Dead-cell discrimination in a different light

SYTOX® RED DEAD CELL STAIN.

Thousands of flow cytometry runs every day rely on dead-cell discrimination either as an endpoint result or as one parameter in a broader experimental query. Fluorescent markers for dead-cell staining are therefore indispensable reagents for flow cytometric analysis.

Propidium iodide (PI) has widely been utilized as a stain to differentiate live and dead cells in flow cytometry experiments. Because of its broad emission spectrum, multiple detection channels on the flow cytometer are occupied when PI is used, which limits the number of parameters that can be detected in a given experiment. In addition, the emission of PI directly overlaps the emission of R-phycoerythrin (R-PE), making PI incompatible with R-PE–labeled probes. Dead-cell stains that can be excited by light sources other than the 488 nm laser are highly sought-after reagents because they allow the 488 nm laser to be reserved for bright fluorochromes on antibodies directed against challenging antigens.

SYTOX® Red dead cell stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes, but will not cross intact cell membranes. After a brief incubation with SYTOX® Red stain (excitation/emission ~640/658 nm), the nucleic acids of dead cells fluoresce bright red when excited with 633 or 635 nm laser light. And because SYTOX® Red dead cell stain does not rely on the 488 nm laser and has an emission signal limited to one channel, adding it to other dyes in multicolor flow cytometry experiments is easy. For example, staining an ammonium chloride–lysed whole blood sample with mouse anti–human CD4 R-PE Alexa Fluor® 700, mouse anti–human CD8 R-PE, and mouse anti–human CD3 Alexa Fluor® 488 for 30 minutes, followed by 5 µM SYTOX® Red stain, allowed easy gating on the live-cell population and clear visualization of the various immunophenotypes (Figure 1). These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® Red dead cell stain a simple and quantitative single-step dead-cell indicator for use with red laser–equipped flow cytometers. ■

Product	Quantity	Cat. no.
SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 ml	S34859

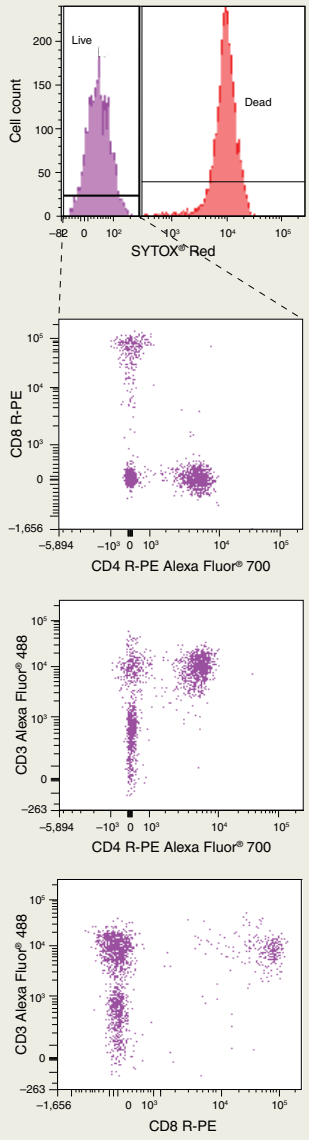

Figure 1—Illuminate your samples with better dead-cell discrimination. The blood cell sample was stained as described in the text. Using a BD™ LSR II system (BD Biosciences), the sample was gated on lymphocytes and on cells negative for SYTOX® Red stain. Fluorescence was monitored using 488 and 633 nm excitation and 530/30, 585/42, 720/20, and 660/20 nm bandpass filters.