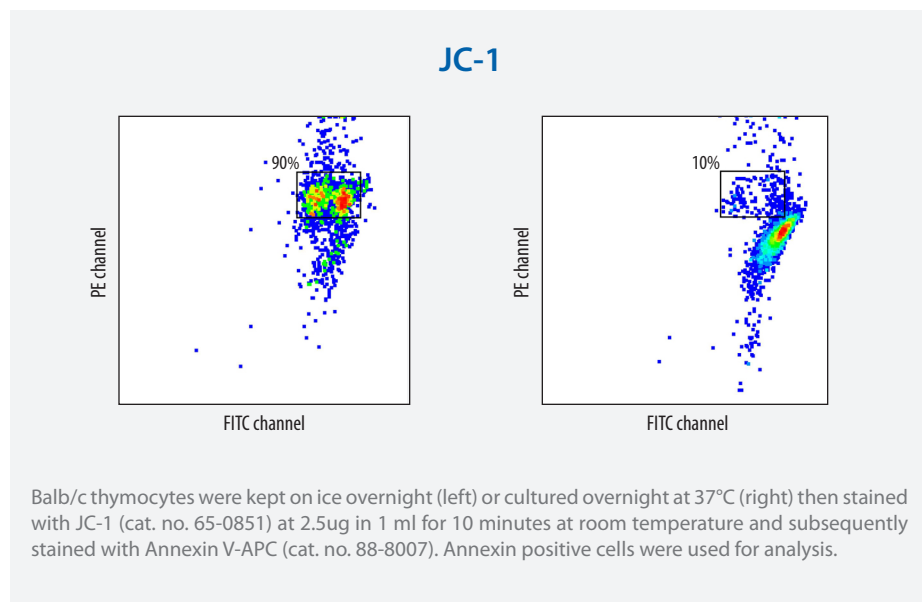


Cell Functional Dyes

NEW Tools for the Analysis of Cellular Function

Mitochondrial Membrane Potential Dye

JC-1 is a membrane permeable dye widely used for determining mitochondrial membrane potential in flow cytometry and fluorescent microscopy. This dye can selectively enter the mitochondria where it reversibly changes color as membrane potentials increase (over values of 80-100 mV). This property is due to the reversible formation of J-aggregates upon membrane polarization that causes shifts in emitted light from 530 nm (i.e., emission of JC-1 monomeric form) to 590 nm (i.e., emission of J-aggregate) when excited at 488 nm. Both colors can be detected using filters for FITC and PE/rhodamine, respectively. JC-1 is both qualitative, in regards to the shift from green to orange fluorescence emission, and quantitative, as measured by fluorescence intensity, in both filter sets. JC-1 can be used to indicate the initiation of apoptosis. As cells undergo apoptosis the mitochondrial membrane potential decreases and the JC-1 undergoes a shift from orange to green fluorescence.



eBioscience New Releases

August | 2009

FEATURING

MITOCHONDRIAL MEMBRANE POTENTIAL DYE

- JC-1

FLUORESCENT CELL VIABILITY DYES

- Calcein AM (UltraPure Grade)
- Calcein Blue AM
- Calcein Violet 450 AM
- Propidium Iodide
- 7-AAD

CALCIUM SENSING DYES

- Fura-2 AM
- Indo-1 AM
- eFluor™ 514 Calcium Sensor Dye

CELL TRACKING & PROLIFERATION DYE

- CFSE

Mitochondrial Membrane Potential Dye

Product	Cat. No.	MW (kDa)	Application notes	Excitation (nm)	Emission (nm)	Size
JC-1	65-0851	652.2	Used in flow cytometry to detect changes in mitochondrial membrane potential which can occur during apoptosis or in metabolically stressed cells	515	529 & 590	1 x 5 mg

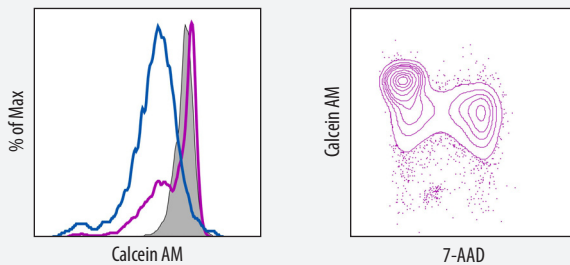
Fluorescent Cell Viability Dyes

Calcein AM is a green fluorescent viability dye that has been engineered with acetoxymethyl ester (AM) to make it hydrophobic and suitable for uptake across cell membranes in live cells. Calcein AM becomes highly fluorescent when the acetoxymethyl ester is hydrolyzed by cytoplasmic esterases thereby releasing the hydrophilic highly fluorescent cell-bound calcein. Since dead cells do not contain active esterases, dead cells are not labeled. Calcein AM is not toxic so it can also be used for short-term cell tracing studies. Calcein Blue AM is a UV excited alternative to Calcein AM, having an excitation similar to DAPI, Hoechst, and AMCA. Calcein Violet

450 AM, is a violet laser (405 nm) excited equivalent to Calcein AM. Co-staining with Annexin V or 7-AAD is recommended to allow the greatest resolution between live and dead/apoptotic cells. This series of calcein dyes can be used to label live cells and enumerate them using flow cytometry or by fluorescent microscopy.

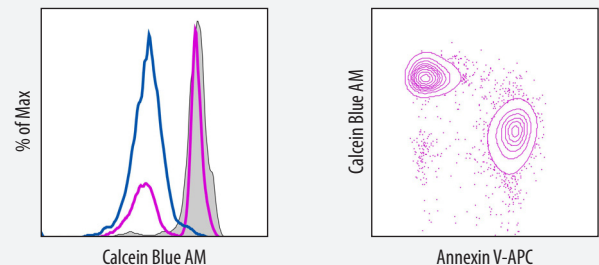
Propidium iodide (PI) and 7-amino-actinomycin D (7-AAD) are both fluorescent viability dyes that can be used to measure plasma membrane integrity; only cells with compromised plasma membranes will stain with PI and 7-AAD.

Calcein AM



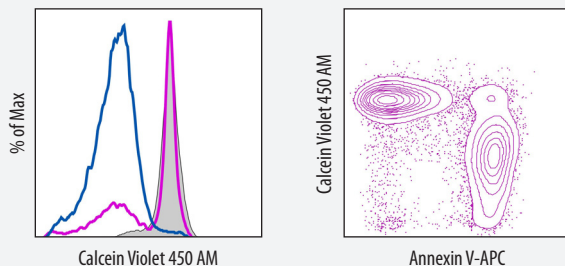
Balb/c thymocytes were stained with 12.5 nM Calcein AM (cat. no. 65-0853) for 30 minutes at room temperature (left). Thymocytes were kept on ice overnight (shaded histogram) or cultured overnight at 37°C without (purple) or with (blue) 1 μM dexamethasone. Thymocytes cultured overnight without dexamethasone were also stained with 7-AAD (cat. no. 00-6993) allowing further discrimination between live and dead cells (right). Total cells were used for analysis.

Calcein Blue AM



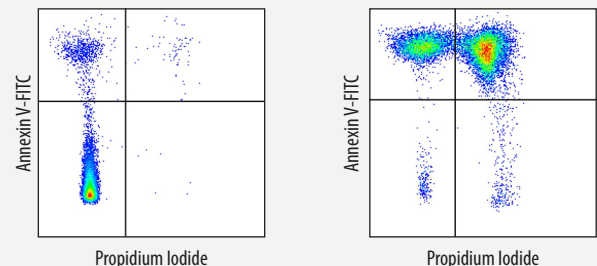
Balb/c thymocytes were stained with 1 μM Calcein Blue AM (cat. no. 65-0855) for 30 minutes at room temperature (left). Thymocytes were kept on ice overnight (shaded histogram) or cultured overnight at 37°C without (purple) or with (blue) 1 μM dexamethasone. Thymocytes cultured overnight without dexamethasone were also stained with Annexin V-APC (cat. no. 88-8007) allowing further discrimination between live and dead cells (right). Total cells were used for analysis.

Calcein Violet 450 AM



Balb/c thymocytes were stained with 1 μM Calcein Violet 450 AM (cat. no. 65-0854) for 30 minutes at room temperature (left). Thymocytes were kept on ice overnight (shaded histogram) or cultured overnight at 37°C without (purple) or with (blue) 1 μM dexamethasone. Thymocytes cultured overnight without dexamethasone were also stained with Annexin V-APC (cat. no. 88-8007) allowing further discrimination between live and dead cells (right). Total cells were used for analysis.

Propidium Iodide



Mouse thymocytes were prepared as a single cell suspension and incubated overnight at 37°C in medium (left) or medium with 1 μM dexamethasone (right). Cells were harvested and stained using the Annexin V-FITC Apoptosis Detection Kit (cat. no. 88-8005) and Propidium Iodide Staining Solution (cat. no. 00-6990).

Fluorescent Cell Viability Dyes						
Product	Cat. No.	MW (kDa)	Application notes	Excitation (nm)	Emission (nm)	Size
Calcein AM (UltraPure Grade)	65-0853	994.8	Label live cells; suitable for use in microscopy and flow cytometry when using appropriate filter sets	495	515	20 x 50 µg 1 x 1 mg
Calcein Blue AM	65-0855	465.4	Label live cells; suitable for use in microscopy and flow cytometry when using appropriate filter sets	360	445	1 x 1 mg
Calcein Violet 450 AM	65-0854	600	Label live cells; suitable for use in microscopy and flow cytometry when using appropriate filter sets	408	450	1 x 1 mg
Propidium Iodide	00-6990	668.4	Distinguish live (unstained) from dead cells; suitable for use in flow cytometry	536	617	2.0 ml
7-AAD	00-6993	1270.4	Distinguish live (unstained) from dead cells; suitable for use in flow cytometry	555	655	2.0 ml

Calcium Sensor Dyes

Intracellular Ca^{2+} is a key second messenger in signaling processes in the cell. The ability to measure changes in intracellular free Ca^{2+} through the use of fluorescent Ca^{2+} indicators has dramatically advanced the understanding of Ca^{2+} signaling in both normal and disease processes. eBioscience is pleased to offer three new Ca^{2+} indicator dyes, Fura-2, Indo-1 and eFluor™ 514 Calcium Sensor Dye, all of which have been engineered with acetoxymethyl esters (AM) to make them hydrophobic and suitable for loading into live cells. Once in the cell, the AM groups are removed by cellular esterases. Fura-2 and Indo-1 are two UV excitable Ca^{2+} indicators, while eFluor™ 514 Calcium Sensor Dye is excited by the 488 nm laser.

Fura-2 is the preferred dye for ratiometric imaging microscopy with digital image analysis, especially when the alteration of excitation wavelengths is more practical than the detection of multiple emission wavelengths. Upon binding Ca^{2+} , the excitation spectrum of Fura-2 shifts to shorter wavelengths between 300 and 400 nm, while the peak emission remains steady around 510 nm. In contrast, Indo-1 AM is a single excitation and dual emission Ca^{2+} indicator. Unbound Indo-1 has a peak emission at 485 nm. Upon binding calcium, the peak emission shifts down to 410 nm. In flow cytometry

this can be measured over time and can be represented as a ratio of the two emission wavelengths. To optimize the ratio between the two emissions, unbound Indo-1 fluorescence should be collected using a filter above 485 nm (525 nm is a good option) while bound Indo-1 fluorescence should be collected using a filter below 400 nm. Peak absorption occurs at ~350 nm, while peak emission is at ~400 nm and ~480 nm in the Ca^{2+} bound and unbound state, respectively.

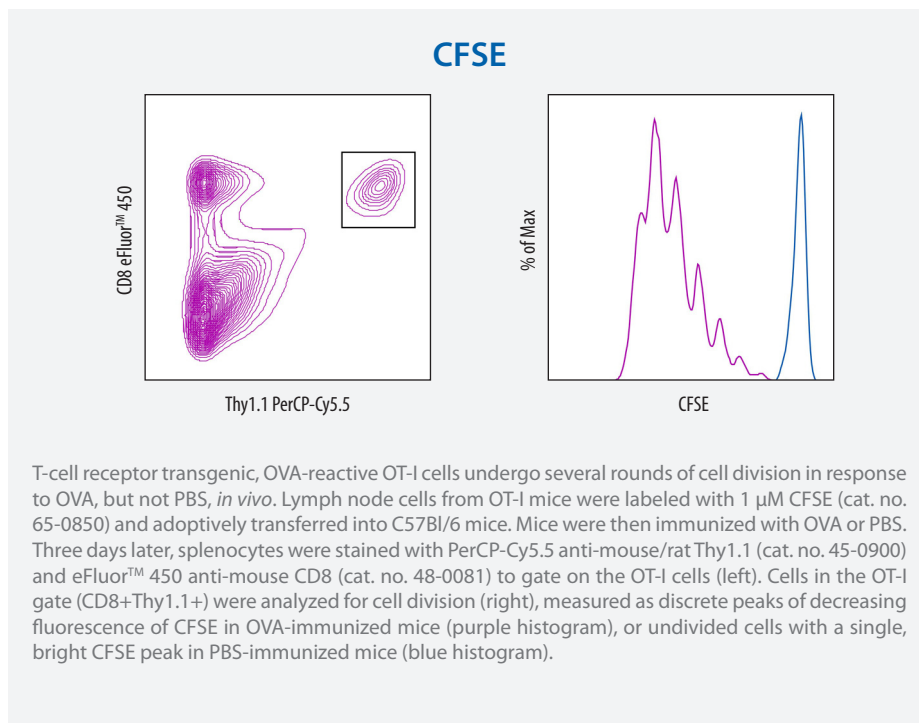
eFluor™ 514 Calcium Sensor Dye is a membrane-permeable dye used for monitoring changes in intracellular free Ca^{2+} concentrations using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Once eFluor™ 514 Calcium Sensor Dye enters the cell, cellular esterases cleave the AM group yielding a membrane-impermeable dye fluorescing at ~520 nm. eFluor™ 514 Calcium Sensor Dye, like Fluo-3 and Fluo-4, is a commonly used dye among the visible light-excitable Ca^{2+} indicators but with increased cellular uptake (even at room temperature) and brightness. Since there is not a significant shift in emission and excitation wavelength upon binding Ca^{2+} , this dye cannot be used for ratiometric measurements. Increases in free Ca^{2+} are observed as an increase in the fluorescence intensity of eFluor™ 514 Calcium Sensor Dye.

Calcium Sensor Dyes						
Product	Cat. No.	MW (kDa)	Application notes	Excitation (nm)	Emission (nm)	Size
Fura-2 AM	65-0858	1001.8	Ratio-imaging microscopy on live cells	300-400	510	1 x 1 mg
Indo-1 AM	65-0857	1009.9	Ratiometric indicator by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers	346	~410 (Ca^{2+} bound) ~485 (Ca^{2+} free)	20 x 50 µg
eFluor™ 514 Calcium Sensor Dye	65-0859	1100	Fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers	490	514	1 x 1 mg 10 x 50 µg

Cell Tracking & Proliferation Dye

CFSE [5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester] is widely used for cell tracking and proliferation studies. It has also been used in CTL assays and cell motility studies. CFSE readily crosses intact cell membranes. Once inside the cells, intracellular esterases cleave the acetate groups to yield

the fluorescent carboxyfluorescein molecule. The succinimidyl ester group reacts with primary amines, crosslinking the dye to intracellular proteins. Cell division can be measured as successive halving of the fluorescence intensity of CFSE.



Cell Tracking & Proliferation Dye

Product	Cat. No.	MW (kDa)	Application notes	Excitation (nm)	Emission (nm)	Size
CFSE	65-0850	557.4	CFSE is used for cell tracking and proliferation studies by flow cytometry	494	521	5 x 500 μ g