

Functional stains that can be excited at 488 nm and are detectable in basic instruments with three or four fluorescence channels<sup>1</sup>.

Channel <sup>2</sup>	Fluorochrome <sup>3</sup>	Use	Excitation (nm)	Emission (nm)
<b>FL1</b>	Acridine orange	DNA	500	<b>526</b>
		RNA	460	<b>650</b>
	BCECF	Membrane integrity; pH (load as AM ester)	~505	<b>525:640</b>
	Calcein	Membrane integrity (load as AM ester)	494	<b>517</b>
	Carboxyfluorescein diacetate	Membrane integrity (load as AM ester)	511	<b>534</b>
	2',7'-dichlorofluorescein diacetate	Metabolic burst, oxidative metabolism	510	<b>532</b>
	DiBAC <sub>4</sub> (3)	Trans-membrane potential	493	<b>516</b>
	DiOC <sub>6</sub> (3)	Trans-membrane potential	484	<b>501</b>
	Fluorescein diacetate	Membrane integrity (load as AM ester)	475	<b>530</b>
	JC-1	Mitochondrial trans-membrane potential	485-585	<b>590<sup>5</sup></b>
	PicoGreen <sup>6</sup>	Highly selective for DNA	502	<b>523</b>
	Rhodamine 123	Mitochondrial trans-membrane potential	507	<b>529</b>
	SYBR® Green I <sup>6</sup>	High sensitivity DNA stain	494	<b>521</b>
	SYTOX® Green	Cell impermeant nucleic acid	504	<b>523</b>
	Thiazole orange	Nucleic acid	453,510	<b>480,</b>
	TOTO®-1	High affinity DNA stain	514	<b>533</b>
	YOYO®-1	High affinity DNA stain	491	<b>509</b>
<b>FL2</b>	Dihydroethidium (Hydroethidine™) <sup>4</sup>	Metabolic burst, oxidative metabolism	518	<b>605</b>
	Ethidium bromide	Cell impermeant nucleic acid stain; apoptosis	518	<b>610</b>
	Ethidium monoazide	Fluorescent photoaffinity nucleic acid label compatible with fixation	464	<b>625</b>
	Fluo-3, Fluo-4, Fluo-5	Calcium (load as AM ester)	464	<b>526</b>
	Propidium iodide	Cell impermeant nucleic acid stain; viability	520	<b>610</b>
	SNARF-1	Intracellular pH (load as AM ester)	488-530	<b>580:640</b>
<b>FL3</b>	7-AAD	Generally cell impermeant nucleic acid stain	546	<b>647</b>
	Fura Red™	Calcium (load as AM ester; fluorescence decreases on binding)	450-500	<b>~660</b>
<b>FL4</b>	LDS-751	Nuclear DNA	<b>543</b>	<b>712</b>
	DRAQ5	Nuclear DNA		<b>Deep red</b>

<sup>1</sup>Where there are only three fluorescence detectors, FL3 will normally detect fluorochromes emitting in the red and the long red wavelengths. <sup>2</sup>Filters as described in Table 3.1. <sup>3</sup>7-AAD = 7-aminoactinomycin D; AM = acetoxymethyl; BCECF = 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein; DiBAC<sub>4</sub>(3) = bis-(1,3-

dibutylbarbituric acid) trimethine oxonol; DiOC<sub>6</sub> (3) = 3,3'-dihexyloxacarbocyanine iodide; SNARF-1 = a seminaphthorhoda-fluor dye. <sup>4</sup>After oxidation to ethidium. <sup>5</sup>As aggregates. <sup>6</sup>Originally used in solution assays but have been used in flow cytometry.

The newer instruments have more lasers that are able to excite more fluorochromes