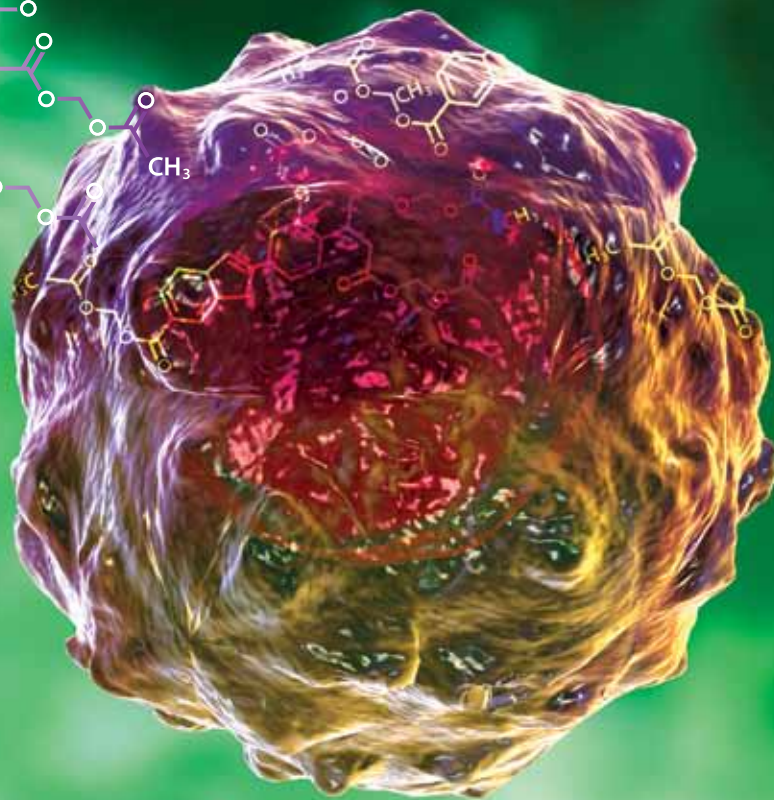
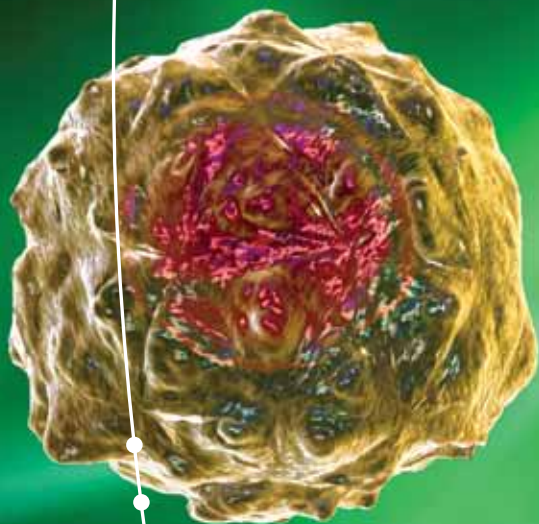
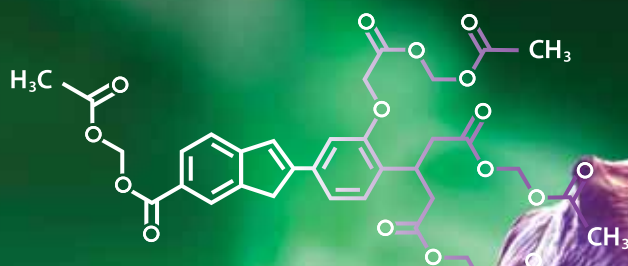


# Cell Biology

## Dyes and Staining Reagents

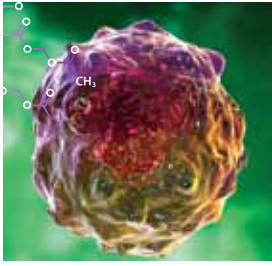




*eBioscience is committed to developing and manufacturing high-quality, innovative reagents in an ISO certified facility. As a provider of more than 10,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.*

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# Cell Biology

## Dyes and Staining Reagents

eBioscience is an industry-leading provider of antibodies against both novel and well-established targets. With the addition of our Cell Proliferation Dye eFluor® 670, eBioscience now offers our widest selection of dyes and fluorometric reagents for cell functional analysis, featuring reagents for:

### Cell Viability

#### Fixable Viability eFluor® Dyes

- Assess viability of cells by flow cytometry intracellular and surface staining
- Signal maintained in cryopreserved samples

#### Propidium Iodide and 7-Aminoactinomycin D (7-AAD)

- Measure cell viability and plasma membrane integrity
- For flow cytometry

### Cell Proliferation and Division

#### Cell Proliferation Dye eFluor® 670

- Monitor cell proliferation *in vitro* and *in vivo*
- For flow cytometry - excited by the red laser and emits at 670 nm

#### CFSE

- Monitor cell proliferation *in vitro* and *in vivo*
- For fluorescence microscopy and flow cytometry

#### Nuclear RED (DRAQ5™) and Nuclear ORANGE (CyTRAK Orange™)

- Nuclear labeling of DNA for ploidy and cell cycle analysis
- For fluorescence microscopy and flow cytometry

## Cell Function

### CellVue® Dyes

- Cell membrane labeling
- For fluorescence microscopy and flow cytometry

### Calcein AM, Calcein Blue AM and Calcein Violet AM

- Non-toxic live cell labeling
- For fluorescence microscopy and flow cytometry

### Fura-2 AM, Indo-1 AM and eFluor® 514 Calcium Sensor Dye

- Calcium sensing reagents
- For microscopy, fluorescence microscopy, and flow cytometry

## Cell Death

### JC-1

- Analyze mitochondrial membrane potential
- For fluorescence microscopy and flow cytometry

### Annexin V Apoptosis Detection Kits

- Identify apoptotic cells by flow cytometry

# Cell Viability

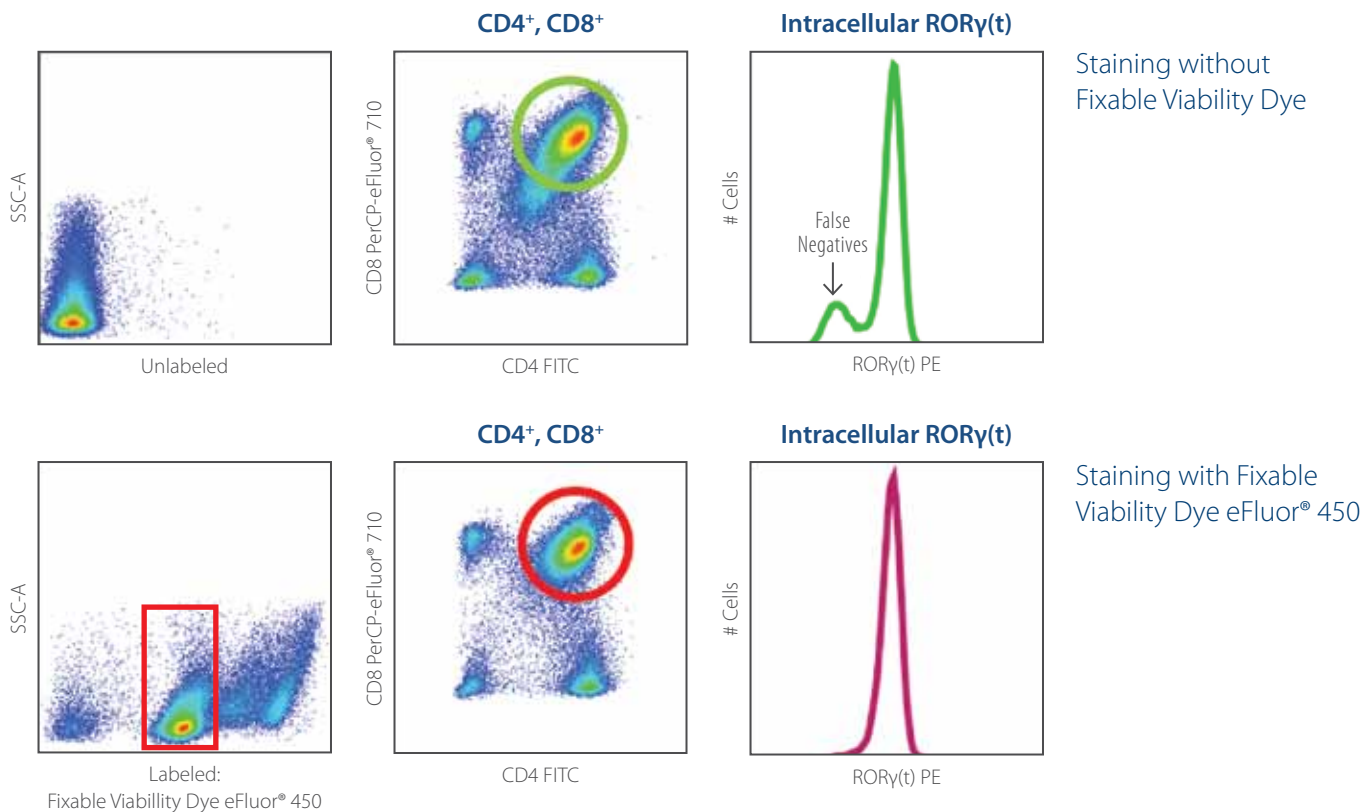
- Fixable Viability eFluor® Dyes
- Propidium Iodide and 7-Aminoactinomycin D (7-AAD)

## Fixable Viability eFluor® Dyes for Flow Cytometry:

- Fixable Viability Dye eFluor® 450
- Fixable Viability Dye eFluor® 660
- Fixable Viability Dye eFluor® 780

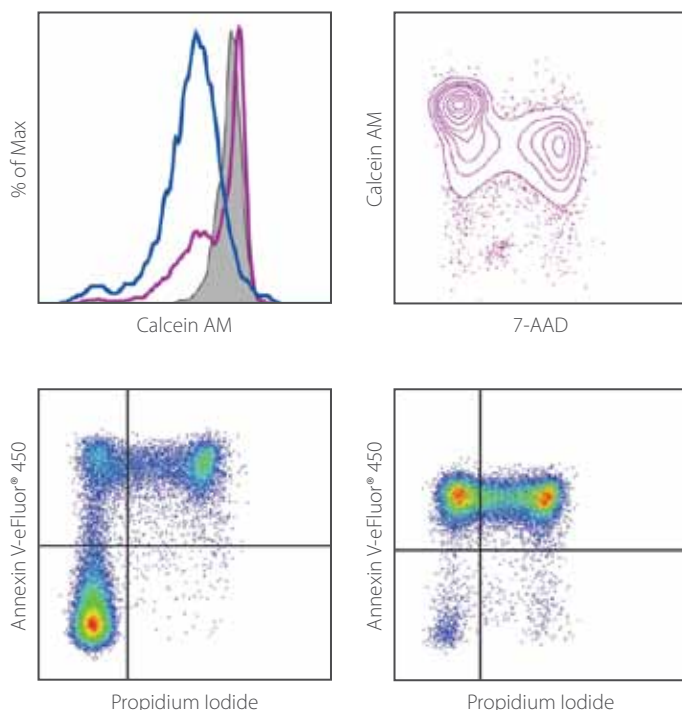
**Fixable Viability eFluor® Dyes** have the significant advantage over conventional viability dyes of irreversibly labeling dead cells prior to the fixation and permeabilization steps that are required for staining intracellular targets. These are amine-reactive fluorescent reagents, excited by the violet or red laser, that bind free amine groups on proteins. They are not membrane permeable and therefore only minimally label live cells. However, dead cells with compromised membranes allow access of the dye to the interior of the cell resulting in a very bright fluorescence of the dead cell population. Unlike 7-AAD and Propidium Iodide, Fixable Viability eFluor® Dyes are retained by dead cells throughout fixation, permeabilization and cryopreservation.

Excluding dead cells from analysis is a recommended procedure for all intracellular staining protocols including detection of nuclear factors, transcription factors, cytosolic signaling molecules and secreted growth factors and cytokines.



## Propidium Iodide (PI) and 7-Aminoactinomycin D (7-AAD)

For assessing cell viability by flow cytometry, PI and 7-AAD dyes are ready-to-use solutions for the exclusion of nonviable cells in flow cytometric analysis. **PI** binds to double stranded DNA, but is excluded from cells with intact plasma membranes. PI can be detected with the PerCP tandem dye channel for viability exclusion, but should be analyzed in the PE channel when used as a counterstain for FITC Annexin V. **7-AAD** can be used in place of PI. The advantage of 7-AAD over PI is that there is minimal spectral overlap between these emissions. Fluorescence is detected in the far red range of the spectrum (650 nm long-pass filter). PI and 7-AAD can also be used for flow cytometric analysis of the cell cycle.



### 7-AAD

*Balb/c thymocytes were stained with 12.5 nM Calcein AM 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.*

### 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-eFluor® 450 Apoptosis Detection Kit and Propidium Iodide Staining Solution (cat. no. 00-6990).*

## Products for Evaluation of Cell Viability

| Description                       | Catalog Number | Sizes                     | Excitation (nm) | Peak Emission (nm) |
|-----------------------------------|----------------|---------------------------|-----------------|--------------------|
| Fixable Viability Dye eFluor® 450 | 65-0863-14     | 100 tests                 | Violet (405)    | 450                |
|                                   | 65-0863-18     | 500 tests (5 X 100 tests) |                 |                    |
| Fixable Viability Dye eFluor® 660 | 65-0864-14     | 100 tests                 | Red (633)       | 660                |
|                                   | 65-0864-18     | 500 tests (5 X 100 tests) |                 |                    |
| Fixable Viability Dye eFluor® 780 | 65-0865-14     | 100 tests                 | Red (633)       | 780                |
|                                   | 65-0865-18     | 500 tests (5 X 100 tests) |                 |                    |
| Propidium Iodide                  | 00-6990-50     | 2.0 ml                    | Blue (488)      | 617                |
| 7-AAD                             | 00-6993-50     | 2.0 ml                    | Blue (488)      | 655                |

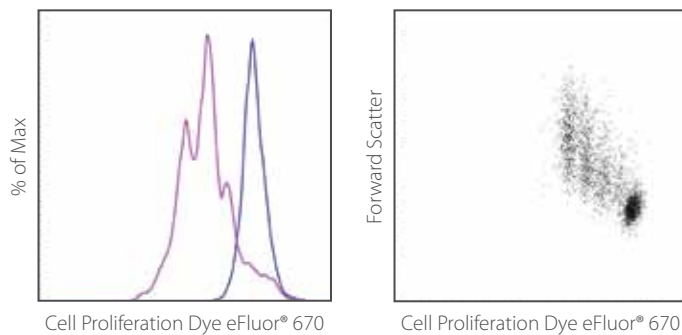
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# Cell Proliferation and Division

- Cell Proliferation Dye eFluor® 670
- CFSE
- Nuclear RED (DRAQ5™) and Nuclear ORANGE (CyTRAK Orange™)

## Cell Proliferation Dye eFluor® 670

Use **Cell Proliferation Dye eFluor® 670** for tracking cell proliferation as an alternative to CFSE. This dye is ideally suited for situations where CFSE cannot be used due to the presence of FITC or GFP. Cell Proliferation Dye eFluor® 670 is excited by the red laser and emits at 670 nm. As with CFSE, Cell Proliferation Dye eFluor® 670 accurately indicates cell division by partitioning equally into daughter cells during successive cell divisions, and can be used for both *in vitro* and *in vivo* tracking of cell proliferation.

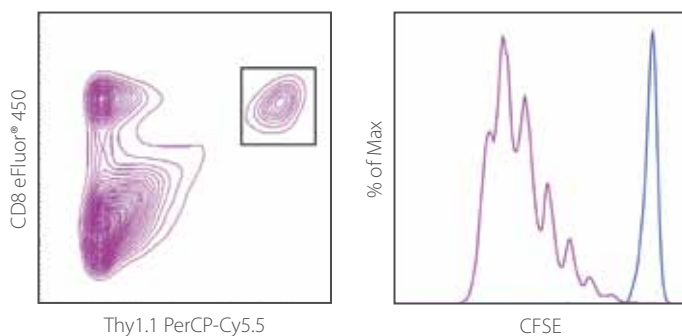


### Cell Proliferation Dye eFluor® 670

Mouse splenocytes were labeled with 5  $\mu$ M Cell Proliferation Dye eFluor® 670 (cat. no. 65-0840) and adoptively transferred into C57Bl/6 mice. Three days later, cells were analyzed for cell division, measured as discrete peaks of decreasing fluorescence of dye in stimulated (pink histogram), or undivided cells with a single, bright peak (purple histogram).

## CFSE [5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester]

**CFSE** is the gold standard for analyzing cell proliferation and is also useful for 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. After the acetate groups are cleaved, it has a peak excitation of 494 nm and peak emission of 521 nm.

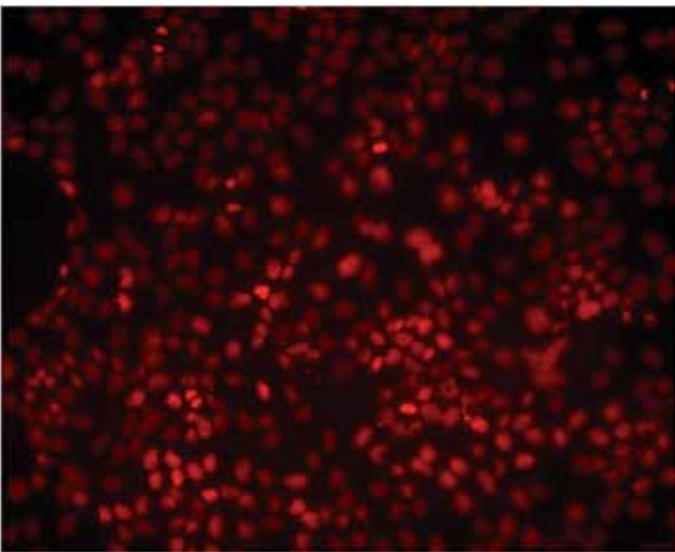


### CFSE

T-cell receptor transgenic, OVA-reactive OT-I cells undergo several rounds of cell division in response to OVA, but not PBS, *in vivo*. Lymph node cells from OT-I mice were labeled with 1  $\mu$ M CFSE (cat. no. 65-0850) and adoptively transferred into C57Bl/6 mice. Mice were then immunized with OVA or PBS. Three days later, splenocytes were stained with PerCP-Cy5.5 anti-mouse/rat Thy1.1 and eFluor® 450 anti-mouse CD8 to gate on the OT-I cells (left). Cells in the OT-I gate (CD8+Thy1.1+) were analyzed for cell division (right), measured as discrete peaks of decreasing fluorescence of CFSE in OVA-immunized mice (purple histogram), or undivided cells with a single, bright CFSE peak in PBS-immunized mice (blue histogram).

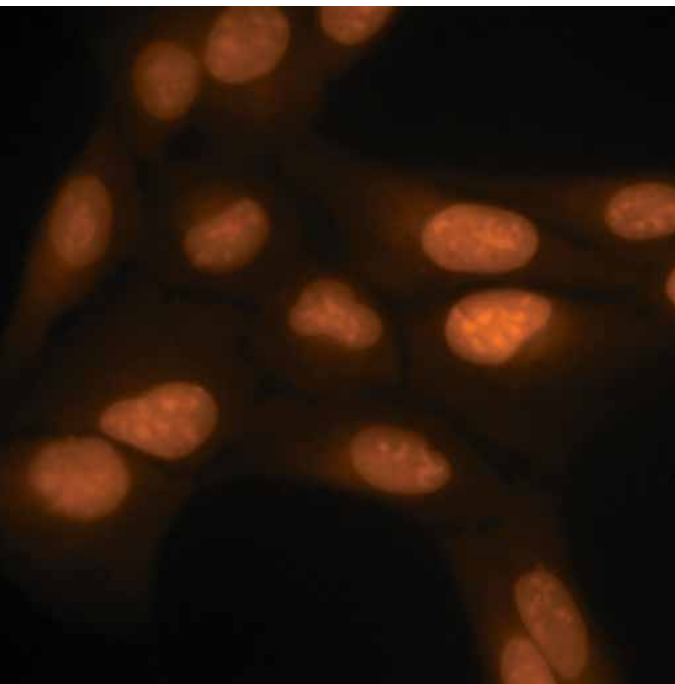
## Nuclear RED (DRAQ5™) and Nuclear ORANGE (CyTRAK Orange™)

These are membrane-permeable anthraquinone dyes with high affinity for double-stranded DNA. They are membrane-permeable, and can label live or fixed/dead cells. **Nuclear RED (DRAQ5™)** can be incorporated into fluorescence microscopy applications as a useful nuclear counterstain for distinguishing nucleated and non-nucleated cells. **Nuclear ORANGE (CyTRAK Orange™)** is ideal for fluorescent microscopy; it can be used to identify and discriminate the nucleus and cytoplasm without the need for a second dye due to its high intensity staining of the nucleus and low intensity staining of the cytoplasm. (*Tissue Transglutaminase Activation Modulates Inflammation in Cystic Fibrosis via PPAR-g Down-Regulation*. Luigi Maiuri, et al. *J. Immunol.* 2008;180;7697-7705)



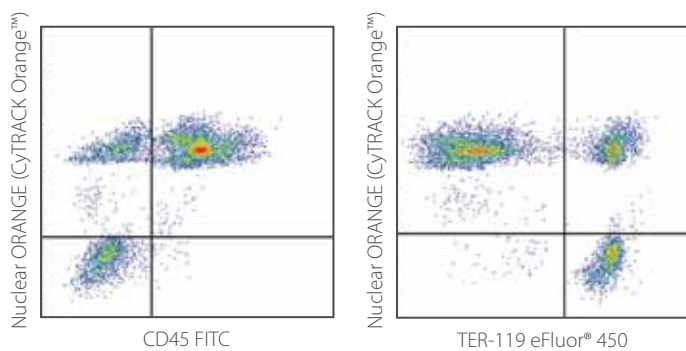
Nuclear RED (DRAQ5™)

*Fixed and permeabilized MDCK cells stained with 10 nM Nuclear RED (DRAQ5™) nuclear stain (cat. no. 65-0880) (red), 20X.*



Nuclear ORANGE (CyTRAK Orange™)

*U2-OS human osteosarcoma cells, counterstained with Nuclear ORANGE (CyTRAK Orange™) (courtesy of Biostatus).*



## Nuclear ORANGE (CyTRAK Orange™)

*C57Bl/6 bone marrow cells were stained with FITC anti-mouse CD45 (30-F11) (cat. no. 11-0451) (left) and eFluor® 450 anti-mouse TER-119 (cat. no. 48-5921) (right), followed by staining with 5 µM Nuclear ORANGE (CyTRAK Orange™) for 15 minutes at room temperature. Total viable cells were used for analysis.*

## Products for Evaluation of Cell Proliferation and Division

| Description                        | Catalog Number | Sizes               | Excitation (nm) | Detector | Peak Emission (nm) |
|------------------------------------|----------------|---------------------|-----------------|----------|--------------------|
| CFSE                               | 65-0850-84     | 1 Pack (5 x 500 µg) | Blue (488)      | FITC     | 521                |
| Cell Proliferation Dye eFluor® 670 | 65-0840-90     | 1 Pack (5 x 400 µg) | Red (633)       | APC      | 670                |
| Nuclear RED (DRAQ5™)               | 65-0880-92     | 50 µl               | 488-647         | APC      | 660 - 710          |
|                                    | 65-0880-96     | 200 µl              |                 |          |                    |
| Nuclear ORANGE (CyTRAK Orange™)    | 65-0881-92     | 50 µl               | 488-550         | PE       | 610                |
|                                    | 65-0881-96     | 200 µl              |                 |          |                    |



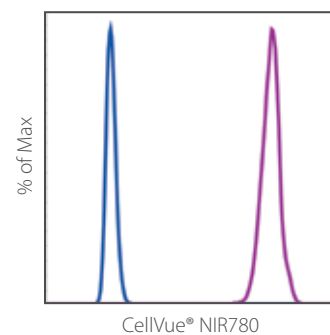
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# Cell Function

- CellVue® Dyes
- Calcein AM, Calcein Blue AM and Calcein Violet AM
- Fura-2 AM, Indo-1 AM and eFluor® 514 Calcium Sensor Dye

## CellVue® Dyes

**CellVue®** lipophilic dyes can be used to label the cell membrane of live cells. Cell labeling is rapid and stable and can be combined with fluorescently labeled antibodies and other markers of cellular function for flow cytometric analysis and fluorescent microscopy.

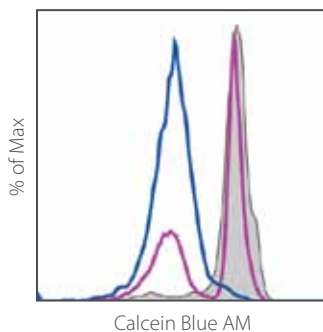


### CellVue® NIR780

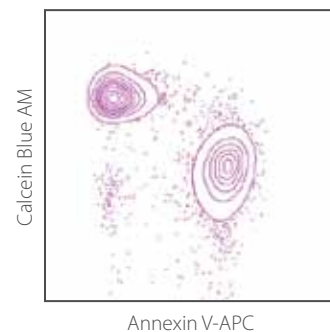
Labeling of mouse spleen cells with 2  $\mu\text{M}$  CellVue® NIR780 for 5 minutes at room temperature in Diluent C (purple histogram). Unlabeled cells are shown for comparison (blue histogram). Total viable cells were used for analysis.

## Calcein AM, Calcein Blue AM and Calcein Violet AM

Calcein labeling dyes easily cross the cell membrane and selectively label live cells for analysis in flow cytometry or fluorescence microscopy. **Calcein AM** is non-toxic and may be used for short-term cell tracing. **Calcein Blue AM**, a UV excited alternative to calcein AM, has excitation characteristics similar to DAPI, Hoechst, and AMCA dyes. **Calcein Violet 450**, is a violet laser (405 nm) 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.



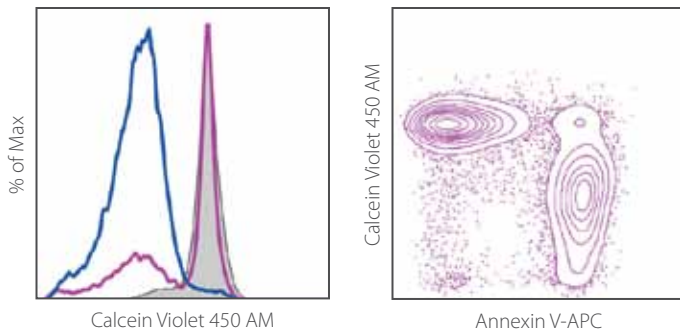
Calcein Blue AM



Annexin V-APC

### Calcein Blue AM

Balb/c thymocytes were stained with 1  $\mu\text{M}$  Calcein Blue AM 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  $\mu\text{M}$  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  $\mu$ M Calcein Violet 450 AM 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  $\mu$ M 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.*

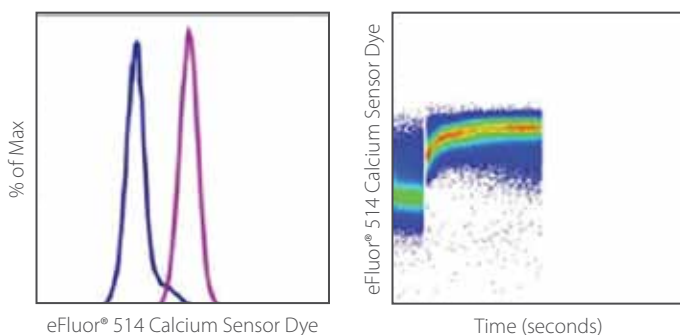
## Calcium Sensing Reagents

### Fura-2 AM, Indo-1 AM, and eFluor® 514 Calcium Sensor Dye

The ability to measure changes in intracellular  $Ca^{2+}$  through the use of fluorescent  $Ca^{2+}$  indicators has dramatically advanced our understanding of  $Ca^{2+}$  signaling in normal and disease processes. Fura-2 AM, Indo-1 AM, and eFluor® 514 Calcium Sensor Dyes contain acetoxymethyl esters (AM) which allow these dyes to cross the cell membrane for easy loading of live cells. Fura-2 AM and Indo-1 AM are UV excitable  $Ca^{2+}$  indicators, while eFluor® 514 is excited by visible light.

**Fura-2 AM** is the preferred dye for ratiometric imaging microscopy with digital image analysis. Upon binding  $Ca^{2+}$ , the excitation spectrum of Fura-2 shifts to shorter wavelengths (300 to 400 nm), while its peak emission remains steady (~510 nm). Conversely, **Indo-1 AM** is a single excitation / dual emission  $Ca^{2+}$  indicator. Unbound Indo-1 has a peak emission at 485 nm, which shifts to 410 nm upon  $Ca^{2+}$  binding. In flow cytometry, this shift can be measured over time and represented as a ratio of the two emission wavelengths.

**eFluor® 514 Calcium Sensor Dye** is a useful indicator for monitoring changes in intracellular free calcium concentrations using flow cytometry, fluorescence microscopy, fluorescence spectroscopy, or fluorescence microplate readers. eFluor® 514 is cleaved by intracellular esterases, removing its AM moiety to create a membrane-impermeable dye fluorescing at ~520 nm. In comparison to other visible light-excitable calcium indicators, eFluor® 514 provides increased cellular uptake and brightness. Since peak emission and excitation wavelength does not change upon  $Ca^{2+}$  binding by eFluor® 514, this dye is not recommended for ratiometric measurements (calcium binding affinity:  $K_d = 232$  nM).



### eFluor® 514 Calcium Sensor Dye

*Jurkat cells were harvested, washed and loaded with eFluor® 514 Calcium Sensor Dye for 30 minutes at 37°C. The left panel shows cells that were washed and analyzed by flow cytometry as unstimulated (blue histogram) or stimulated with 1  $\mu$ g/ml ionomycin (purple histogram). The right panel shows Jurkat cells loaded with eFluor® 514 Calcium Sensor Dye that were acquired on a flow cytometer for 1 minute and then removed for the addition of 1  $\mu$ g/ml ionomycin and immediately placed back on the flow cytometer for continued acquisition.*

## Products for Analysis of Cell Function

| Description                    | Catalog Number | Application Notes  | Excitation (nm) | Emission (nm)   | Size                    |
|--------------------------------|----------------|--|-----------------|---|-------------------------|
| CellVue® Maroon                | 88-0870        | Cannot be combined with APC, Alexa Fluor® 700, APC-eFluor® 780   | 647             | 667   | Mini, Midi              |
| CellVue® Plum                  | 88-0871        | Cannot be combined with APC, Alexa Fluor® 700, APC-eFluor® 780   | 652             | 671   | Mini, Midi              |
| CellVue® Burgundy              | 88-0872        | Cannot be combined with Alexa Fluor® 700, APC-eFluor® 780  | 683             | 707   | Mini, Midi              |
| CellVue® Lavender              | 88-0873        | Cannot be combined with eFluor® 450 or Pacific Blue®   | 420             | 461   | Mini, Midi              |
| CellVue® NIR815                | 88-0874        | Useful for short-term <i>in vivo</i> tracking studies  | 786             | 814   | Mini, Midi              |
| CellVue® NIR780                | 88-0875        | Cannot be combined with APC-eFluor® 780 or APC-Alexa Fluor® 750  | 633             | 776   | Mini, Midi              |
| CellVue® Jade                  | 88-0876        | Cannot be combined with FITC or Alexa Fluor® 488   | 478             | 508   | Mini, Midi              |
| Calcein, AM (Ultra Grade)      | 65-0853        | Label live cells, for microscopy and flow cytometry with appropriate filters   | 495             | 515   | 20 x 50 ug<br>1 x 50 ug |
| Calcein Blue, AM               | 65-0855        | Label live cells, for microscopy and flow cytometry with appropriate filters   | 360             | 445   | 1 x 1 mg                |
| Calcein Violet 450, AM         | 65-0854        | Label live cells, for microscopy and flow cytometry with appropriate filters   | 408             | 450   | 1 x 1 mg                |
| Fura-2, AM                     | 65-0858        | Ratiometric imaging microscopy on live cells   | 300-400         | 510   | 1 x 1 mg                |
| Indo-1, AM                     | 65-0857        | Ratiometric indicator for fluorescence microscopy, flow cytometry, fluorescence spectroscopy & fluorescence microplate readers | 346             | ~410 (Ca <sup>2+</sup> bound)<br>~485 (Ca <sup>2+</sup> free) | 1 x 50 ug<br>20 x 50 ug |
| eFluor® 514 Calcium Sensor Dye | 65-0859        | Fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers                         | 490             | 514   | 1 x 1 mg<br>10 x 50 ug  |

Mini CellVue® Kits: contain 1 vial of dye stock (1 mM in ethanol) and 1 vial of Diluent C (the labeling vehicle)  
Midi CellVue® Kits contain two vials of dye stock (1 mM in ethanol) and six vials of Diluent C (the labeling vehicle).

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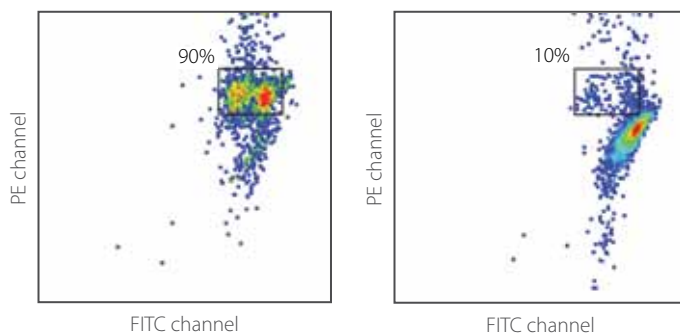
# Cell Death

- JC-1
- Annexin V

## JC-1 Mitochondrial Membrane Potential Dye

**JC-1** is an important tool for determining the loss of mitochondrial membrane potential associated with apoptosis or cell stress. In healthy cells, JC-1 accumulates in mitochondria and subsequently forms aggregates which display red fluorescence. In apoptotic cells, the loss of mitochondrial membrane potential deters the formation of these aggregates, and results in accumulation of green-fluorescent, monomeric JC-1 in the cytoplasm. This visible change from red to green is a valuable, qualitative index of apoptosis for use in fluorescence microscopy.

For quantitative analysis by flow cytometry, particularly in conjunction with other apoptotic indicators such as Annexin V, JC-1 is ideal and is easily measured as a shift in emitted light (JC-1 monomeric form ~530 nm, whereas emission of J-aggregate ~590 nm) when excited at 488 nm.

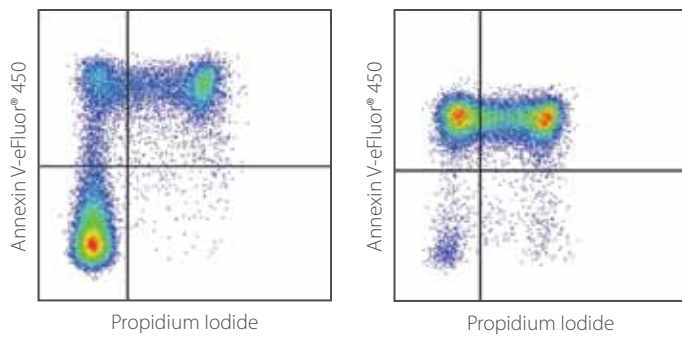


### JC-1

*Balb/c thymocytes were kept on ice overnight (left) or cultured overnight at 37°C (right) then stained with JC-1 at 2.5 ug/ml for 10 minutes at room temperature and subsequently stained with Annexin V-APC (cat. no. 88-8007). Annexin positive cells were used for analysis.*

## Annexin V Apoptosis Detection Kits

Annexins are a family of calcium-dependent phospholipid-binding proteins, which bind to phosphatidylserine (PS) to identify apoptotic cells. In healthy cells, PS is predominantly located along the cytosolic side of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution in the phospholipid bilayer and translocates to the extracellular membrane, which is detectable with fluorescently labeled **Annexin V**. In early stages of apoptosis, the plasma membrane excludes viability dyes such as propidium iodide and 7-AAD, therefore cells which display only Annexin V staining (PI/7-AAD negative) are in early stages of apoptosis. During late-stage apoptosis, loss of cell membrane integrity allows Annexin V binding to cytosolic PS, as well as cell uptake of PI and 7-AAD. Annexin V staining, paired with 7-AAD or propidium iodide (PI) is widely used to identify apoptotic stages by flow cytometry.



### Annexin V-eFluor® 450

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-eFluor® 450 Apoptosis Detection Kit and Propidium Iodide Staining Solution (cat. no. 00-6990).

### Products for Analysis of Cell Death

| Description                                   | Catalog Number | Sizes                              |
|---|----------------|------------------------------------|
| JC-1 Mitochondrial Membrane Potential Dye     | 0851           | 5 mg                               |
| Annexin V-APC Apoptosis Detection Kit         | 8007           | 50 tests<br>100 tests              |
| Annexin V-Biotin Apoptosis Detection Kit      | BMS306BT       | 20 tests<br>100 tests<br>300 tests |
| Annexin V-eFluor® 450 Apoptosis Detection Kit | 8006           | 50 tests<br>200 tests              |
| Annexin V-FITC Apoptosis Detection Kit        | 8005           | 50 tests<br>200 tests              |
| Annexin V-PE Recombinant Protein              | BMS306PE       | 20 tests<br>100 tests              |

 New products are launched regularly. **Discover more at [www.eBioscience.com](http://www.eBioscience.com).**



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