion catalog</u> e.g. <u>Biocolor cellular adhesion compounds assays (collagen, fastin, elastin, sGAG)</u> <u>SHU23e, List</u>

## Anti cytoskeletton and celular matrices antibodies

See PrimAbs chapter D – over 130 000 primary antibodies.



## Acidic organelles probes

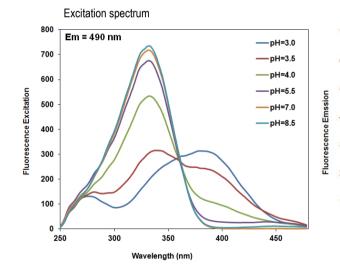
The existing pH probes are ill-adapted to study acidic organelles because their fluorescence is significantly reduced at lower pH. In addition, most of the existing pH probes (such as BCECF and SNARF) are not selectively localized in acidic organelles. Following are efficient tracking dyes for these applications.

The Sensor dyes are acidotropic pH probe that selectively labels acidic organelles such as lysosomes, endosomes, exosomes, spermatozoa and acrosomes. They demonstrate pH-dependent fluorescence, but unlike most of the existing fluorescent dyes, their fluorescence intensity dramatically increases as pH decreases from neutral to acid. The lack of fluorescence outside the cell eliminates the wash steps. This enables, together its bright fluorescence in acidic compartments, the specific detection of cellular acidic compartments with reduced signal variability and improved accuracy for imaging or flow applications. 3 colors are available: 450/540Ratiometric Yellow/Blue (PDMPO, DNP-160, 500-Green and 600-Red (Lys oSen sor dyes)

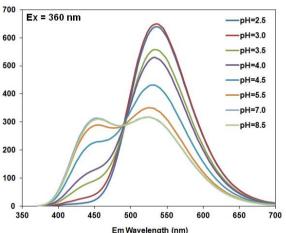
In peculiar, PDMPO has dual-excitation and dual-emission and emits intense yellow fluorescence at lower pH to intense blue fluorescence at higher pH. This unique pH-dependent fluorescence makes PDMPO an ideal pH probe for acidic organelles with pKa = 4.2-4.5, to monitor the pH fluctuations of live cells in ratio measurements. Additionally, its very large Stokes shift and excellent photostability make PDMPO an excellent fluorescence acidotropic reagent for fluorescence imaging. PDMPO can be well excited by the violet laser at 405 nm for flow cytometric applications.

PDMPO is available as a dextran conjugate, and other conjugates can be prepared using the Succinimidyl Ester (SE) derivate. Bioconjugates allow for specific detection of phagocytosis and endocytosis with reduced signal variability and improved accuracy, and for multiplexing cell functional analysis with complementar colors.

Cat #		Probe	Abs * (nm)	Em * (nm)	рКа
FP-44201A		Acidic pH Sensor 450/540Ratiometric,	384 to 329*	540 to 440*	3.9
		Yellow/Blue PDMPO, DNP-160			
FP-022900		(SE ester)			
FP-024010		(Dextran 10K)			
FP-JQ7870		Acidic pH Sensor 500, Green	443	505	ND
FP-JQ7880		(SE ester)			
FP-JQ7890		(Dextran complex)			
FP-GCZ160		Acidic pH Sensor 600, Red	575	597	ND
FP-GDA090		(SE ester)			
FP-GDA090		(Latex beads)			
	See also	Acidic-pH <i>Tracking</i> dyes <u>FT-97578A</u>			
		(Lys o Tra cker dyes: Blue DND-122, Green DND26,			
		Yellow HCK123, Red DND99)			

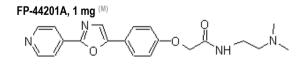


### Emission spectrum (with exc.@360nm)



FP-JQ7870, 1 mg

FP-GCZ160, 1 mg



Soluble in DMSO: A<sub>exc</sub>W<sub>em</sub> (MeOH) = 405 / 550 nm - Use ratio: Ex= 360 nm, and Em= 450 and 540 nm Also available as:

Lvs oSen sor ™Yellow/Blue DNP160. RatioWorks™ PDMPO: MW= 366.42

-Succinimidyl ester (SE, FP-022900): amine reactive

-Dextran conjugate (FP-024010: ~10K, soluble/water)

#### Sensor 500-ratiom. Yellow/Blue for Acidic pH - PDMPO, DNP-160

MW= 398.46; λ<sub>exc</sub>\λ<sub>em</sub> (MeOH) = 443 / 505 nm Also available as: -Succinimidyl ester (SE, FP-JQ7880); MW: 539.54

-Dextran complex (FP-JQ7890)

Sensor 450/540-ratiom. Yellow/Blue for Acidic pH - PDMPO, DNP-160

2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl)methoxy) phenyl)oxazole; CAS:

Sensor 600-ratiom. Yellow/Blue for Acidic pH – PDMPO, DNP-160 MW= 984.03; Aexc\Aem (MeOH) = 575 / 597 nm

Also available as:

-Succinimidyl ester (SE, FP-GDA080); MW:953.06 Contact your local distributor -Latex Beads conjugate (FP-GDA090) InterBioTech, powered by



interbiotech@interchim.com

## Lysosyme assay

Lysosomes are acidic cytoplasmic organelles that are present in all nucleated mammalian cells. Lysosomes have been found to be involved in a variety of cellular processes including repair of the plasma membrane, defense against pathogens, cholesterol homeostasis, bone remodeling, metabolism, apoptosis, and cell signaling. Defects in lysosomal enzyme activity have been associated with a variety of diseases including Parkinson's, Tay-Sachs, Sandhoff, Krabbe, Wolman, and Gaucher syndromes.

#### LysoLive™ Lysosomal Metabolic Health Assay Kit M1910, 1Kit

Utilizes a sensitive substrate for esterase to stain only actively metabolizing lysosomes. The kit allows for labeling lysosomes in a live-cell format and is capable of monitoring lysosomal metabolic activity.

References Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, Golland P, Sabatini DM (2006) "CellProfiler: image analysis software for identifying and quantifying cell phenotypes." Genome Biology 7:R100.

Michihara A, Toda K, Kubo T, Fujiwara Y, Akasaki K, Tsuji H. (2005) "Disruptive effectof chloroquine on lysosomes in cultured rat hepatocytes." Biol Pharm Bull. 28(6):947-51. Karageorgos LE, Isaac EL, Brooks DA, Ravenscroft EM, Davey R, Hopwood JJ, Meikle PJ (1997) "Lysosomal Biogenesis in Lysosomal Storage Disorders" Experimental Cell Research 234, 85-97.

#### SophiaGreen<sup>™</sup> 421

M1931, 50µl

M2018, 1Kit

Excellent green membrane-impermeant counterstain for DNA and chromatin, providing high increase in fluorescence upon binding to nucleic acid membrane impermeant, does not stain live cells

## RE / Golgi study

Fluorescent Golgi Labeling Kit

Convenient kit for labeling Golgi apparatus using fluorescence microscopy. Technical sheet.

## ■ Labeled Dextrans (Fluorescent, Biotin)

Inquire / in progress

## Lectins

Biotin Lectin -ConA	FP-MS9690, mg	FT-MS969.0.pdf
Canavalia Ensiformis Lectin (Jackbean) is specific of gD-Mannos	se, aD-Glucose, Branched mannose.	-
WGA Lectin -SR101	FP-MS9540, mg	FT-MS954.0.doc
Wheat Germ Agglutinin (WGA) is specific of (GlcNAc-β-(1,4)-Gl	cNAc) <sub>1-4</sub> >β-GlcNAc>Neu5Ac.	
GS-I Lectin - FITC	FP-MS9020, 2mg	FT-MS9020.pdf

Pure Griffonia simplicifolia lectin is specific for Melibiose, a-D-Galactose (calcium is required); Working range: 20-30µg/ml (blood cells agglutination)

Please inquire at Fluoprobes@fluoprobes.com for other lectins, conjugated to Biotin, FITC, or AP (Alckaline Phosphatase):

Please inquire at <u>Fluoprobes@fluoprobes.com</u> for oth	er lectins, conjug	ated to Biotin, FITC, or AP (Alckaline Phosphatase):	
Abrus Precatorius Lectin (Jequirity Bean)	-APA-	Lens Culinaris Lectin (Lentil)	-LCH-
Aegopodium Podagraria Lectin (Ground Elder)	-APP-	Limax Flavus Lectin (Garden Slug)	-LFA-
Agaricus Bisporus Lectin (Mushroom)	-ABA-	Limulus Polyphemus Lectin (Horseshoe Crab)	-LPA-
Allium Sativum Lectin (Garlic)	-ASA-	Lotus Tetragonolobus Lectin (Asparagus Pea)	-LOTUS-
Anguilla Anguilla Lectin (Fresh Water Eel)	-AAA-	Lris Hybrid Lectin (Dutch Iris)	-IRA-
Arachis Hypogaea Lectin (Peanut)	-PNA-	Lycopersicon Esculentum Lectin (Tomato)	-LEA-
Artocarpus Integrifolia Lectin (Jackfruit) – Jacalin		Maackia Amurensis Lectin	-MAA-
Bauhinia Purpurea Lectin (Camel's Foot Tree)	-BPA-	Maclura Pomifera Lectin (Osage Orange)	-MPA-
Bauhinia Purpurea Lectin	-BPA-	Marasmium Oreades Agglutinin Lectin (Mushroom)	-MOA-
Bryonia Dioica Lectin (White Bryony)	-BDA-	Morniga G Lectin (Black Mulberry)	-MNA-G-
Calystega Sepiem Lectin (Hedge Bindweed Rhizomes)	-CALSEPA-	Morniga M Lectin (Black Mulberry)	-MNA-M-
Canavalia Ensiformis Lectin (Jackbean)	-CON A-	Narcissus Pseudonarcissus Lectin (Daffodil)	-NPA-
Cancer Antennarius Lectin (California Crab)	-CCA-	Phaseolus Lunatus Lectin (Lima Bean)	-LBA-
Caragana Arborescens Lectin (Pea Tree)	-CAA-	Phaseolus Vulgaris Lectin (Red Kidney Bean)	-PHA-E-
Cicer Arietinum Lectin (Chick Pea)	-CPA-	Phaseolus Vulgaris Lectin (Red Kidney Bean)	-PHA-L-
Colchicum Autumnale Lectin (Meadow Saffron)	-CA-	Phytolacca Americana Lectin (Pokeweed)	-PWM-
Cytisus Sessilifolius Lectin (Portugal Broom)	-CSA-	Pisum Sativum Lectin (Garden Pea)	-PEA-
Datura Stramonium Lectin (Jimson Weed)	-DSA-	Polygonatum Mulitiflorum Lectin (Commom Solomon's Seal)	-PMA-
Dioclea Grandiflora Lectin (Legume)	-DGL-	Polyporus Squamosus Lectin (Mushroom)	-PSL-
Dolichos Biflorus Lectin (Horse Gram)	-DBA-	Ricinus Communis Lectin (Castor Bean)	-RCA-I-
Erythrina Cristagalli Lectin (Coral Tree)	-ECA-	Ricinus Communis Lectin (Castor Bean)	-RCA-II-
Euonymus Europaeus Lectin (Spindle Tree)	-EEA-	Triticum Vulgare Lectin (Wheat Germ)	-WGA-
Galanthus Nivalis Lectin (Snowdrop Bulb)	-GNA-	Tulipa Sp. Lectin (Tulip)	-TL-
Glechoma Hederacea Lectin (Ground Ivy)	-GHA-	Ulex Europaeus Lectin (Gorse)	
Glycine Max Lectin (Soybean)	-SBA-	Ulex Europaeus Lectin (Gorse)	
Griffonia Simplicifolia Lectin	-GS-I-	Urtica Dioica Lectin (Stinging Nettle)	-UDA-
Griffonia Simplicifolia Lectin	-GS-II-	Vicia Fava Lectin (Fava Bean)	-VFA-
Helix Aspersa Lectin (Garden Snail)	-HAA-	Vicia Villosa Lectin (Hairy Vetch)	-VVA-
Helix Pomatia Lectin (Edible Snail)	-HPA-	Viscum Album Lectin (Mistletoe)	-VAA-
Hippeastrum Hybrid Lectin (Amaryllis)	-HHA-	Wisteria Floribunda Lectin (Japanese Wisteria)	-WFA-

Contact your local distributor



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## Lysochrome dyes (Sudan dyes, Oil red)

#### A lysochrome is a fat soluble dye that have high affinity to fats, therefore are used for biochemical staining of triglycerides, fatty acids, and lipoproteins. They also may be useful for staining lipoproteins, and other lipids. Some examples are Sudan IV. Oil Red O and Amido Black. Technical sheet

#### Sudan IV

N13862, 100g

Sudan R, Lipid Crimson, Oil Red, Oil Red BB, Fat Red B, Oil Red IV, Scarlet Red, Scarlet Red N.F, Scarlet Red Scharlach, Scarlet R; CAS: [85-83-6]; MW: 380.45 λabs = 513-529 nm (red); Soluble in EtOH (0.09%). (Z)

Sudan IV is a diazo dye used for the staining of lipids, triglycerides and lipoproteins on frozen paraffin sections.

#### Sudan III

08002A, 25g Other names: Rouge Sudan ; rouge Ceresin ; CI 26100; CI Solvent Red 23; CAS:[85-86-9]; MW: 352.40

λabs = 503-510 nm (red); Soluble in EtOH (0.15%). (Z)

Sudan III is similarly to Sudan IV. Applications include staining lipidic inclusions or fat globules in histological section, and the determination the level of fecal fat to diagnose steatorrhea. It is less popular than oil red O as it has a more orange shade.

#### Sudan Black B

Sudan Black; Fat Black HB; Solvent Black 3; C.I. 26150; CAS: [4197-25-5]; MW: 456.54  $\lambda abs = 596-605nm$  (blue-black). (Z)

Sudan Black B is a diazo dye used for staining (blue-black) neutral triglycerides and lipids on frozen sections and some lipoproteins on paraffin sections. It can be used to stain some other materials than the other Sudan dyes, as it is not so specific to lipids. In differentiating haematological disorders Sudan black will stain myeloblasts but not lymphoblasts.

AR7910, 100tests stain for lipids granules

#### Oil Red O

N13002, 100g

279040, 25g

Solvent Red 27, Sudan Red 5B, C.I. 26125; CAS: [1320-06-5 ]; MW: 408.51

λabs = 518(359) nm (red); Soluble in EtOH (moderate) (Z)

Oil Red O is a diazo dye used for staining of neutral triglycerides and lipids on frozen sections and some lipoproteins on paraffin sections. It has maximum absorption at 518(359) nm. Oil Red O provides much deeper red color than Sudan III and Sudan IV hence replace them for more visible image

## Enzymes probes

See also section Enzymes assays and sections dedicated to each cell biology application in which the enzyme is involved (i.e. Apoptosis section for capases, Reporter assays for  $\beta$ -Galactosidase,...)

#### Fluorescent labeled Glucosides

cat.number	CAS	Product
M1312, 5mg		Resorufin maltotriose
		α-amylase substrate with red emission
M1340, 5mg		Resorufin-α-D-mannopyranoside
, 0		Red fluorogenic substrate for monitoring intracellular mannosidase activity
M1940, 50mg.	242-736-7	4-Methylumbelliferyl β-D-glucopyranoside
		Fluorescent substrate for analysis of beta-glucosidase activity, exhibits bright blue fluorescence
M0095, 10mg	5894-59-7	alpha-DGalU(1-4) DGalU, Digalacturonic Acid
M0203, 10mg	95079-19-9	Resorufin beta-D-galactopyranoside (Res-Gal)
Ŭ		Long Wavelength (Red), Stable Fluorescent beta-Galactosidase Substrate

## Counter staining, AutoFluorescence quenching

See following product lines: BE144q TrueBlack Lipofuscin Autofluorescence Quencher (superior alternative to Sudan Black B)

