

Probes for mitochondria and ER

Mitochondria assumes in cells first energy production through oxidative phosphorylation (OxPhos) and lipid oxidation, but also other metabolic pathways (urea production, heme, non-heme iron and steroid biogenesis), making their study important in many applications, including apoptosis. We provide several fluorescent tools for visualization of mitochondria morphology, localization and abundance, typically by microscopy, and to study biochemical activity (measurement of potential, as well as reactive oxygen) typically by cytometry. Unlike mitochondria or ER specific antibodies, they can be used in living cells without interfering with their functions.

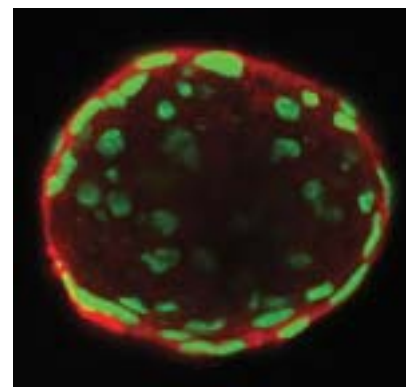
Rhodamine 123 probe has been popularized for mitochondria, but several new rhodamines and carbocyanines have been shown more interesting in several applications.

Our selection of most useful probes for mitochondria visualization, includes :

- ◆ Potential independent distribution dye for fixed slides, as **nonyl acridine orange**,
- ◆ pH, potential or oxygen reactive probes, especially the **JC-1** and **TMRE** for living cells,
- ◆ **DASPEI** for mitochondria potential dynamic activity.
- ◆ The amphiphilic probe DiOC₆ that FluoProbes® is proud to introduce formulated with our superior technology, **DiOC₆ FluoCD™**.



Due to light diffraction limit, and out-of-focus light, fluorescence detection may be insufficiently resolvable for microscopy analysis of fine structures as Mitochondria and ER, even in confocal microscopy. Resolution might be improved by several mathematical algorithms to deconvolute images (Ultramicroscopy 2001, 87, 155).

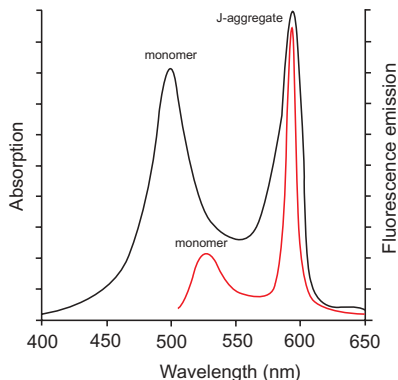


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Membrane potential probes for mitochondria (JC-1, DiOC, DASPEI, TMRE, TMRM)

Potential probes are useful for studies of mitochondry activity, notably energy production. This apply to living cell samples typically by cytometry or in microplate cell assays.



Absorption and fluorescence emission (excited at 488 nm) spectra of JC-1 in pH 8.2 buffer containing 1 % (v/v) DMSO.

JC-1

A useful probe for mitochondria potential studies, notably for cell viability and apoptosis.

JC-1 monomer is in equilibrium with so-called J-aggregates, which are favored at concentrations above 0.1 μM in aqueous solutions (pH 8.0) or higher membrane potential.

JC-1 allows sensitive measurements of membrane potential ratioing the green-fluorescent JC-1 monomer (abs/em. maxima $\sim 510/527$ nm in water) and the red J-aggregate that has a broad excitation spectrum (max at 585 nm) and an emission maximum at 590 nm. One can detect both forms either simultaneously, or separately. The red to green fluorescence ratio is only dependent on the membrane potential, and compared to other dyes giving single-component fluorescence signals, is not affected by mitochondrial size, shape and density. Applications are very wide as shown by the numerous literatures, combined or not with other probes.

Smiley, S.T., et al. Proc. Natl. Acad. Sci. **88**, 3671(1991)Reers, M., et al. Biochemistry **30**, 4480(1991).

abs em. JC-1

$\text{C}_{25}\text{H}_{27}\text{Cl}_4\text{N}_4$ MW : 652.24

Soluble in DMF, DMSO

Store at 4°C

$\lambda_{\text{exc.}}/\lambda_{\text{em.}}$: 514/529 nm (mon.), 590 nm (aggr.) ; EC : 190 000 $\text{Mol}^{-1} \text{cm}^{-1}$

Description	Cat.#	Qty
JC-1	FP-52314A	5 mg
	FP-52314B	100 tests (100 x in DMSO buffer)

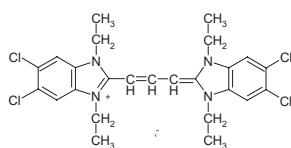
abs em. MitoRed stain

9-[2-(4'-Methylcoumarin-7'-oxycarbonyl)phenyl]-3,6-bis(diethylamino)xanthylium chloride (L)

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

MitoRed is a cell membrane permeable dye. It is loaded into cells by simple incubation with concentrations between 20 and 200 nM at 37°C for 30 min to 1 hour. 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. Cells can be observed with a fluorescence microscope with a rhodamine filter

Description	Cat.#	Qty
MitoRed stain	FP-T32842	8 x 50 μg



JC-1 is also available as a iodide salt.

DiOC dyes (Carbocyanines)

DiOC dyes are sensitive amphiphilic fluorescent probes of membrane-potential, suitable for mitochondria and ER studies. They accumulate in the lipidic bilayer of hyperpolarized membranes. The most popular is **DiOC₆(3)**, now available with our great technology FluoCD™.

abs. em. DiOC₆(3)

3,3'-dihexyloxycarbocyanine iodide

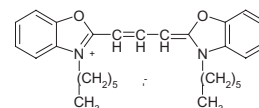
C₂₉H₃₇N₂O₂ MW : 572.53

Soluble in DMSO or DMF

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$: 484/501 nm (in MeOH) ; EC : 150 000 cm⁻¹M⁻¹ (in MeOH)

The most popular dye for mitochondria in live yeast and other eukaryotic cells (see the section "Mitochondria"), but also for other membrane potential measurements sarcoplasmic reticulum. Photolysis of bound DiOC₆(3) specifically destroys the cells microtubules allowing organelle motility studies.



Description	Cat.#	Qty
DiOC ₆ (3)	FP-46764A	100 mg

JUMBO : Custom pack. & Bulk on inquire

DiOC₆(3) FluoCD™stain for FCM

Equivalent to a 200-300 nM DiOC₆ solution.

The same properties than classic DiOC₆ plus the benefits of our FluoCD™ technology (see technical tip and application). Formulation optimized for Cytometry applications:

- ◆ Ready-to-dissolve with water : gain time and reproductibility
- ◆ More sensitive : 2-3 higher signals
- ◆ Cost effective : less DiOC₆ is used

Description	Cat.#	Qty
DiOC ₆ (3) FluoCD™stain for FCM	FP-BC1001	1 ml

DiOC₆(3) FluoCD™stain for Microscopy

Equivalent to a 5 - 7 μM DiOC₆ solution.

The same properties than classic DiOC₆ plus benefits of FluoCD™ adjuvant (see Technical tip). Formulation optimized for Microscopy applications :

- ◆ Ready-to-dissolve with water : gain time and reproductibility
- ◆ No artifacts due to precipitates
- ◆ More sensitive / cost effective

Description	Cat.#	Qty
DiOC ₆ (3) FluoCD™stain	FP-BC1011	1 ml

Technical tip

FluoCD™ technology

Our new **FluoCD™ technology** adds great value to many fluorescent probes. It is based on a proprietary cyclodextrin with definite structure that shows incredible properties, as shown with several dyes (see application). Great benefits :

- ◆ Solves solubilization concerns – no more dissolution step, nor organic solvents use ! – Gains time and reproductibility!
- ◆ Increases fluorescence intensity by 2-3 fold (or reduce required dye material)
- ◆ Prevents dye aggregation in situ (artifacts)
- ◆ No cytotoxicity
- ◆ Improved multiple labeling (better overlay figures)

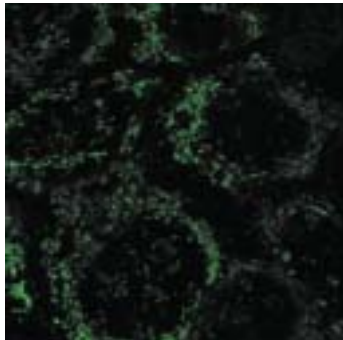
See also FluoCD™ wash solution for FCM (FP-BC099).

Cell Biology - Study/Probes

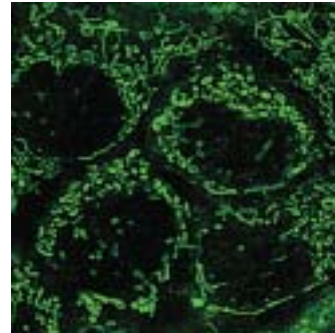
Probes for Mitochondria and Endoplasmic Reticulum (ER)

Application

The efficiency of our **FluoCD™** technology is shown as follow for the lipophilic DiOC6(3) dye, but applies also to other dyes including Synaptracetr™3-2, Calcein and Fluo3 (under testing). Please inquire.

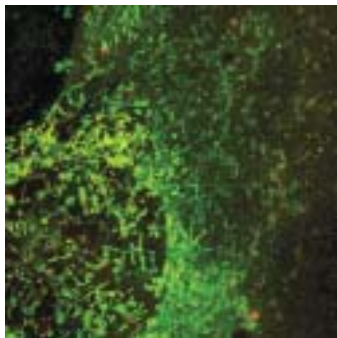


(A) DiOC₆ (100 nM)

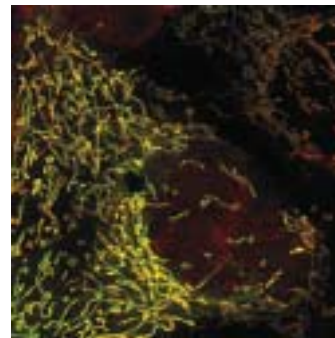


(B) Fluo CD™ : DiOC₆ (100 nM)

Non-Fixed Caco-2 cells were labeled in same conditions by DiOC₆(3) with (B) or without (A) FluoCD™ technology, and observed by confocal microscopy (each image represents 0.16 x 0.16 μm). FluoCD™: DiOC6 formulation clearly improves the detection signal whithout modifying the labeling specificity.



(C) CMTR / DiOC₆ (100 nM)



(D) CMTR / FluoCD™ : DiOC₆ (100 nM)

Caco-2 cells were co-labelled by CMTR and DiOC₆ (2 fluorophores with same targets), with (C) or without FluoCD™ technology (D). Observation by confocal microscopy (depicted is a multiplan view, 0.16x0.16 μm) shows that FluoCD™ technology, **do not alter DiOC₆ probing specificity**, and better visualize mitochondria by overlay of red fluorecence (CMTR) and green fluorecence (DiOC₆).

Other membrane potential sensors (DASPEI, TMRE, TMRM)

- ◆ Some styryl dyes that have a large fluorescence Stokes shift and are taken up relatively slowly as a function of membrane potential. DASPEI is the most popular. It has been useful for mitochondria in living cells, yeast and neurobiology studies.
- ◆ TMRE and TMRM are rhodamine based dyes for membrane potential probing with higher kinetic.

abs em. DASPEI

(2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide)

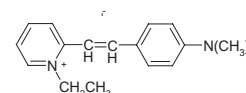
$C_{17}H_{21}IN_2$ MW : 380.27

Soluble in DMF

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$: 461/589 nm (MeOH) ; EC : 39 000 $M^{-1}cm^{-1}$

Description	Cat.#	Qty
DASPEI	FP-46797A	25 mg



abs em. TMRE

(Tetramethylrhodamine Ethyl Ester, perchlorate)

$C_{26}H_{27}ClN_2O_7$ MW : 514.96

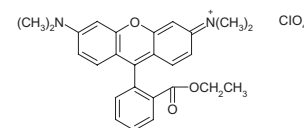
Soluble in DMSO, EtOH

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$: 549/574 nm (MeOH) ; EC : (549 nm) : 109 000 $M^{-1}cm^{-1}$

Quickly loaded in mitochondria, that makes it ideal for dynamic and *in situ* quantitative measurements of mitochondria depolarization (better than rhodamine 123). May be use combined with Ca^{2+} indicators (cytosolic transients).

Description	Cat.#	Qty
TMRE	FP-41391A	25 mg



abs em. TMRM

(Tetramethylrhodamine Methyl Ester, perchlorate)

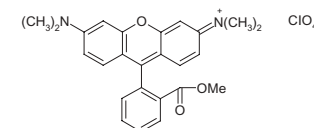
$C_{25}H_{25}ClN_2O_7$ MW : 500.93

Soluble in DMSO, MeOH Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$: 548/573 nm (MeOH) ; EC (549 nm) : 115 000 $M^{-1}cm^{-1}$

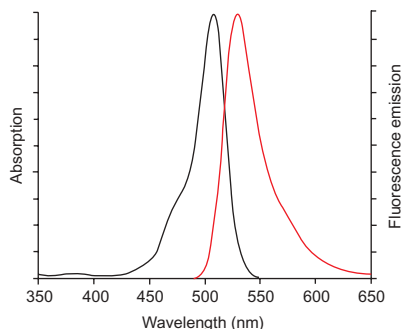
Useful in dual color imaging with Fluorescein. See description in section "potential indicators".

Description	Cat.#	Qty
TMRM	FP-21089A	25 mg

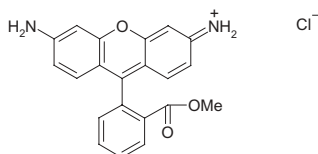


Cell Biology - Study/Probes

Probes for Mitochondria and Endoplasmic Reticulum (ER)



Absorption and emission spectra of rhodamine 123 in MeOH.



Reactive oxygen probes for mitochondria

Rhodamine 123 dyes

Rhodamine 123 is a commonly used probe for Endoplasmic Reticulum and Golgi apparatus, but also mitochondria.

Rhodamine 123 accumulates within a few minutes in mitochondria without cytotoxic effects. It does not stain ER unlike most potential indicators, but is merely released by washes of many cells.

The reduced form is generally preferred, because it stains mitochondria of viable cells.

abs em. Rhodamine 123

$C_{21}H_{17}ClN_2O_3$ MW : 380.83

Soluble in MeOH and DMF

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 505/534 nm ; EC (505 nm) : 97 000 $M^{-1}cm^{-1}$

Description

Cat.#

Qty

Rhodamine 123

FP-47372A

50 mg

abs em. Dihydrorhodamine 123

$C_{21}H_{18}N_2O_3$ MW : 346.39

Soluble in DMSO and DMF

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 289 nm/ none ; EC : 7 100 $M^{-1}cm^{-1}$

$\lambda_{exc.}/\lambda_{em.}$ (oxidized) : see Rhodamine 123

The reduced form of Rhodamine 123. Dihydrorhodamine 123 itself is nonfluorescent, but it is oxidized by oxidative species or by cellular redox systems to the fluorescent rhodamine 123 that accumulates in mitochondrial membranes.

Dihydrorhodamine 123 is also useful for reactive oxygen species detecting including superoxide (in the presence of peroxidase or cytochrome c) and peroxynitrite.

Description

Cat.#

Qty

Dihydrorhodamine 123

FP-83775A

10 mg

abs em. Dihydrorhodamine Dihydrochloride salt

$C_{21}H_{20}Cl_2N_2O_3$ MW : 419

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 289 nm/ none ; EC : 71 000 $M^{-1}cm^{-1}$

$\lambda_{exc.}/\lambda_{em.}$ (oxidized) : see Rhodamine 123

The HCl salt form of dihydrorhodamine 123. Functionally equivalent to it. But more water soluble and more stable to air oxidation. Thus, dihydrorhodamine 123 dihydrochloride is easier to use and can be stored longer than dihydrorhodamine 123.

Description

Cat.#

Qty

Dihydrochloride salt

FP-AM352A

10 mg

Nonyl acridine orange

This mitochondrial dye is uptaken by mitochondria and well retained, but is not dependent on mitochondrial membrane potential, unlike JC-1 and rhodamine 123. It is useful for long term studies, as mitochondria isolation, drug screening, drug resistance, and apoptosis. It is however toxic at high concentration and binds to cardiolipin.

abs em. Nonyl acridine orange

$C_{26}H_{38}BrN_3$ MW : 472.52

Soluble in DMSO and EtOH

Store at 4°C Toxic

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 495/522 nm ; EC : 63 000 $M^{-1}cm^{-1}$

Description

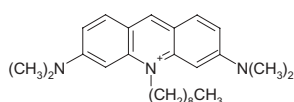
Cat.#

Qty

Nonyl acridine orange

FP-58566A

50 mg



See other acridine dyes pages E125-E127.

Immunocapture kits of OXPHOS complexes

OXPHOS are essential protein complexes inserted in mitochondria membrane acting as redox carrier between O_2 and ATP (respiratory chain) once associated to other components (UQ, ELTH,...). Protons are pumped from the inside (matrix space) to the outside of mitochondria (cytosol), and creates substantial pH and electrical gradients across the mitochondrial inner membrane. These protons eventually re-enter the matrix space via the F1 ATPase, driving the synthesis of ATP as they return.

Mitochondria play an important role in several common conditions, and about 150 different types of hereditary mitochondrial defects have been reported as well as other diseases (*mitopathies*). OXPHOS are involved in many respiratory defect mitopathies, as well as in auto-immune mitopathies (auto-abs against mitochondrial proteins) and permeability transition (loss of the normal membrane potential and ATP production failure) occurring in. Interchim provides purification kits to isolate the 5 OXPHOS complexes for their study (i.e. defective components), and further drugs screening assays.

All kits contains 250 µg, 500 µg or 750 µg monoclonal antibody irreversibly crosslinked to protein G-agarose beads which can immunocapture OXPHOS Complexes.

MS101 Complex I Immunocapture Kit

250 µg, 500 µg or 750 µg monoclonal antibody irreversibly crosslinked to protein G-agarose beads which can immunocapture ~60 µg, ~120 µg or ~180 µg respectively of Complex I (NADH dehydrogenase) from heart mitochondria. Also included are 2 mg of bovine heart mitochondria for control immunocapture.

Description	Cat.#	Qty
MS101 Complex I Immunocapture Kit	BP1970	1 kit (small)
	BP1971	1 kit (medium)
	BP1972	1 kit (large)

MS201 Complex II Immunocapture Kit

250 µg, 500 µg or 750 µg monoclonal antibodies irreversibly crosslinked to protein G-agarose beads which can immunocapture ~13 µg, ~26 µg or ~39 µg respectively of Complex II from heart mitochondria. Also included are 2 mg of bovine heart mitochondria for control immunocapture.

Description	Cat.#	Qty
MS201 Complex II Immunocapture Kit	BP1980	1 kit (small)
	BP1981	1 kit (medium)
	BP1982	1 kit (large)

MS301 Complex III Immunocapture Kit

250 µg, 500 µg or 750 µg monoclonal antibodies irreversibly crosslinked to protein G-agarose beads which can immunocapture ~25 µg, ~50 µg or ~75 µg respectively of Complex III from heart mitochondria. Also included are 2 mg of bovine heart mitochondria for control immunocapture.

Description	Cat.#	Qty
MS301 Complex III Immunocapture Kit	BP1990	1 kit (small)
	BP1991	1 kit (medium)
	BP1992	1 kit (large)

MS401 Complex IV Immunocapture Kit

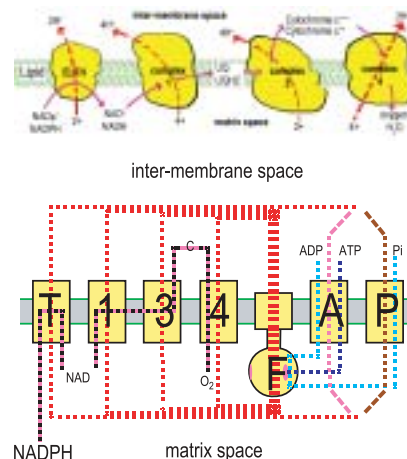
250 µg, 500 µg or 750 µg monoclonal antibodies irreversibly crosslinked to protein G-agarose beads which can immunocapture ~25 µg, ~50 µg or ~75 µg respectively of Complex IV from heart mitochondria. Also included are 2 mg of bovine heart mitochondria for control immunocapture.

Description	Cat.#	Qty
MS401 Complex IV Immunocapture Kit	BP2000	1 kit (small)
	BP2001	1 kit (medium)
	BP2002	1 kit (large)

MS501 Complex V Immunocapture Kit

250 µg, 500 µg or 750 µg of monoclonal antibodies irreversibly crosslinked to protein G-agarose beads which can immunocapture ~25 µg, ~50 µg or ~75 µg respectively of Complex V from heart mitochondria. Also included are 2 mg of bovine heart mitochondria for control immunocapture.

Description	Cat.#	Qty
MS501 Complex V Immunocapture Kit	BP1610	1 kit (small)
	BP1611	1 kit (medium)
	BP1612	1 kit (large)



F : F₀F₁-ATPase
 A : electrical ATP₄-ADP₃-exchange (inhibited by atractyloside bongkreikic acid)
 P : electroneutral exchange of H₂PO₄- for OH- (inhibited by Nem, Mersalyl)
 complex I : NADH dehydrogenase
 complex II : succinate dehydrogenase
 complex III : ubiquinol : cytochrome c reductase
 complex IV : cytochrome c oxidase

See also OXPHOS ImmunoCytoChemistry kits page E201.