



# BD FACSAria™ III Cell Sorter

**The BD FACSAria™ III cell sorter** is built on the solid foundation of patented technologies, multicolor performance and renowned ease-of-use that has led to the unparalleled success of the BD FACSAria™ cell sorter portfolio. 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 five 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 include 488-nm, 633-nm, 405-nm, 561-nm and 355-nm lasers. Mount up to 20 detectors and measure a maximum of 18 colors simultaneously.



# Technical Specifications

## Optics

### Excitation optics

#### Optical platform

Fixed optical alignment of all Class IIb 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

355 nm: >15 mW, elliptical shape

405 nm: >50 mW, elliptical shape

488 nm: >20 mW, elliptical shape

561 nm: >50 mW, elliptical shape

633 nm: >18 mW, elliptical shape

### Emission optics

#### Steering optics

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

UV (355-nm) laser: Air-launched and focused on the cuvette flow cell at the same location as the 405-nm lasers.

#### 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.

#### Side scatter detector

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

#### Fluorescence detectors and filters

Four fixed-fiber apertures. The 488-nm, 633-nm and 561-nm lasers have their own aperture and fiber. The 405-nm and 355-nm lasers share an aperture and fiber.

An octagon technology detector array enables user-defined detection configurations.

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

#### 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-Cy™5.5 or PI or

675/20-nm PerCP

760/60-nm PE-Cy™7

#### Wavelengths detected from 488-nm laser with 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 Horizon™ V450, Pacific Blue™, 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-Cy™5 or 710/50-nm PE-Cy5.5

780/60-nm PE-Cy7

#### Wavelengths detected from 355-nm laser

450/20-nm Hoechst Blue, DAPI

670 LP Hoechst Red, PI

## Fluidics

### General operation

The 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- $\mu\text{m}$ , 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°C to 120°C

### Automated cell deposition unit (ACDU)

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 SPHERO™ 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- $\mu$ m 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

### Instrument 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)

See Site Preparation Guide for space and clearance requirement.

### 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

355 nm

405 nm

561 nm

BD optical filters for BD Horizon Brilliant™ polymer conjugates available for the BD FACSAria™ Family.

## 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

Class 1 Laser Product.

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