BD FACSuite™ Research Assays Integrating Hardware, Software, and Reagent Solutions for

Cell Cycle Research

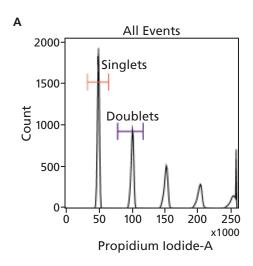
Features

Take advantage of robust instrument setup for increased reproducibility

Intelligent experimental setup reduces time

Quickly generate reports of results

Optimized for BD Cycletest™ Plus and BD Pharmingen™ **BrdU flow kits**



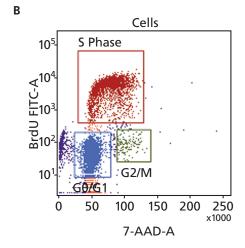


Figure 1.

- A. Chicken erythrocyte nuclei (CEN) stained with the BD CycleTest Plus assay kit and acquired on the BD FACSVerse flow cytometer.
- B. Mouse splenocytes incubated with recombinant IL-2 for three days and subsequently stained with the FITC BrdU Flow Kit and acquired on the BD FACSVerse flow cytometer.

Hardware and Software

The BD FACSVerse™ flow cytometer equipped with BD FACSuite™ software can standardize routine applications. BD FACSuite Research Assays consist of defined acquisition workspaces that include all of the data plots, gates, and statistics needed to acquire and analyze data that is consistent with the reagent manual or technical data sheet. After acquisition, results are displayed on formatted reports that include specific plots and statistics for each tube as well as summarized results for side-byside comparison of data for related tubes. Coupled with a novel setup function in BD FACSuite software, these research assays provide a new level of standardization for cell cycle studies.

Reagent Kits

The BD Cycletest Plus reagent kit includes propidium iodide (PI) for DNA staining and reagents to prepare cell nuclei for a precise DNA measurement. The samples are subsequently analyzed using flow cytometry to assess ploidy and to determine the cell cycle phase distribution of the population.

The BrdU kits, which are designed for the detection of cell cycle in proliferating cells by measurement of bromodeoxyuridine (BrdU), an analog of the DNA precursor thymidine, are used to measure de novo DNA synthesis. During the S phase of the cell cycle, BrdU is incorporated into the newly synthesized DNA and can be readily detected by anti-BrdU specific antibodies. Combination with 7-AAD enables classification of cells into G0/ G1, S, and G2/M phases of the cell cycle.

Results Reports

The BD Cycletest Plus assay directly measures the amount of nuclear DNA content by assessing the amount of PI that is incorporated into the nucleus. PI binds in a stoichiometric manner. Therefore diploid cells (2N) in the early stages of the cell cycle (eg, G0/G1) will have half the amount of DNA and therefore half the fluorescent signal of tetraploid (4N) cells that are preparing to undergo mitosis. Treatment with different compounds can have an effect on the number of cells in a given phase of the cell cycle. To assess the efficacy of a given treatment, the assay compares a treated sample to an untreated sample. The results table (Table 1) compares these samples and summarizes the overall number of cells as well as the percentages of cells in the sub-G0/G1 (apoptotic), G0/G1 (2N), S, G2/M (4N), and >4N stages of the cell cycle.

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BD FACSuite™ Research Assays Solutions for Cell Cycle Research

Since BrdU loading conditions are of great importance in determination of the cell cycle phases, the BrdU assays compare a sample that has been pulsed with BrdU to the same sample that has not been pulsed. This allows for determination of background effects of staining with the anti-BrdU antibody. The results table (Table 2) compares the pulsed and unpulsed samples side-by-side to look at the overall number of cells as

well as the percentages of cells that are in the various cell cycle phases: G0/G1, S, and G2/M.

Users can customize many properties of results reports, including requiring approvals and e-signatures, as well as data export and printing preferences.

Table 1. Example Results Summary from the BD Cycletest Plus Assay.

| Results Summary | , | |
|-----------------|-------------|-------------|
| Label | Test Sample | DNA Control |
| Nuclei events | 15134 | 15435 |
| Singlet events | 13092 | 11987 |
| % sub G0/G1 | 1.01 | 2.41 |
| % G0/G1 | 51.37 | 67.35 |
| % S | 25.29 | 17.97 |
| % G2/M | 21.88 | 11.61 |
| % >4N | 0.45 | 0.66 |

Table 2. Example Results Summary from the BrdU Assay.

| Results Summary | | | |
|-------------------|----------|---------|--|
| Label | Unpulsed | Pulsed | |
| Events Acquired | 10000.0 | 10000.0 | |
| % Cells | 81.3 | 81.6 | |
| % G0/G1 | 40.8 | 45.7 | |
| % S Phase (BrdU+) | 0.0 | 23.4 | |
| % G2/M | 12.2 | 1.3 | |

Product List

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|-------------------------------------|--|-----------|----------|--|--|
| Description | Contents | Size | Cat. No. | | |
| BD FACSuite Research Assay Software | Installation DVD | 1 disc | 651363 | | |
| BD Cycletest Plus DNA Reagent Kit | Solution A: trypsin; Solution B: RNase A and trypsin inhibitor; Solution C: Propidium Iodide (PI); Buffer Solution: dimethylsulfoxide (DMSO) | 40 tests | 340242 | | |
| BD Pharmingen FITC BrdU Flow Kit | FITC anti-BrdU antibody, 7-AAD, DNase, BrdU solution, BD Cytofix/Cytoperm™ Buffer, BD Perm/Wash™ Buffer, | 50 tests | 559619 | | |
| | BD Cytoperm™ Plus Permeabilization Buffer | 200 tests | 557891 | | |
| BD Pharmingen APC BrdU Flow Kit | APC anti-BrdU antibody, 7-AAD, DNase, BrdU solution, BD Cytofix/Cytoperm Buffer, BD Perm/Wash Buffer, BD Cytoperm Plus Permeabilization Buffer | 50 tests | 552598 | | |
| | | 200 tests | 557892 | | |



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