BD Cycletest™ Plus DNA Reagent Kit

DNA Analysis on the BD Accuri™ C6 Flow Cytometer

Features

- Precise DNA ploidy and cell cycle measurements using isolated cell nuclei
- Compatible with solid tissue or cell suspension samples
- Designed for compatibility with the easy-to-use BD Accuri™ C6 flow cytometer

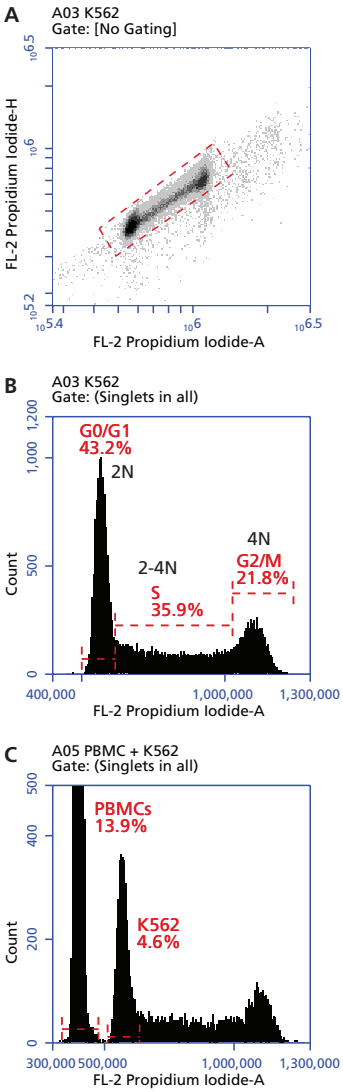

Figure 1. Cell cycle and ploidy analysis with the BD Cycletest Plus DNA Reagent Kit.

K562 leukemia cells (incorporating the Philadelphia translocation) were cultured, stained with the BD Cycletest Plus DNA Reagent Kit, acquired, and analyzed on the BD Accuri C6. **A.** K562 cells were gated to exclude aggregates on a PI FL2-A vs PI FL2-H plot. **B.** A PI histogram of K562 cells (gated on singlets, Panel A) distinguishes cells at the G_0/G_1, S, and G_2+M cycle phases (Table 1). **C.** Staining and analyzing normal PBMCs along with the K562 cells can quantify their aneuploidy by gating on their G_0/G_1 peaks (Table 2).

The BD Cycletest™ Plus DNA Reagent Kit contains all necessary reagents to prepare nuclei for DNA analysis, including ploidy and cell cycle, by flow cytometry. After a series of incubations, nuclei are isolated from either tissue samples or cell suspensions and stained with propidium iodide (PI), a dye that binds stoichiometrically to DNA. The stained nuclei are then analyzed on a flow cytometer to quantify the PI bound to the DNA.

On a histogram plot of PI (FL-2A) fluorescence, diploid (2N) and tetraploid (4N) cells can be identified by the mean fluorescence intensities (MFIs) of their peaks. Diploid cells in the early phase of the cell cycle (G_0/G_1) will contain half the amount of DNA and therefore half the fluorescence of tetraploid cells that are preparing to undergo mitosis (G_2/M). To determine aneuploidy of abnormal cells, normal or peripheral blood mononuclear cells (PBMCs) can be stained simultaneously as a reference. The DNA Index (DI) can then be calculated as the MFI ratio of the abnormal G_0/G_1 population to the normal G_0/G_1 population.

The BD Accuri C6 personal flow cytometer brings DNA analysis to your benchtop. The system is easy to use, simple to maintain, and affordable. The BD Accuri C6 is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. Compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use, and a non-pressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory offers automated sampling from 24-tube racks or multiwell plates.

The BD Cycletest Plus DNA Reagent Kit is designed to be compatible with the BD Accuri C6 flow cytometer. Its straightforward format is suitable for either fresh or frozen samples and enables precise DNA measurement using a single stain.

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Table 1. Diploid G_0/G_1 cells have half the MFI of tetraploid G_2+M cells.

Cell Cycle Phase	Ploidy	Mean Fluorescence Intensity (MFI)
G_0/G_1	2N	567,989
S	2–4N	810,744
G_2/M	4N	1,104,293

Table 2. Aneuploidy (DNA Index) is the ratio of abnormal to normal G_0/G_1 MFIs.

Sample	Mean Fluorescence Intensity (MFI)	DNA Index
K562 G_0/G_1	571,768	1.4
PBMC G_0/G_1	396,087	–

Ordering Information

Description	Quantity	Number of Tests	Cat. No.
BD Cycletest Plus DNA Reagent Kit containing:			
Solution A: Trypsin in spermine tetrahydrochloride detergent buffer	10 mL	40 tests	**340242**
Solution B: RNase A and trypsin inhibitor in spermine buffer	8 mL		
Solution C: Propidium iodide (PI) in spermine buffer	8 mL		
Buffer Solution: Dimethyl sulfoxide (DMSO) in sucrose-sodium citrate	3 x 50 mL		

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