BD Influx™ Cell Sorter

The BD Influx™ cell sorter is a flexible flow cytometry platform that easily adapts to a researcher's application or environmental requirements. The system features a modular architecture and a powerful combination of detection capabilities, hands-on controls, and high performance which allows researchers to configure the BD Influx system to their site and application needs.

The optical system offers up to ten lasers to adapt to application requirements. Each laser can be customized with detectors and filters best suited to your research. A variety of exchangeable detector options is also offered. To meet needed speed and accuracy, the BD Influx system can handle a throughput rate of up to 200,000 events per second.

A unique fluidics system protects cells and addresses contamination. The nozzle assembly is designed to produce high droplet frequencies at relatively low pressures, enabling high-speed sorting while maintaining cell viability and functionality. To simplify drop-delay determination, patented BD FACS™ Accudrop technology allows researchers to quickly and easily see the best drop-delay value.

The BD Influx system supports up to six-way sorting to maximize efficiency, and plate sorting to accommodate isolation of single cells. To support aseptic sorting, disposable fluidics allow researchers to replace a sample line or the complete fluidics path, from sheath tank to nozzle tip.

To protect operators and samples, a Class II Type A2 biosafety cabinet specifically designed for the BD Influx system is available as an option.

BD FACS™ Sortware sorter software gives researchers comprehensive control of the sorter from configuration and compensation setup to acquisition, sorting, and analysis. BD FACS Sortware supports sorting by capturing all information about an event and making it available to researchers on demand for later analysis. To visualize experiment data, rich output formats including histograms, density, and contour plots, are available to support analysis.

Technical specifications are contained on the following pages.

BD

Technical Specifications

Optics

Excitation Optics

Optical Platform
Lasers are mounted on a standard optical breadboard. Laser beams are aligned independently by adjustable mirrors. Each laser beam has its own final focus lens mounted on a dedicated translational stage for fine adjustments. For systems with more than five lasers, the laser paths have one or more collinear beams through the steering optics and focusing lens, but intercept the stream at up to seven spatially separated points.

Lasers
A selection of laser wavelengths and powers is available. New laser combinations may require custom engineering of the optical platform.

Power Out of the Laser Head
355 nm: >100 mW, >250 mW
405 nm: >100 mW
445 nm: >100 mW
457 nm: >300 mW
488 nm: >200 mW
515 nm: >100 mW
532 nm: >150 mW
552 nm: >200 mW
561 nm: >75 mW, >150 mW
594 nm: >100 mW
640 nm: >120 mW
785 nm: >40 mW

Beam-shaping optics provide elliptical beam spots for selected laser paths (3:1 ratio with a typical beam height of 15–20 µm).

Emission Optics

Emission light is collected through a 20X, 0.6 NA microscope objective (90°). Light is focused on three, five, or seven spatially separated mirror pinholes depending on the number of lasers. Modular detector blocks allow for a user-defined detection configuration (see the *BD Influx Cell Sorter Filter Guide*).

Simultaneous video observation of the stream, the pinholes, and the laser intercepts allows for fast and intuitive alignment.

Forward scatter is detected using 75- and 50-mm lenses, an aperture, and a photomultiplier tube (PMT). Resolution for the standard forward scatter detector is >0.5 µm (measured using beads). Collection angle is 2°–17°.

Side scatter is collected through the 90° collection lens and measured using a PMT. Side scatter resolution is >0.2 µm (measured using beads and 0.1-µm filtered sheath fluid).

The forward scatter Small Particle Option (SPO) incorporates a 20X, 0.42 NA microscope objective, a mirror pinhole, and a pinhole camera. Resolution for the SPO is >0.2 mm (measured using beads and 0.1-mm filtered sheath fluid). Collection angle is 2°–30°.

Fluidics

General Operation
- Laboratory air pressure and/or vacuum can be used for operation.
- An optional air pressure supply and/or vacuum pump is available.
- Sheath pressure is adjustable from 1–90 psi (0.07–6.2 bar).

Fluidics Reservoirs
Autoclavable 7-L sheath and waste containers, equipped with pressure and vacuum readout, are provided.

Fluidics Control
- Sheath, sample, and boost pressure can be individually adjusted.
- A sample flow fine adjustment is provided for precise regulation of sample flow.
- Purge, pulse, rinse, and run buttons are provided for quick stream startup and bubble removal.

Replaceable Fluidics Path
- The fluidics path, including the nozzle assembly, can be exchanged. There are no inline valves. Only pinch valves are used.
- The sample line can also be exchanged.

Bubble Detector
A bubble detector in the sample line detects air bubbles from the sample tube and stops sample flow when the sample tube is empty, preventing air bubbles from reaching the nozzle assembly.

Sample Input
12 x 75-mm tubes, polypropylene

Temperature Control
Sample input and sort collection tubes can be cooled or heated by an optional circulating water bath.

Performance

Example Fluorescence Sensitivity
Measured using SPHERO™ Rainbow Calibration Particles (RCP-30-5A) according to the manufacturer's specifications:

Sheath pressure:	33 psi
Drop dive:	On, ~68 kHz
Excitation:	488 nm, 200 mW
Emission:	530/40 nm for FITC 580/30 nm for PE

FITC: 100 molecules of equivalent soluble fluorochrome (MESF-FITC)
PE: 100 molecules of equivalent soluble fluorochrome (MESF-PE)

Example Fluorescence Resolution
Measured using propidium iodide (PI)–stained chicken erythrocyte nuclei (CEN):

Sheath pressure:	33 psi
Drop drive:	On, ~68 kHz
Excitation:	488 nm, 200 mW
Emission:	610/20 nm for PI

Coefficient of variation (CV) of PI: <3%, full G_0/G_1 peak

Example Fluorescence Linearity
Measured using PI-stained CEN:

Sheath pressure:	33 psi
Drop drive:	On, ~68 kHz
Excitation:	488 nm, 200 mW
Emission:	610/20 nm for PI

Doublet/singlet ratio: 1.95–2.05

Sort Performance

Drop Drive Frequency
Adjustable 9–180 kHz

Purity and Yield
At 60 psi and 100 kHz with an average threshold rate of 25,000 events per second, a four-way sort achieved a purity of 98% and a yield >80% of Poisson's expected yield for all four populations. Higher threshold rates of up to 70,000 events per second can be achieved without affecting purity; however, yield will decrease based on Poisson's statistics.

Viability
As shown in published literature, sorts performed using murine[1-4] and human[5] cells and/or cell lines[6] demonstrated good recovery and viability in several experimental systems. Optimal sort conditions need to be established for different cell types.

Nozzles
Supplied: 70, 86, 100, and 140 µm

Optional: 200 µm

Sort Collection Devices
All collection devices are designed to fit on the Computerized Cell Deposition Unit (CCDU). The CCDU is standard on all instruments.

- Two-way sorting: 1.5-mL, 5-mL, 15-mL, and 50-mL tubes, and 25-mm round filter paper
- Three-way sorting: One 50-mL tube and two 5-mL tubes
- Four-way sorting: 1.5-mL and 5-mL tubes
- Six-way sorting: 5-mL tubes
- Plates and slides: 6, 24, 48, 96, and 384-well plates; slides; and user-defined collection devices

Temperature Control
Water recirculator for refrigeration or heating of collection tubes (optional).

Sort Monitoring
- Live video feed of waste collection and side streams.
- Live video feed of breakoff point.
- Drop-delay determination is achieved with BD FACS™ Accudrop technology. The drop-delay value can be adjusted while viewing BD Accudrop beads which are illuminated by a red diode laser in the center and side sort streams.

Signal Processing

Data Acquisition Channels
- Up to 5-laser systems: 16 channels, usually 14 colors plus forward and side scatter
- Systems with 6 lasers or more: 24 channels, usually 22 colors plus forward and side scatter

Signal Processing
- 16-bit analog-to-digital conversion, 65,536 channels
- Parallel data stream with channel ID and integrity check
- Less than 1 correlation error per 10^8 events

Acquisition Rate
Dead time is 0 µs. The maximum throughput rate is 200,000 events per second, independent of the number of parameters.

Fluorescence Compensation
- Up to 5-laser systems: 16 x 16 digital compensation matrix.
- Systems with 6 lasers or more: 24 x 24 digital compensation matrix.
- Compensated parameters are added to the bus as separate parameters.

Pulse Processing
- All signals are height (peak) by default.
- Pulse processor electronics add area and width measurements for a maximum of 8 parameters to the bus.
- Width measurement on the trigger parameter is standard.

Time
Time can be correlated to any parameter for kinetic experiments or other applications.

Channel Threshold
- Any parameter can be used as the threshold from the primary laser.
- Lasers and detectors can easily be switched to change laser sequence.

Workstation

Workstation
PC workstation with Intel® Quad Core (3.06 GHz or faster), Windows® 7 Professional 64-bit operating system

Memory
8 GB of RAM

Data Storage
- 2 x 500 GB hard drives, RAID 1 (mirrored) configured
- 16x CD/DVD +/- RW
- 2 USB 2.0/3.0

Networking
10/100/1000 GB Ethernet

Monitor
23-inch LED, 1920 x 1080 resolution

Data File Structure
Flow cytometry standard (FCS) 3.0

Software
BD FACS™ Sortware v1.0 or later

Technical Specifications

Installation Requirements

Dimensions (H x W x D)
Cytometer: 72.0 x 62.0 x 48.0 in. (182.9 x 157.5 x 121.9 cm)

Cytometer with specially modified Baker BioPROtect® IV biological safety cabinet, operational footprint 143.9 x 94.0 x 84.0 in. (365.5 x 238.8 x 213.4 cm)

Operational footprint (W x D): 84.0 x 84.0 in. (213.4 x 213.4 cm)

Weight
Cytometer: 229 lb (104 kg)

Temperature Operating Range
59°F–73°F (15°C–23°C)
Rapid temperature changes, such as greater than 3.6°F (2°C) per hour, may require system alignment to be verified.

Heat Dissipation
6,200 BTU/h maximum, dependent on laser choice

Electrical Requirements

Power for the BD Influx
One dedicated line is required for the cytometer and electronics. An appropriate power transformer will be delivered depending on region-specific settings. Multiple peripherals may require additional line(s). See the following table.

Region	VAC	Hz	A
North America	120 ±10%	50/60	15
Outside North America	220 ±10%	50/60	10
Japan	100 ±10%	50/60	15

Power for the BD Influx with BSC
Two dedicated lines are required for the cytometer and electronics. Multiple peripherals may require additional line(s). See the following table.

Region	VAC	Hz	A
North America	120 ±10%	50/60	20
Outside North America	220 ±10%	50/60	20
Japan	100 ±10%	50/60	20

Power
Power requirements are between 1,080 watts (3 lasers) and 1,800 watts (5 lasers). Standby status is not applicable.

Humidity
55% ±10% relative humidity

Noise
<80 dBA from all running equipment

Air Supply
90 psi (6.2 bar) regulated. The source of compressed air must deliver clean (less than 5 ppm), dry-filtered (oil-free) air, and stable pressures.

Vacuum Supply
A vacuum supply is delivered with the system. A laboratory vacuum supply between 5 and 15 in. Hg at 1 CFM can also be used.

Options

Small particle option

Specially modified Baker BioPROtect® IV, Class II, Type A2 biological safety cabinet verified by The Baker Company to meet personnel and product protection standards for a Class II Type A2 biosafety cabinet and the National Sanitation Foundation International Standard 49. No verification to other aspects of this or other standards has been made.

Aerosol evacuation

Sample temperature control

Polarization detector

Air compressor

Compliance with Safety Standards

UL 61010 (US)

IEC 61010 and IEC 60825 (Europe)

CSA Electrical Safety Standards (Canada)

References

1. Chen HL, Pistollato F, Hoeppner DJ, Ni HT, McKay RD, Panchision DM. Oxygen tension regulates survival and fate of mouse central nervous system precursors at multiple levels. *Stem Cells.* 2007;25:2291-2301.
2. Kumar R, Fossati V, Israel M, Snoeck HW. Lin-Sca1+kit-bone marrow cells contain early lymphoid-committed precursors that are distinct from common lymphoid progenitors. *J Immunol.* 2008;181:7507-7513.
3. Mar L, Hoodless PA. Embryonic fibroblasts from mice lacking Tgif were defective in cell cycling. *Mol Cell Biol.* 2006;26:4302-4310.
4. Seamons A, Perchellet A, Goverman J. Endogenous myelin basic protein is presented in the periphery by both dendritic cells and resting B cells with different functional consequences. *J Immunol.* 2006;177:2097-2106.
5. Pistollato F, Chen HL, Schwartz PH, Basso G, Panchision DM. Oxygen tension controls the expansion of human CNS precursors and the generation of astrocytes and oligodendrocytes. *Mol Cell Neurosci.* 2007;35:424-435.
6. Panchision DM, Chen HL, Pistollato F, Papini D, Ni HT, Hawley TS. Optimized flow cytometric analysis of central nervous system tissue reveals novel functional relationships among cells expressing CD133, CD15, and CD24. *Stem Cells.* 2007;25:1560-1570.

23-10501-06

BD Biosciences
bdbiosciences.com