BD Biosciences Nucleic Acid Dyes

Features

- Applicable for DNA content analysis, viability analysis, and as nuclear counterstains
- Multitude of colors allows for easier panel design for multicolor flow cytometry and multiparameter immunofluorescence imaging

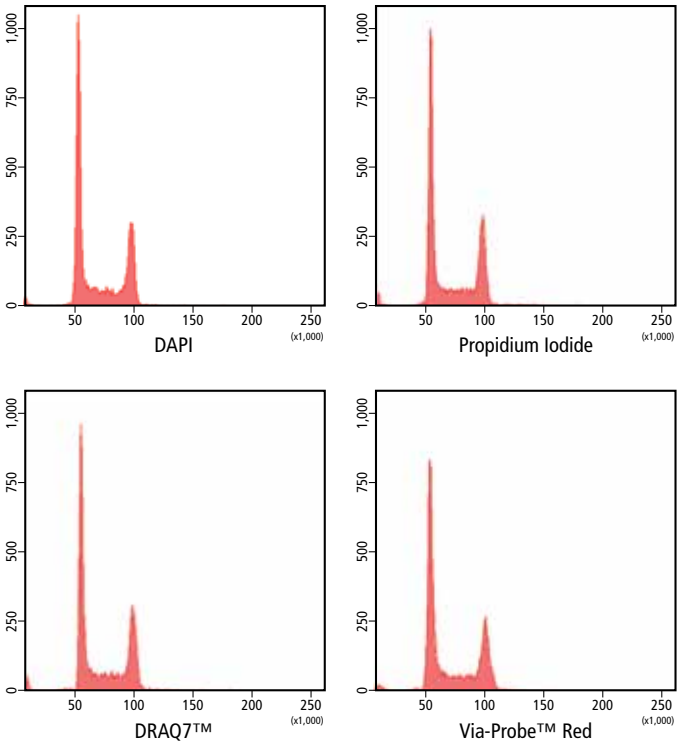

Figure 1. DNA Content Analysis in Jurkat Cells

Jurkat cells (ATCC, TIB-152) were fixed and permeabilized with ice-cold, 70% ethanol and then stained with BD Pharmingen™ DAPI Solution (Cat. No. 564907), BD Pharmingen™ Propidium Iodide Staining Solution (Cat. No. 556463), BD Pharmingen™ DRAQ7™ (Cat. No. 564904), or BD Via-Probe™ Red (Cat. No. 565804) in PBS with 0.25 mg/mL of RNAse, and then analyzed using the violet (BV421 filter set), blue (FITC filter set), yellow-green (PE-Cy™7 filter set), or red (APC filter set) lasers, respectively. All samples were acquired using a BD LSRFortessa™ cell analyzer, and histograms were derived from gated events based on light scattering characteristics of cells. Cells with 2N DNA content (first peak) are in the G0 or G1 phases, cells with 4N DNA content (second peak) are in the G2 or M phases, and cells with DNA content between 2N and 4N are in the S phase. All four dyes provide resolution of cell cycle status, enabling analysis across multiple laser lines. For multiplexing with specificities or fluorochromes that might be denatured by ethanol treatment, equivalent DNA content histograms can also be generated using the BD Pharmingen™ Transcription Factor Buffer Set (Cat. Nos. 562725, 562574) (data not shown).

Available for multiple laser lines

BD Biosciences offers a rainbow of nucleic acid dyes for DNA content analysis, viability analysis, and immunofluorescence imaging for use with multiple laser lines (Table 1).
BD Pharmingen™ DAPI Solution and BD Pharmingen™ Hoechst 33342 Solution are available for the UV or violet lasers.
BD Via-Probe™ Green is available for the blue laser.
BD Pharmingen™ Propidium Iodide Staining Solution (PI) and BD Pharmingen™ 7-Amino-Actinomycin D (7-AAD) are available for the yellow-green or blue lasers. BD Pharmingen™ DRAQ5™ and DRAQ7™ are excited by both the yellow-green and red lasers, and BD Via-Probe™ Red is available for the red laser. Together, these dyes provide maximum flexibility for multicolor flow cytometry and immunofluorescence imaging.

Tools to monitor cell cycle and DNA content

In fixed and permeabilized samples, BD Biosciences nucleic acid dyes bind stoichiometrically to DNA, allowing a quantitative assessment of DNA content and correlation with the cell cycle phase. Combined with the power of single cell analysis provided by flow cytometry, a heterogeneous population of cells can be assessed for the cell cycle status of individual cells (Figure 1). Membrane-permeable nucleic acid dyes, such as Hoechst 33342 and DRAQ5, can also be used to assess DNA content in unfixed cells.

Increased flexibility in viability analysis

BD Biosciences membrane-impermeable nucleic acid dyes include DAPI, BD Via-Probe Green, PI, 7-AAD, DRAQ7, and BD Via-Probe Red. Because these dyes cannot cross intact cell membranes, they are excluded from live cells and stain dead cells brightly. This allows the dyes to be used for viability analysis conveniently and easily, in a no-wash flow cytometry assay amenable to multiple laser lines. The dyes also can be used for multiplexing with surface marker antibodies and other apoptosis and viability probes (Figure 2).

Compatible with high parameter immunofluorescence imaging

The breadth of nucleic acid dyes offered, combined with our BD Horizon Brilliant™ Violet dyes, enables easy immunofluorescence imaging of up to five colors simultaneously (Figure 3). BD Biosciences nucleic acid dyes also are compatible with common imaging fluorochromes such as the Alexa Fluor® dyes. This enables robust high parameter immunofluorescence imaging.

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

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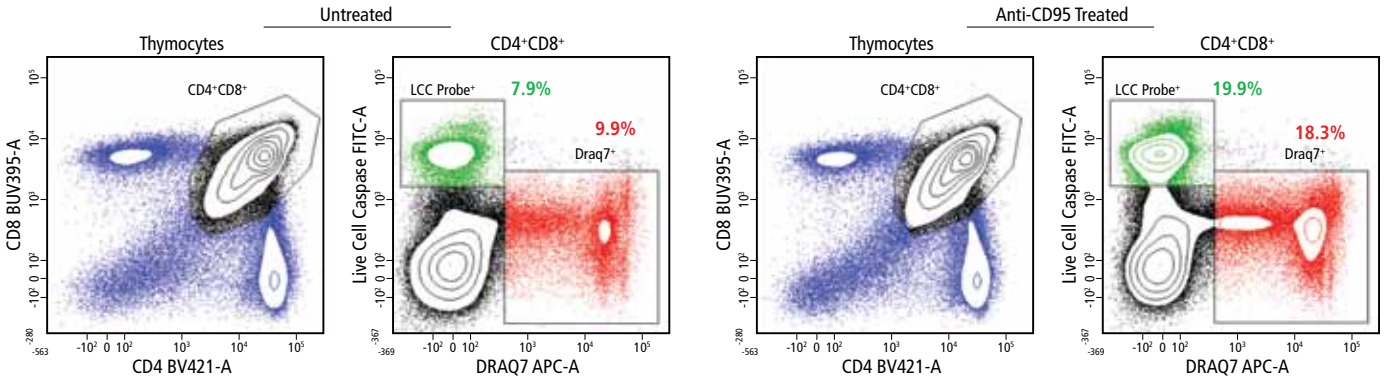

Figure 2. Multicolor Flow Cytometric Analysis of Viability in Mouse Thymocytes

Balb/c thymocytes were incubated with (right, treated) or without (left, untreated) BD Pharmingen™ Purified NA/LE Hamster Anti-Mouse CD95 (anti-FAS, Cat. No. 554254) for 6 hours in culture, followed by staining with BD Pharmingen™ Blue Live Cell Caspase Probe (Cat. No. 565519). Cells were washed and stained with BD Horizon™ BV421 Rat Anti-Mouse CD4 (Cat. No. 562891) and BD Horizon™ BUV395 Rat Anti-Mouse CD8a (Cat. No. 563786), followed by staining with 1 µM of DRAQ7. Samples were analyzed on a BD LSRFortessa cell analyzer and gated based on scatter properties to exclude debris. Anti-FAS-treated cells show an increase in the amount of $CD4^+CD8^+$ apoptotic and dead cells, as assessed by staining with Blue Live Cell Caspase Probe and DRAQ7, respectively.

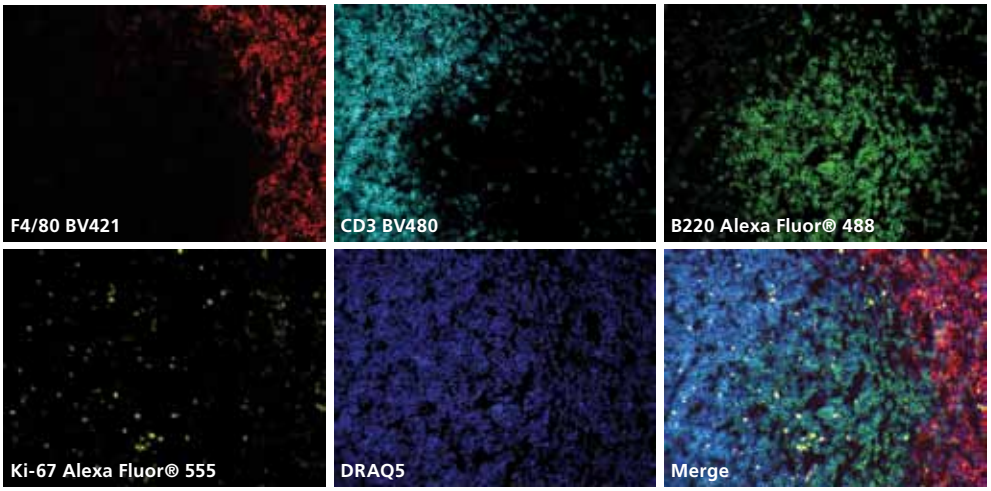

Figure 3. Multicolor Fluorescence Immunohistochemistry Staining of Mouse Spleen Tissue

C57BL/6 spleen cryosections (5 µm) from wild-type mice were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), blocked with 5% goat serum and 1% BSA diluted in 1X PBS, and stained with BD Horizon™ BV421 Rat Anti-Mouse F4/80 (Cat. No. 565411, pseudo-colored red), BD Horizon™ BV480 Rat Anti-Mouse CD3 Molecular Complex (Cat. No. 565642, pseudo-colored cyan), Alexa Fluor® 488 Rat Anti-Mouse CD45R/B220 (Cat No. 557669, pseudo-colored green), and Alexa Fluor® 555 Mouse Anti-Human Ki-67 (Cat. No. 558617, pseudo-colored yellow). Slides were nuclear counterstained with DRAQ5™ (Cat. Nos. 564902/564903, pseudo-colored blue) and mounted with ProLong® Gold. T lymphocytes ($CD3^+$), B lymphocytes ($B220^+$), macrophages (F4/80), and cycling cells (Ki-67) can be clearly distinguished in a single panel. The images were captured on a BD Pathway™ 435 cell analyzer (epifluorescence microscope) and merged using BD Attovision™ software. Original magnification: 20x.

Description	Flow Cytometry			Imaging		Excitation Laser†	Fluorescence Channel	Ex (nm)	Em (nm)‡	Cat. No.
	DNA Content		Viability							
	Live	Fixed	Live*	Live	Fixed					
BD Pharmingen Hoechst 33342 Solution	✔	✔		✔	✔	UV/Violet	DAPI (UV)/BV421 (V)	350	461	**561908**
BD Pharmingen DRAQ5™	✔	✔		✔	✔	Red/YG	Alexa Fluor® 700/APC/PE-Cy7	600/646	697	**564902, 564903**
BD Pharmingen DAPI Solution		✔	✔		✔	UV/Violet	DAPI (UV)/BV421 (V)	358	461	**564907**
BD Via-Probe Green Nucleic Acid Stain		✔	✔		✔	Blue	FITC	503	526	**565799, 565802**
BD Pharmingen Propidium Iodide Staining Solution		✔	✔		✔	YG/Blue	PE	535	617	**556463, 550825**
BD Pharmingen 7-AAD		✔	✔		✔	YG/Blue	PerCP-Cy™5.5	546	647	**559925, 555815, 555806**
BD Via-Probe Red Nucleic Acid Stain		✔	✔		✔	Red	APC	642	660	**565803, 565804**
BD Pharmingen DRAQ7™		✔	✔		✔	Red/YG	Alexa Fluor® 700/APC/PE-Cy7	599/644	694	**564904**

Table 1. Nucleic Acid Stains Offered by BD Biosciences

*For viability in fixed cells, please see our BD Horizon™ Fixable Viability Stains.

†DRAQ5 and DRAQ7 are also suboptimally excited by the blue laser.

‡When bound to dsDNA.

23-17881-00

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