

Supplementary Table S1 Commonly used nucleic acid binding dyes in flow cytometry¹

Dye category	Dye	Binding target		Cell membrane permeability ^a	Use
		Major	Minor		
Minor groove binding	DAPI	DNA	–	Yes	DNA ploidy and cell cycle
	Hoechst 33342	ds DNA	–	Yes	Cell cycle
	Hoechst 33258	ds DNA	–	No	Cell cycle
	FxCycle violet	ds DNA	–	Yes	DNA ploidy and cell cycle
Interbase-intercalating	7-AAD	DNA	RNA	Weak	Viability analysis
	EtBr	ds DNA	RNA	No	Cell cycle
	Propidium iodide	DNA	RNA	No	DNA ploidy and cell cycle
	DRAQ5	DNA	RNA	Yes	Cell cycle
Dual binding	SYTOX green	DNA	RNA	No	Cell cycle and viability analysis
	TO-PRO-1	DNA	RNA	No	Cell cycle
	TO-PRO-3	DNA	RNA	No	Cell cycle
	TOTO-1	DNA	RNA	No	Cell cycle
	TOTO-3	DNA	RNA	No	Cell cycle
	YO-PRO-1	DNA	RNA	No	Cell cycle
	YOYO-1	DNA	RNA	No	Cell cycle
Nucleic acid–precipitating and metachromatic	Acridine orange	RNA + DNA	–		DNA/RNA discrimination
	Pyronin Y	RNA + DNA	–	No	DNA/RNA discrimination

Abbreviations: 7-AAD, 7-amino actinomycin-D; DAPI, 4',6-diamidino-2-phenylindole; EtBr, ethidium bromide.

^aCell membrane permeability: Cell impermeable nucleic acid binding dyes cannot percolate intact cell membrane and can bind only to extracellular DNA/RNA. Cell cycle analysis using these dyes requires treatment of cells with a permeabilizing agent (usually a detergent) to facilitate access to intracellular DNA/RNA. However, such permeabilizing reagents are not needed while using cell membrane permeable nucleic acid binding dyes.²

Supplementary Table S2 Cytogenetic ploidy status and the corresponding flow cytometric DNA index and modal chromosome numbers^{3–5}

Ploidy status	DNA Index	Modal Chromosomes
Near haploid	0.55–0.69	24–29
Low hypodiploid	0.70–0.88	31–39
High hypodiploid	0.89–0.95	40–45
Diploid	0.96–1.05	46
Low hyperdiploid	1.06–1.15	47–50
High hyperdiploid	1.16–1.39	51–65
Near triploid	1.40–1.79	66–80
Near tetraploid	1.80–2.28	81–102

References

- 1 Poot M. Nucleic acid probes. *Curr Protoc Cytom* 2003;26(01): 4.3.1–4.3.10
- 2 Kim KH, Sederstrom JM. Assaying cell cycle status using flow cytometry. *Curr Protoc Mol Biol* 2015;111(01):28.6.1–28.6.11
- 3 Gupta N, Parihar M, Banerjee S, et al. FxCycle™ based ploidy correlates with cytogenetic ploidy in B-cell acute lymphoblastic leukemia and is able to detect the aneuploid minimal residual disease clone. *Cytometry B Clin Cytom* 2019;96(05):359–367
- 4 Smets LA, Slater R, van Wering ER, et al. DNA index and %S-phase cells determined in acute lymphoblastic leukemia of children: a report from studies ALL V, ALL VI, and ALL VII (1979-1991) of the Dutch Childhood Leukemia Study Group and the Netherlands Workgroup on cancer genetics and cytogenetics. *Med Pediatr Oncol* 1995;25(06):437–444
- 5 Rachieru-Sourisseau P, Baranger L, Dastugue N, et al. DNA index in childhood acute lymphoblastic leukaemia: a karyotypic method to validate the flow cytometric measurement. *Int J Lab Hematol* 2010;32(03):288–298