BD Horizon™ Cell Proliferation Dyes

Features

- Measure cell proliferation in a variety of cell types
- Detectable post cell fixation and permeabilization, increasing experimental flexibility
- Available for both the violet and blue lasers, allowing flexibility in multicolor panel design

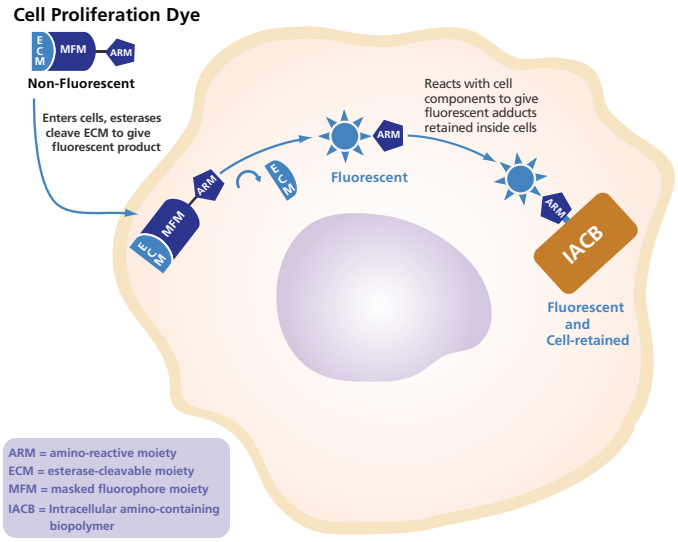

Figure 1. BD Horizon™ cell proliferation dyes freely enter a cell. Once inside the cell, the dyes are cleaved by nonspecific esterases and release a fluorescent molecule, which becomes trapped inside the cell.

Cell proliferation is an increase in the number of cells as a result of growth and division. The balance of cell proliferation and apoptosis is important for both development and normal tissue homeostasis. Multiple stimuli, such as cytokine treatment, can affect cell proliferation.

How the dye works

The BD Horizon™ cell proliferation dyes are non-fluorescent molecules that passively diffuse into cells. Once inside the cell, they are hydrolyzed by intracellular non-specific esterases to become fluorescent products. After hydrolysis, the dye becomes trapped inside the cell.

BD Horizon™ Violet Proliferation Dye 450 (VPD450) is excited by the violet laser and emits at 450 nm (V450 channel). BD Horizon™ CFSE is excited by the blue laser, and emits at 521 nm (FITC channel). These reagents allow for flexibility in multicolor panel design.

Stimulated cells loaded with a BD Horizon cell proliferation dye will divide and form new generations of cells. Each new population will have approximately half the fluorescence intensity of the previous division. These divisions can be counted by displaying a fluorescence histogram, where each peak corresponds to a separate generation of cellular division. Combining cell generations with specific cell surface and intracellular markers allows the determination of the timing of key events in experimental systems.

Other tools for the study of cell proliferation

BD has a broad portfolio of tools for the study of cell proliferation in a variety of samples by multiple detection methods. These tools are summarized in Table 1.

Table 1. Other cell proliferation tools

Reagents	Measures	Mechanism	Measured By
Propidium iodide (PI), 7-aminoactinomycin D (7-AAD), Hoechst 33342, DAPI	DNA Content	Intercalation into double stranded DNA	Flow cytometry
BrdU incorporation	DNA Synthesis	Bromodeoxyuridine replaces thymidine (T) in dividing DNA and is then detected by antibodies to BrdU	Flow cytometry, cell imaging, immunohistochemistry
Antibodies to Ki-67, PCNA	Protein level	Levels increase as a result of proliferation	Flow cytometry, cell imaging, immunohistochemistry, Western blot

Visit **bdbiosciences.com** for more information.

BD Horizon™ Cell Proliferation Dyes

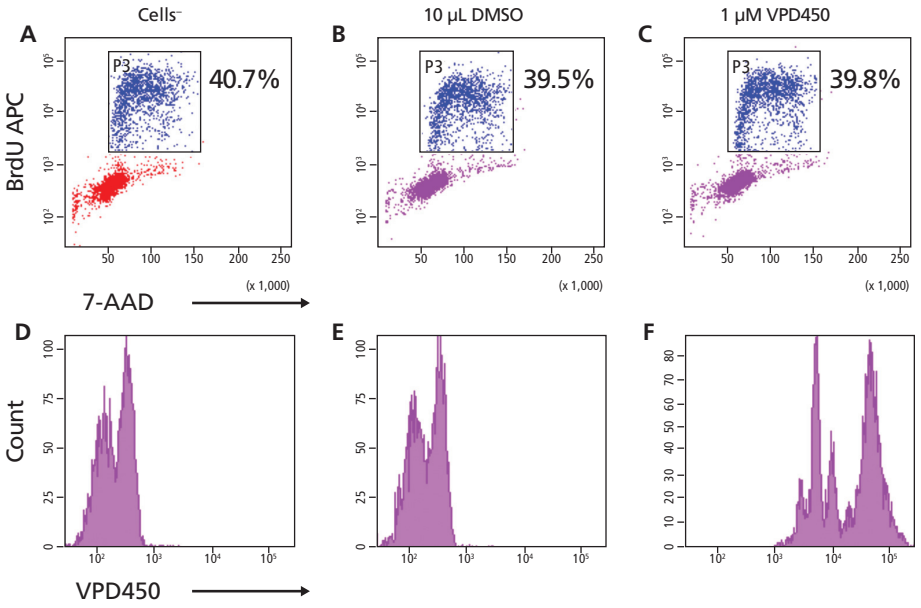

Figure 2. Concentration of VPD450 and cell-cycle kinetics on mouse spleen stimulated with anti-CD3e and anti-CD28.

C57 Black/6 splenocytes were either loaded with varying concentrations of BD Horizon VPD450, DMSO, or left as untreated controls, then stimulated with anti-CD3e and anti-CD28 for two days. Cells were pulsed with BrdU prior to harvesting, then stained with APC anti-BrdU and 7-AAD (Cat. No. 552598). The top panels (A–C) illustrate APC anti-BrdU and 7-AAD staining. The bottom panels (D–F) illustrate the corresponding VPD450 histograms. The control cells (Cells⁻) (Panel A) and the 1-µM VPD450-loaded cell population (Panel C) demonstrated a similar percentage of BrdU⁺ cells (40.7% and 39.8%, respectively). Higher concentrations of dye can negatively impact cell proliferation (data not shown). To confirm that the DMSO (which is used as a solvent for VPD450) is not responsible for a decrease in proliferation, a DMSO group was included (Panel B). DMSO-treated cells incorporated a similar percentage of BrdU compared to the Cells⁻ group and the 1-µM VPD450-loaded cell populations.

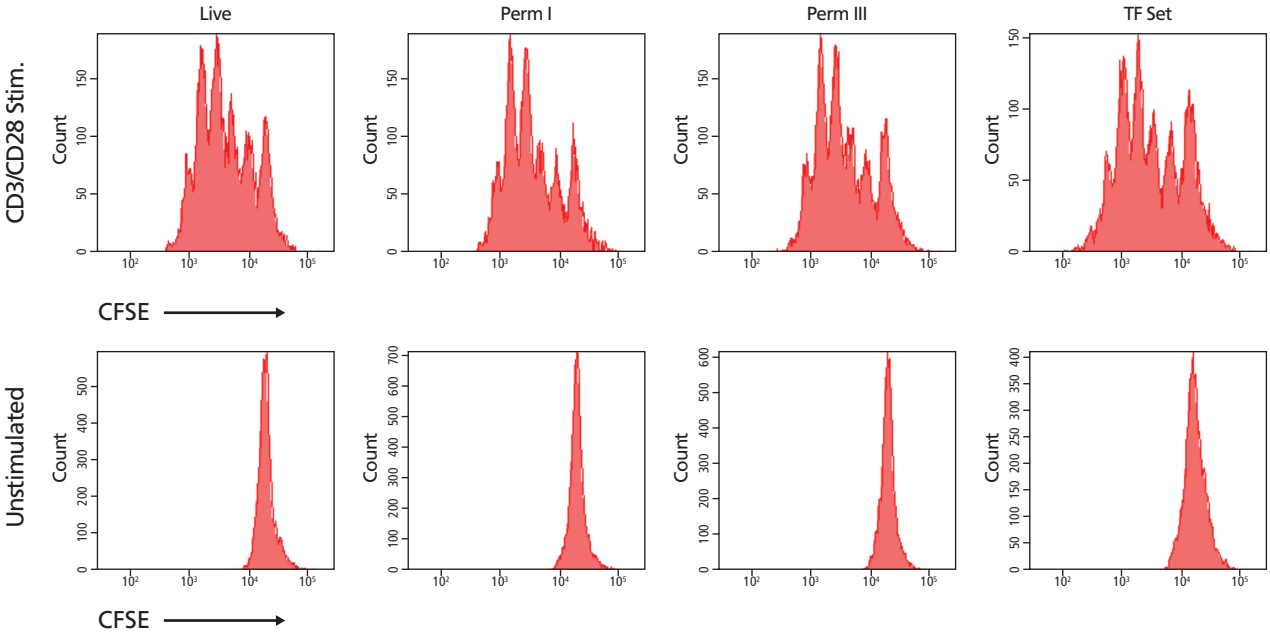

Figure 3. Fluorescent staining of BD Horizon CFSE in live or fixed and permeabilized mouse splenocytes.

Balb/c splenocytes were loaded with 1-µM BD Horizon CFSE, and then left unstimulated or stimulated with anti-CD3 and anti-CD28 for 3 days. Cells were analyzed live or fixed and permeabilized with BD Phosflow™ Perm Wash Buffer I (Cat. No. 557885) , BD Phosflow™ Perm Buffer III (Cat. No. 558050), or BD Pharmingen™ Transcription Factor Buffer Set (TF set) (Cat. No. 562574). All samples were acquired using a BD™ LSRFortessa flow cytometry system and histograms were derived from gated events based on light scattering characteristics of Balb/c lymphocytes. CFSE fluorescence profile and resolution of proliferating generations is maintained after fixation and permeabilization by the Perm I, Perm III, and TF Buffer Set protocols.

Ordering Information

Description	Size	Cat. No.
BD Horizon Violet Proliferation Dye 450	1 mg	**562158**
BD Horizon CFSE	1 mg	**565082**

23-13105-01

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