



BD FACSAria III Cell Sorter

Technical Specifications

The BD FACSAria™ III is built on the solid foundation of patented technologies, superior multicolor performance, and renowned ease-of-use that has led to the unparalleled success of the BD FACSAria. Since its introduction in 2003, each successive generation of the BD FACSAria has opened the complex world of cell sorting to a broader audience of researchers and wider range of applications. Now, the BD FACSAria III system is even more powerful, dependable, and easier to use.

The BD FACSAria III delivers proven multicolor performance. Its fluidics and optical systems include unique design innovations precisely integrated to maximize signal detection. These innovations include the laser excitation optics, the patented flow cell with gel-coupled cuvette, and the highly efficient, patented octagon and trigon modules. Together, these systems allow the BD FACSAria III to achieve unrivaled sensitivity and resolution.

The BD FACSAria III is powerful and expandable. The system can mount up to six lasers, so you can choose the configuration that meets application, budget, and site requirements. An innovative new X-mount optical plate makes this possible. It accommodates easy expansion to six lasers and four spatially separated beam spots. Wavelength choices now include 561-nm and 445-nm lasers, as well as the 488-nm, 633-nm, 405-nm, and 375-nm lasers. Mount up to 20 detectors, and measure a maximum of 18 colors simultaneously.

The BD FACSAria III is a solid long term investment. Unlike other instruments, the BD FACSAria enables you to upgrade your existing instrument to the next-generation platform instead of purchasing a new system. A field upgrade can bring your BD FACSAria or BD FACSAria II system up to the capabilities of the BD FACSAria III.

Optics

Excitation Optics

Optical Platform

Fixed optical alignment of all Class IIIB lasers upon the cuvette flow cell. The 488-nm and 633-nm lasers come standard. All other laser choices are optional. All are solid state except the 633 nm, which is gas.

Beam height: $9 \pm 3 \mu\text{m}$

Beam width: $65 \pm 7 \mu\text{m}$

Power Out of the Laser Head

375 nm: >7 mW, top hat shape

405 nm: >50 mW, elliptical shape

445 nm: >20 mW, elliptical shape

488 nm: >20 mW, elliptical shape

561 nm: >50 mW, elliptical shape

633 nm: >18 mW, elliptical shape

Emission Optics

Optical Coupling

The quartz cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescent objective lens for optimal collection efficiency.

Numerical aperture: 1.2

Forward Scatter Detector and Filters

Photodiode with 488/10 bandpass filter for the 488-nm laser and 462/52 bandpass filter for the 445-nm laser.

Side Scatter Detector

Photomultiplier with a 488/10 bandpass filter for the 488-nm laser and a 445/15 bandpass filter for the 445-nm laser.

Fluorescence Detectors and Filters

Five fixed-fiber apertures for the 488, 633, 561, 445, and 405-nm lasers

The 445-nm laser focuses on the flow cell at the same location as the 488-nm laser. The 375-nm laser is air launched and focuses on the flow cell at the same location as the 405-nm laser. An octagon technology detector array enables user-defined detection configurations.

Wavelengths detected from 488-nm laser without 561-nm laser installed

- 530/30-nm FITC
- 585/42-nm PE
- 616/23-nm PE-Texas Red®
- 695/40-nm PerCP-CyTM5.5 or PI or 675/20-nm PerCP-Cy7
- 760/60-nm PE-CyTM7

Wavelengths detected from 488-nm laser when 561-nm laser installed

- 530/30-nm FITC
- 695/40-nm PerCP-Cy5.5 or PI or 675/20-nm PerCP

Wavelengths detected from 633-nm laser

- 660/20-nm APC
- 780/60-nm APC-Cy7 or APC-H7

Wavelengths detected from 405-nm laser

- 450/50-nm BD HorizonTM V450, Pacific BlueTM, DAPI
- 510/50-nm BD Horizon V500, AmCyan

Wavelengths detected from 561-nm laser

- 582/15-nm PE
- 610/20-nm PE-Texas Red®, Living Colors®, mCherry, propidium iodide
- 670/14-nm PE-CyTM5 or 710/50-nm PE-Cy5.5
- 780/60-nm PE-Cy7

Wavelength detected from 445-nm laser

- 510/80-nm, cyan fluorescent protein (CFP)

Wavelengths detected from 375-nm laser

- 450/20-nm Hoechst Blue, DAPI
- 670 LP Hoechst Red, PI

Filters and mirrors are user changeable. Additional detectors up to a total of 18 wavelengths can be added to the arrays.

Steering Optics

488, 633, 561, 445, and 405-nm laser: Fiber optics steer the fixed alignment laser beams onto the expansion prisms to focus them on the cuvette flow cell.

Near UV (375-nm) laser: Air launched and focused on the cuvette flow cell

Fluidics

General Operation

Fluidics cart provides sheath and cleaning fluids to the instrument and receives waste. No air or vacuum required. Room air can be used if desired. Sheath pressure is adjustable from 5 to 75 psi.

Fluidic Reservoirs

Autoclavable 10-L sheath and waste containers and 5-L cleaning reservoirs provided.

Sample Flow Rates

Adjustable dynamic range of sample flow rates

Fluidic Cleaning Modes Included (Software)

- Automated startup and shutdown
- Clean flow cell
- Prepare for aseptic sort

Nozzles

70, 85, 100, and 130- μm , removable and can be sonicated.

A registered key-fit position at the bottom of the cuvette provides fixed stream alignment.

Sample Collection Cooling/Heating

Refrigerator/heater option is available to provide cooling or heating for sort collection into tube holders, multiwell plates, and slides.

Operating temperature range: -20 to 120°C

Automatic Cell Deposition Unit

ACDU for slide and plate sorting: 6, 24, 48, 96, and 384-well plates

Performance

Fluorescence Sensitivity

Measurements performed at 70 psi and 90 kHz using SPHEROTM Rainbow Calibration Particles (RCP-30-5A)

FITC: 85 molecules of equivalent soluble fluorochrome (MESF-FITC)

PE: 29 molecules of equivalent soluble fluorochrome (MESF-PE)

Fluorescence Resolution

Coefficient of variation (CV)

PI: Area, <3.0%, full G_0/G_1 peak for propidium iodide (PI)-stained chicken erythrocyte nuclei (CEN)

Hoechst: Area, <3.5%, full G_0/G_1 peak for Hoechst-stained CEN

Fluorescence Linearity

Doublet/singlet ratio:

CEN stained with PI: 1.95–2.05

(488-nm laser) or

Hoechst: 1.95–2.05 (405-nm laser)

Forward and Side Scatter Sensitivity

Sensitivity enables separation of fixed platelets from noise, identification of bacteria, and detection of 0.5- μm beads.

Forward and Side Scatter Resolution

Scatter performance is optimized for resolving lymphocytes, monocytes, and granulocytes.

Sample Acquisition Rate

Maximum acquisition rate (events per second) with 12 compensation pairs and 8 parameters: 70,000.

Sort Performance

Drop Drive Frequency

Range 1–100,000 Hz

Purity and Yield

At 70 psi and 90 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. Higher threshold rates up to 70,000 events per second can be achieved without affecting purity; however, yield will decrease based on Poisson statistics.

Viability

Sorts were performed at a variety of sheath pressures using cell lines and human peripheral blood mononuclear cells (PBMCs). All sorts resulted in cells that proved viable and proliferated for several days post-sort.

Sort Collection Devices

Two-way sorting: 12 x 75 mm, and 15 mL

Four-way sorting: 1.5 mL microtube and 12 x 75 mm

Sort Collection Cooling

Water recirculator for refrigeration/heating (optional)

BD FACS™ Accudrop

- Red diode laser provided for fully automated drop-delay determination
- Automated drop breakoff monitoring
- Automated clog detection and sort tube protection system using Sweet Spot technology

Signal Processing

Converter

10-MHz Analog-to-Digital converter. Pulse sampling is precisely matched to particle flow rate in the cuvette. Particles travel slower compared to conventional stream-in-air sorters. This increases the light collected, resulting in better sensitivity. High-speed sorting is achieved by accelerating the stream through the nozzle, achieving drop rates comparable to stream-in-air sorters. The flow cell design and electronics are matched to maximize signal while maintaining maximum sort speed, purity, and yield.

Workstation Resolution

262,144 channels

Data Acquisition Channels

20 parameters, 18 fluorescent and 2 scatter

Fluorescent Compensation

No limit to inter- and intra-beam compensation

Pulse Processing

Height, Area, and Width measurements available for any parameter. Ratio measurements are also available.

Time

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

Channel Threshold

Available for any parameter from any lasers with the ability to use multiple thresholds from different lasers simultaneously.

Loading

Sample Input Sizes

Microtubes, 12 x 75 mm, and 15 mL

Polystyrene or polypropylene tubes can be used.

Sample Input Agitation

Adjustable through the software to keep sample constantly suspended

Temperature Control

Sample input, software-adjustable: 4, 20, 37, and 42°C

Sample output for sort collection: water recirculation unit (optional)

Data Management

Workstation

PC workstation with at least Pentium® 4 processor, 3.0 GHz or faster

Memory

>2 GB RAM

Data Storage

80-GB and 250-GB hard drives

16x DVD +/- RW, dual layer

Floppy drive

Networking

10/100/1000 Ethernet

FireWire® serial bus

Monitor

Two 19-inch LCDs, 2560 x 1024 resolution (standard)

One 20-inch LCD, 1600 x 1200 resolution (optional)

One 23-inch LCD, 1920 x 1200 resolution (optional)

Printer

Networkable color-laser printer

Data File Structure

Flow Cytometry Standard (FCS) 3.0 or 2.0

Software

BD FACSDiva™ software v6.1.3 or later

Installation Requirements

Dimensions

BD FACSAria III sensor (cell sorter):
28 H x 28 D x 48 W in.
(71 x 71 x 122 cm)
400 lb (181.4 kg)

BD FACSAria III fluidics cart (wet cart):
26 H x 26 D x 32 W in.
(66 x 66 x 81 cm)
180 lb (81.7 kg)

Temperature Operating Range

Between 17.5°C (63.5°F) and
27.5°C (81.5°F)

Heat Dissipation

5100 BTU per hour

Power

Operation at 100/115/230 VAC and
50 or 60 Hz

Maximum power 1,500 watts

Water Supply

None required

Air Supply

None required

Table (optional)

BD FACSAria III instrument and
computer table:
33.5 H x 53 D x 105 W in.
(85 x 135 x 267 cm)
100 lb (45.4 kg)

Options

Aerosol Management Option (AMO)

The BD FACSAria III features an enclosed pathway from the sample injection chamber to the sort collection tubes. For an added level of aerosol management, the BD™ Aerosol Management Option (AMO) evacuates the sort collection chamber and traps aerosolized particles during sorting. It is equipped with a 0.01-µm size ultra-low penetrating air (ULPA) filter to trap aerosolized particles.

Evacuates the volume of the ACDU chamber area 16 times per minute in normal evacuation mode and 69 times per minute in rapid evacuation mode. When operated under normal and stressed conditions (mimicking a clog), <3 Glo Germ™ particles were identified outside the ACDU sort collection chamber. Glo Germ particles, developed by Glo Germ in Moab, Utah, have been shown to provide good visualization of aerosol deposition in normal and mock failure modes by Oberyszyn and Robertson (*Cytometry*. 43:217-222, 2001).

Available Lasers for Upgrade

- 375 nm
- 405 nm
- 445 nm
- 561 nm

Regulatory Status

CE marked for electrical safety (Europe)

UL Standard for Safety Electrical Equipment for electrical safety (USA)

CSA for electrical safety (Canada)

Class I (1) laser product per CDRH regulations and EN/IEC 60825

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The BD FACSAria III cell sorter is covered by one or more of the following US patents and foreign equivalents:

7,544,326; 7,201,875; 7,129,505; 6,944,338; 6,880,414; 6,809,804; 6,683,314; 6,510,007; 6,372,506; 6,014,904; and 5,700,692.

The BD FACSAria III flow cytometer is For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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