Green Live/Dead Stain
Catalog #6342

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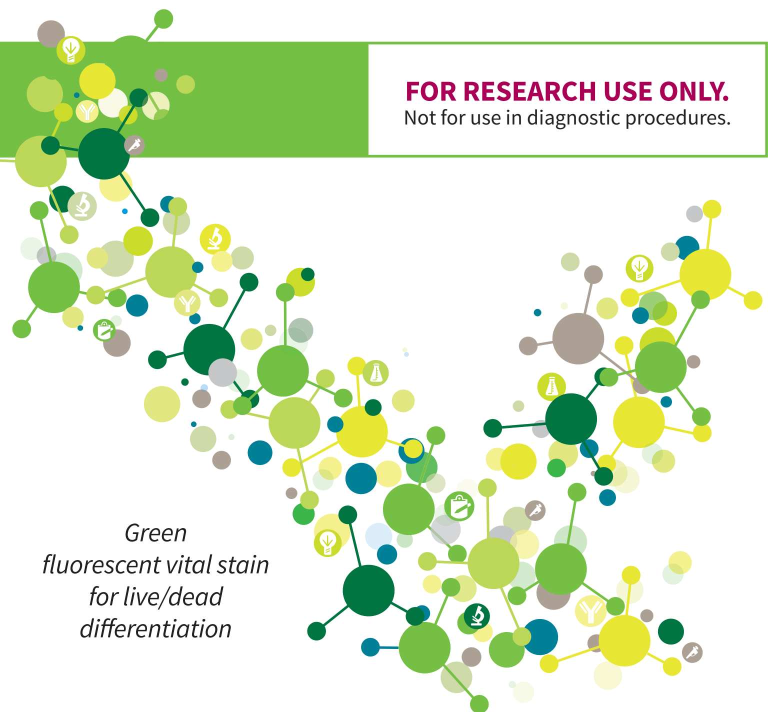

Green fluorescent vital stain for live/dead differentiation

INTRODUCTION

Green Live/Dead Stain (catalog #6342) is a vital dye that exhibits intact cell membrane exclusion properties analogous to the popular red fluorescing vital dyes, Propidium Iodide (PI), 7-aminoactinomycin D (7-AAD) and DRAQ7™. Like the red fluorescing dyes, Green Live/Dead Stain is excluded from intact, healthy cells due to its polar nature. In the presence of cells exhibiting compromised membrane integrity, Green Live/Dead Stain penetrates the cell and nuclear membrane barriers and intercalates tightly to DNA in a manner analogous to PI and 7-AAD. When bound to DNA, it acquires a greatly enhanced fluorescence potential (>2000X) in the green emission range. These important vital dye properties enable Green Live/Dead Stain to be used in flow cytometry-based protocols to assess the percentage of late apoptotic, necrotic, and membrane-compromised cells within a sample cell population.

When bound to nucleic acids, the maximum absorption of Green Live/Dead Stain is 495 nm and the maximum emission is 512 nm (Figure 1). Cells can be viewed through a fluorescence microscope (Figures 2 & 5) or analyzed with a flow cytometer (Figure 4).

Green Live/Dead Stain is provided as a 500 µM concentrated stock solution dissolved in DMSO. For flow cytometry applications, a staining concentration of 50 nM is recommended. Therefore, using sample sizes of 0.5 mL, a single 50 µL vial (catalog #6342) provides enough reagent for 1000 tests. For fluorescence microscopy applications, a usage concentration of 0.5 µM is suggested. In this way, a vial is sufficient for 100 tests (0.45 mL sample sizes). Green Live/Dead Stain can be used with ICT's red FLICA® 660 caspase inhibitor reagents (e.g., catalog #9120) to identify four populations of cells: living; early apoptotic; late apoptotic; and necrotic (Figures 4 & 5).

SPECIFICATIONS

- 50 µL at 500 µM
- Yellow liquid
- Ex/Em: 495 nm/512 nm

STORAGE

- -20°C
- Shelf-life up to 24 months when stored frozen and protected from light.
- Avoid freeze/thaw cycles.

SAFETY

- Contains DMSO
- **Warning!** Combustible liquid. Causes skin irritation. Causes serious eye irritation.
- See Safety Data Sheet (SDS)
- SDS available at www.immunochemistry.com and by calling ICT.
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HOW TO USE

Green Live/Dead Stain is supplied as a 500 µM concentrated stock solution in DMSO. The following protocol is suggested to stain dead and membrane-compromised cells in suspension.

PREPARATION

1. Spin thawed vial briefly in a microcentrifuge to remove any reagent that may have become trapped in the cap.
2. Dilute Green Live/Dead Stain concentrated stock solution (500 µM) 1:100 in PBS to prepare a 5000 nM working solution. For example, add 10 µL stock concentrate to 990 µL PBS. Prepare only what is needed for the experiment. Working solution should be used immediately, and any remaining diluted solution should be discarded.

FOR FLOW CYTOMETRY APPLICATIONS

1. Spike samples with a 1:100 dilution of the 5000 nM working solution (Preparation, step 2). For example, spike 0.495 mL samples with 5 µL working solution.
2. Incubate samples ~ 10 min at RT, protected from light.
3. Analyze with a flow cytometer using a blue laser at 488 nm and a 530/30 (FL1) emission filter setting, or similar.

FOR FLUORESCENCE MICROSCOPY APPLICATIONS

1. Spike samples with a 1:10 dilution of the 5000 nM working solution (Preparation, step 2). For example, spike 0.45 mL samples with 50 µL working solution.
2. Incubate samples ~ 10 min at RT, protected from light.
3. When bound to DNA, the peak absorption of Green Live/Dead Stain is 495 nm and the maximum emission is 512 nm. Visualize with a fluorescence microscope using optical filters that best approximate these settings.

FIGURE 1: FLUORESCENCE SPECTRA

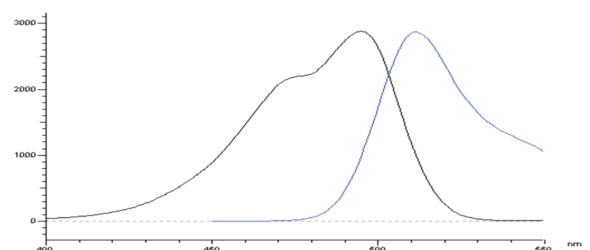

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FIGURE 2: MICROSCOPY IMAGING

Jurkat cells were exposed to 3% formaldehyde for 30 minutes. Following the formaldhehyde treatment, cells were stained with Green Live/Dead stain and then imaged with a Nikon E800 microscope (DIC overlay image, below). Cells with compromised membranes stained green, while cells with intact membranes excluded the Green Live/Dead stain and remained unstained. Data courtesy of Mrs. Tracy Murphy, ICT.

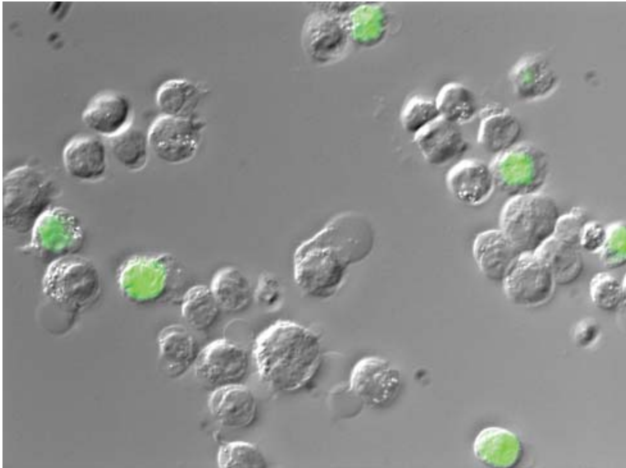

FIGURE 3: CELL VIABILITY IN RESPONSE TO STRESSFUL CULTURE CONDITIONS

Jurkat cells were transferred to a suboptimal culture environment (serum-free DMEM at RT and 0.03% CO_2). Viability results obtained with Green Live/Dead Stain after 3 hours were consistent with the commonly used vital dyes PI, 7-AAD, and DRAQ7.

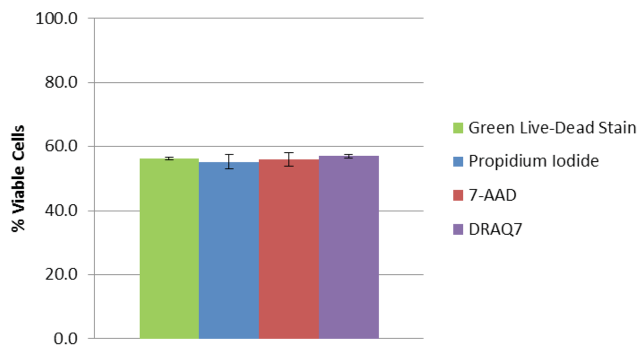

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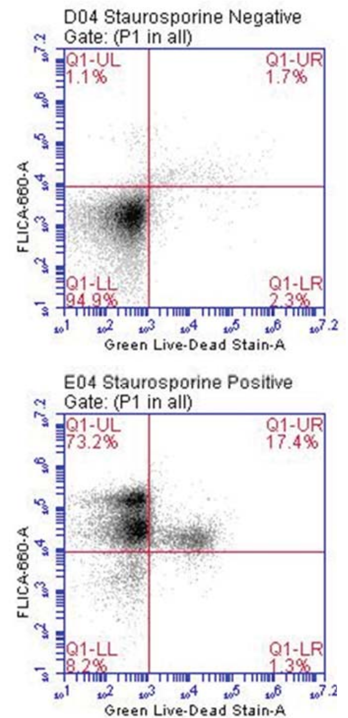
FIGURE 4: APOPTOSIS VS. NECROSIS

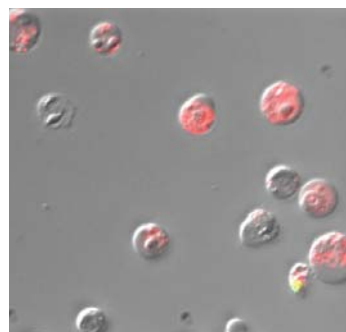
To differentiate cell death pathways, staurosporine-treated Jurkat cells were stained with FLICA® 660 Poly Caspase Assay Kit (catalog #9120) and Green Live/Dead Stain. Cells were analyzed by flow cytometry to detect green on the X-axis (Green Live/Dead Stain, necrosis) and far red on the Y-axis (FLICA® 660, apoptosis).



Four populations of cells were detected: (LL) unstained live cells; (LR) necrotic cells fluoresce green with Green Live/Dead Stain; (UR) cells in late apoptosis are dually stained with red FLICA® 660 and Green Live/Dead Stain (they have active caspases and permeabilized membranes); and (UL) cells in early apoptosis fluoresce red with FLICA® 660. In this experiment, staurosporine primarily triggered programmed cell death via the caspase cascade, not necrosis. The majority of treated cells (73.2%) are in early stage apoptosis (bottom plot, UL). Data courtesy of Mrs. Tracy Murphy, ICT.

FIGURE 5: MULTIPARAMETRIC ANALYSIS - GREEN LIVE/DEAD STAIN WITH FLICA® 660

Green Live/Dead Stain and FLICA® 660 Poly Caspase detection reagent (catalog #9120) were used to assess cell death in staurosporine-treated Jurkat cells. Following a four hour induction period, treated cells were stained with the reagents and imaged with a Nikon E800 microscope (DIC overlay image, left). The majority of cells were in the early stages of apoptosis (active caspases stained red with FLICA® 660). One cell in this field of view (bottom right corner) exhibited both red and green fluorescence, indicating the loss of membrane integrity that is characteristic of late-stage apoptosis.



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ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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