



Flow Cytometry Applications and Techniques

In flow cytometry single particles, most often cells, are analyzed for size, complexity and fluorescence properties as they flow through a beam of light. Multiple lasers are used to detect different fluorescent properties (colors). This analysis can be used to measure cell surface and intracellular biomarkers on cells and to determine cell status and viability. QPS scientists are available to train or assist clinical sites in on-site stimulations or preparations of whole blood or cells.

Instrumentation

The Becton Dickinson (BD) FACSCanto™ II Flow Cytometer provides a flexible work platform, configured with three lasers to detect up to eight colors, maximizing the amount of information acquired from individual samples. Innovations in the fluidics system include a fixed alignment flow cell to minimize startup time and improve reproducibility. The optical system has been designed to maximize sensitivity and resolution for each color. Two Flow Cytometry software packages provide resources and flexibility for acquisition and analysis. The BD FACSCanto™ Clinical Software is used for FDA-approved, *In-Vitro* Diagnostics assays including BD's TBNK analysis kits. The TBNK kits are capable of providing a complete immune panel (T-, Band NK lymphocyte subsets) in a single tube, saving time and resources. Additionally, when used with BD Trucount™ standard tubes, the software is able to determine absolute counts of cells per µL.

Immunophenotyping using BD Trucount™ Beads

Whole blood was collected with K2EDTA, and stained for cell surface markers specific to neutrophils, monocytes and T cells in the presence of BD Trucount™ beads. Each BD Trucount™ tube contains a lyophilized pellet containing a known number of fluorescent beads. During sample preparation, the beads

are released into the sample. BD FACSCanto™ clinical software was used to calculate absolute cell counts for each defined cell population. Calculation to determine absolute cell counts is: (# of events in region containing cell / # of events in absolute count bead region) X (# of beads per test / test volume).

Applications and Techniques

Flow Cytometry Application	
Cell Phenotyping	Quantitation of cell populations (eg., neutrophils, T-cells, B-cells) and subpopulations (eg., cytotoxic T-cells, regulatory T-cells (Treg cells)) as percentages or as number of cells/µL.
Cell Viability	Exclusion of dead cells from analyses.
Cell Surface Expression	Expression levels of key receptors and other cell surface biomarkers.
Intracellular Expression	Permeabilization of the cell membrane allows for intracellular staining. May be performed in combination with cell surface staining.
Absolute Cell Counts using BD Trucount™ Tubes	Numbers of cell populations of interest are expressed in number of cells/µL.
Receptor Occupancy Studies	To measure levels of drug-target binding.
Cytometric Bead Array (CBA)	Bead-based Immunoassays of soluble biomarkers in individual samples (plasma or other biological matrix). Assays can be multiplexed to allow for panel of several biomarkers to be analyzed at once within a small sample volume.
Cell Cycle Analysis	Measures DNA content of cells to determine cell cycle status.
Cell Proliferation Assays	Capable of measuring cell divisions using cell tracer dyes.
Apoptosis Assays	Measurement of regulated cell death using Annexin V staining.

