

Assay Cells in Living Color

When observing a particular facet of eukaryotic cell function, you often need your cells to function throughout your experiment. Our Vybrant fluorescent tracers and fluorescence-based assays are specifically designed for live-cell experiments.

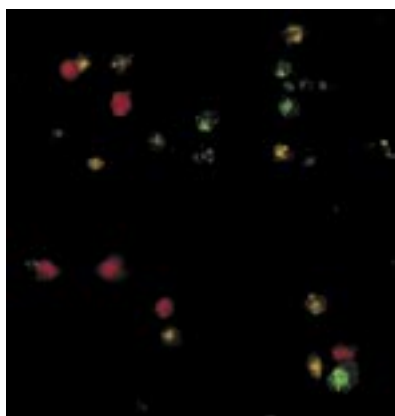


Figure 1. Three populations of P3X cells were mixed after being separately labeled with the Vybrant Dil, DiO, and DiD cell-labeling solutions.

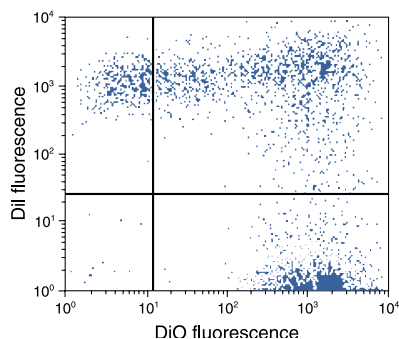


Figure 2. Polyethylene glycol–induced fusion of Jurkat cells detected by flow cytometry. Two populations of Jurkat cells were separately labeled, one with the Vybrant Dil cell-labeling solution and the other with the Vybrant DiO cell-labeling solution. Equal portions (1 mL) of the labeled cell suspensions were combined and treated with poly(ethylene glycol) for 45 seconds to induce fusion. The mixed-cell population was analyzed by flow cytometry. Double-labeled fused cells appear in the upper right quadrant of this bivariate correlation plot.

Trace Live Cells with Membrane Labels

Dil, DiO, and DiD are widely used for membrane tracing because they are highly fluorescent and quite photostable when incorporated into membranes, but only weakly fluorescent in aqueous solutions. However, the highly lipophilic nature of Dil, DiO, and DiD often poses an obstacle to uniform labeling of cells growing in aqueous culture media. Our **Vybrant Dil cell-labeling solution** overcomes this difficulty by providing the orange-fluorescent Dil tracer in a specially formulated solution that can be added directly to normal culture media to uniformly label adherent cells or cells in suspension. We also prepare the complementary **Vybrant DiO cell-labeling solution** and **Vybrant DiD cell-labeling solution**, which allow cell populations to be distinctly labeled with green and red fluorescence, respectively (Figures 1 and 2). For multicolor applications, all three Vybrant cell-labeling solutions are available in our **Vybrant Multicolor Cell-Labeling Kit**.

Trace Live Cells with a Cytoplasmic Label

Our **Vybrant CFDA SE Cell Tracer Kit** was formulated for cell migration, proliferation, and transplantation experiments, as well as other applications requiring a brightly fluorescent polar tracer that is retained in live cells through several generations. Carboxyfluorescein diacetate succinimidyl ester (CFDA SE) is a nonfluorescent amine-reactive tracer that can passively diffuse into cells, where its acetate groups are cleaved by intracellular esterases to yield the highly fluorescent, amine-reactive carboxyfluorescein succinimidyl ester. Upon reaction with intracellular amines, this reactive fluorescein derivative forms a fluorescent dye–protein adduct that is well retained in cells as they move and divide during normal development or after experimental manipulation. This dye–protein adduct can also be fixed with formaldehyde or glutaraldehyde for subsequent labeling experiments or for long-term storage. The Vybrant CFDA SE Cell Tracer Kit provides CFDA SE in ten separate vials that allow you to perform small-scale experiments without preparing excess quantities of the perishable CFDA SE stock solution.

Detect Apoptosis with Annexin V Conjugates

Fluorescent conjugates of annexin V, a Ca^{2+} -dependent phospholipid-binding protein, can serve as quick and reliable indicators of phosphatidylserine flipping, a hallmark of apoptosis. In collaboration with NeXins Research BV, the original developer of fluorescent phosphatidylserine-

binding proteins, we provide the best and brightest annexin V conjugates available (see page 10). These conjugates are included in several of our Vybrant Apoptosis Assay Kits, which also provide a cell-impermeant nucleic acid stain to serve as a marker for necrotic cells.

Our **Vybrant Apoptosis Assay Kit #1** provides recombinant annexin V conjugated to the Alexa Fluor 488 dye, one of our brightest and most photostable green fluorophores, along with a ready-to-use solution of the SYTOX Green nucleic acid stain. After staining a cell population with Alexa Fluor 488 annexin V and SYTOX Green dye in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show a much higher level of green fluorescence, and live cells show little or no fluorescence. These populations can easily be distinguished using a flow cytometer with the 488 nm spectral line of an argon-ion laser for excitation (Figure 3). Both Alexa Fluor 488 annexin and the SYTOX Green dye emit a green fluorescence that can be detected in the FL1 channel, freeing the other channels for the simultaneous detection of additional fluorescent probes with different emission spectra. Indeed, reliable detection of apoptosis often requires a combination of different measurements because no single parameter defines apoptosis in all systems.

Our **Vybrant Apoptosis Assay Kit #2** provides a sensitive two-color assay that employs our green-fluorescent Alexa Fluor 488 annexin and a ready-to-use solution of the red-fluorescent propidium iodide nucleic acid stain. When stained with these two dyes, the apoptotic-cell population shows green fluorescence, the dead-cell population shows red and green fluorescence, and the live-cell population exhibits little or no fluorescence, each of which can be distinguished using a flow cytometer with an argon-ion laser (Figure 4). ➡

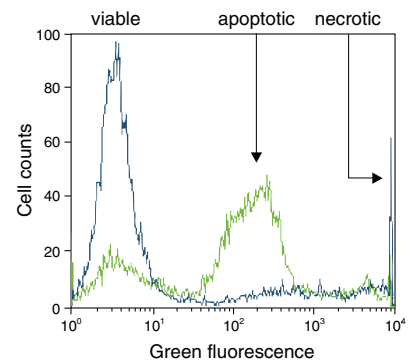


Figure 3. Jurkat human T-cell leukemia cells treated with 10 μ M camptothecin for four hours (green line) or untreated (as control, blue line). Cells were then treated with the reagents in the Vybrant Apoptosis Assay Kit #1, followed by flow cytometric analysis. Note that the camptothecin-treated cells (green line) have a significantly higher percentage of apoptotic cells (intermediate green fluorescence) than the basal level of apoptosis seen in the control cells (blue line).

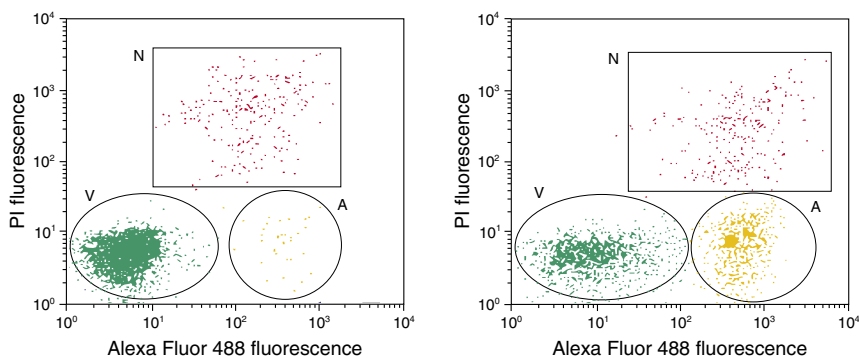


Figure 4. Jurkat human T-cell leukemia cells treated with 10 μ M camptothecin for four hours (right) or untreated (as control, left). Cells were then treated with the reagents in the Vybrant Apoptosis Assay Kit #2, followed by flow cytometric analysis. Note that the camptothecin-treated cells (left) have a significantly higher percentage of apoptotic cells (indicated by an "A") than the basal level of apoptosis seen in the control cells (left). V = viable cells, N = necrotic cells.

Assay Cells in Living Color, continued

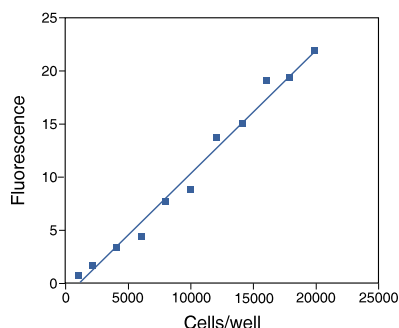


Figure 5. Detection limit of C_{12} -resazurin and linear response with increasing cell number using our Vybrant Cell Metabolic Assay Kit. Jurkat cells were loaded with $5 \mu\text{M}$ C_{12} -resazurin for 15 minutes. The resulting signal was measured in a fluorescence microplate reader with excitation/emission at 530/590 nm. For comparison, the detection limit for alamarBlue in a similar experiment was ~ 8000 cells/well (data not shown).

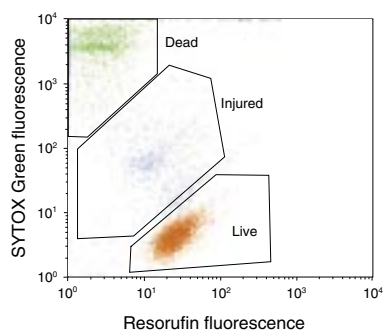


Figure 6. Analysis of Jurkat human T-cell leukemia cells using the LIVE/DEAD Cell Vitality Kit. Cells were treated with $10 \mu\text{M}$ camptothecin for four hours, then incubated with the reagents in the LIVE/DEAD Cell Vitality Assay Kit and analyzed by flow cytometry.

Detect Metabolic Activity and Membrane Permeability in Live Cells

The enhanced uptake, turnover, and retention of our lipophilic resazurin derivative, C_{12} -resazurin, by metabolically active cells translates into brighter signals and better detection limits when compared to conventional metabolic assays that employ resazurin (also known as alamarBlue) alone (Figure 5). The new **LIVE/DEAD Cell Vitality Assay Kit** allows you to assess two important characteristics of a cell's health: metabolic activity and cell membrane permeability. The kit provides C_{12} -resazurin and SYTOX Green nucleic acid stain along with the other key reagents necessary for a single-cell metabolic analysis by flow cytometry. After a short incubation with C_{12} -resazurin, live and actively metabolizing cells generate the red-fluorescent C_{12} -resorufin reduction product, whereas cells with reduced metabolism show little or no fluorescent staining. SYTOX Green stain is impermeant to live and early apoptotic cells, but stains late-stage apoptotic and necrotic cells with intense green fluorescence (Figure 6).

Alternatively, to detect cell metabolic activity using a microplate reader, the **Vybrant Cell Metabolic Assay Kit** is ideal. The kit includes C_{12} -resazurin for assaying metabolic activity and resorufin for generating a standard curve.

Assay Cell Death by Monitoring Enzyme Release

Instead of assaying cell death with probes that gain entry to the interior of the cell through areas of plasma membrane damage, our **Vybrant Cytotoxicity Assay Kit** monitors the release of glucose 6-phosphate dehydrogenase (G6PD) — a ubiquitous, cytosolic enzyme involved in the pentose phosphate pathway — from damaged or dying cells into the surrounding medium. This Vybrant Kit provides all enzymes and substrates required to detect G6PD release via a two-step enzymatic process that leads to the formation of the red-fluorescent resorufin. The resulting fluorescent signal is proportional to the amount of G6PD released into the cell medium, which correlates with the number of dead cells in the sample (Figure 7). This assay can be completed in less than an hour and is effective with as few as 500 cells per sample.

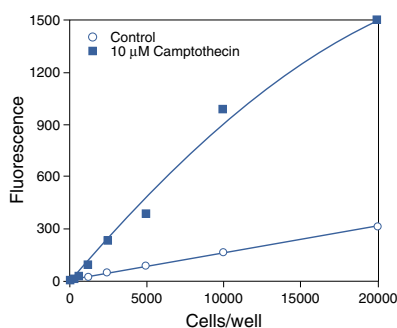


Figure 7. Detection of dead and dying cells using the Vybrant Cytotoxicity Assay Kit. Jurkat cells were treated with $10 \mu\text{M}$ camptothecin for six hours, then assayed for glucose 6-phosphate dehydrogenase release. An untreated control sample is shown for comparison. The fluorescence was measured in a microplate reader (excitation/emission $\sim 530/590$ nm). A background of 55 fluorescence units was subtracted from each value.

Quantitate Phagocytosis in Live Cells

Our **Vybrant Phagocytosis Assay Kit** provides a convenient set of reagents for quantitating phagocytosis and assessing the effects of certain drugs or environmental factors on this important cellular process. In this assay, the cells of interest are incubated first with our green-fluorescent *E. coli* BioParticles, which are chemically killed bacteria that can be internalized by phagocytosis, and then with trypan blue, which quenches the fluorescence of any extracellular *E. coli* BioParticles (Figure 8). The fluorescence emitted by the engulfed particles can then be selectively detected with a fluorescence-based microplate reader. 🧪

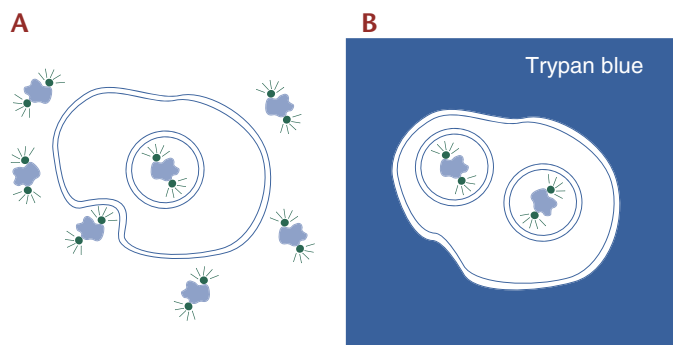


Figure 8. Vybrant Phagocytosis Assay Kit for the simple quantitation of phagocytosis. (A) Phagocytic cells are incubated with green-fluorescent BioParticles. (B) The fluorescence from any noninternalized BioParticles is then quenched by the addition of trypan blue, and the samples are subsequently assayed with a fluorescence-based microplate reader equipped with filters for the detection of fluorescein (FITC).

ORDERING INFORMATION

Cat #	Product Name	Unit Size
NEW L34951	LIVE/DEAD® Cell Vitality Assay Kit *C ₁₂ resazurin/SYTOX® Green* *1000 assays*	1 kit
V13240	Vybrant® Apoptosis Assay Kit #1 *Alexa Fluor® 488 annexin V/SYTOX® Green* *50 assays*	1 kit
V13241	Vybrant® Apoptosis Assay Kit #2 *Alexa Fluor® 488 annexin V/propidium iodide* *50 assays*	1 kit
V23110	Vybrant® Cell Metabolic Assay Kit *with C ₁₂ -resazurin* *500–1000 assays*	1 kit
V12883	Vybrant® CFDA SE Cell Tracer Kit	1 kit
V22888	Vybrant® CM-Dil cell-labeling solution	1 mL
V23111	Vybrant® Cytotoxicity Assay Kit *G6PD release assay* *1000 assays*	1 kit
V22887	Vybrant® DiD cell-labeling solution	1 mL
V22885	Vybrant® Dil cell-labeling solution	1 mL
V22886	Vybrant® DiO cell-labeling solution	1 mL
V22889	Vybrant® Multicolor Cell-Labeling Kit *DiO, Dil, DiD solutions, 1 mL each*	1 kit
V6694	Vybrant® Phagocytosis Assay Kit *250 assays*	1 kit