

# BD FACSAria™ Fusion Cell Sorter

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The BD FACSAria™ Fusion is built on the solid foundation of patented technologies, exceptional multicolor performance, and ease-of-use that was first brought to the world of sorting by the launch of BD FACSAria™ cell sorter in 2003.

Now this sorting know-how is combined with best-in-class biosafety expertise to create the BD FACSAria Fusion, a fully integrated advanced cell sorter and biosafety solution for research laboratories.

Biological safety in flow cytometry is an emerging requirement for core laboratories concerned about the potential accidental exposure of operators to biological samples. To address this requirement, a custom-tailored biosafety cabinet (BSC) for the BD FACSAria Fusion was designed in collaboration with The Baker Company, a leader in biosafety solutions.

The BD FACSAria Fusion has been verified by The Baker Company to meet personnel and product protection standards for a Class II Type A2 biosafety cabinet, the National Sanitation Foundation International Standard 49, the European Standard 12469, and the Australian Standard® AS 2252.2–2009. The BD FACSAria Fusion cell sorter is also available without a BSC and can be easily field upgraded with the cabinet at a later date.

Redundancy is an important aspect of biosafety protection, and the BD FACSAria Fusion has a number of features that further reduce the risk of operator exposure. For example, while the biosafety cabinet protects the operator from aerosol exposure during a sort, the built-in aerosol management system also evacuates aerosols and operates independently of the cabinet for an added measure of safety.

To achieve unrivaled sensitivity and resolution, the fluidic and optical systems are precisely integrated to maximize signal detection. Fiber-launched lasers that improve sensitivity and resolution for each color in a multicolor assay, and innovations such as the patented flow cell with gel-coupled cuvette, ensure performance, safety, and ease-of-use. With the BD FACSAria Fusion, the complex world of cell sorting now is open to a broader audience of researchers and wider range of applications.

Technical specifications are contained on the following pages.



## Technical Specifications

### Optics

#### Excitation Optics

##### Optical Platform

Fixed optical alignment of all Class IIIb lasers with the cuvette flow cell. The 488-nm and 640-nm lasers are standard. All other laser choices are optional. All lasers are solid state.

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

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

Beam shaping: 375-nm laser is top hat, all other lasers are elliptical

##### Power Out of the Laser Head

375 nm: >7 mW

405 nm: >85 mW

445 nm: >30 mW

488 nm: >50 mW

561 nm: >50 mW

640 nm: >100 mW

#### Emission Optics

##### Optical Coupling

The quartz cuvette flow cell is gel-coupled by refractive index-matching optical gel to the fluorescence 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

Four fixed-fiber apertures for the 488, 640, 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 the 488-nm Laser without the 561-nm Laser Installed

- 530/30-nm FITC, Alexa Fluor® 488
- 585/42-nm PE
- 616/23-nm PE-Texas Red®, propidium iodide (PI)
- 695/40-nm PerCP-Cy™5.5 or PI, or 675/20-nm PerCP
- 760/60-nm PE-Cy™7

##### Wavelengths Detected from the 488-nm Laser with the 561-nm Laser Installed

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

##### Wavelengths Detected from the 640-nm Laser

- 670/30-nm APC, Alexa Fluor® 647
- 780/60-nm APC-Cy7 or APC-H7

##### Wavelengths Detected from the 405-nm Laser

- 450/40-nm BD Horizon Brilliant™ Violet 421, V450, VPD450, Pacific Blue™, DAPI
- 525/50-nm BD Horizon Brilliant™ Violet 510 or V500, AmCyan
- 610/20-nm BD Horizon Brilliant™ Violet 605

##### Wavelengths Detected from the 561-nm Laser

- 582/15-nm PE, DsRed
- 610/20-nm PE-Texas Red®, mCherry, PI
- 670/14-nm PE-Cy™5 or 710/50-nm PE-Cy5.5
- 780/60-nm PE-Cy7

##### Wavelength Detected from the 445-nm Laser

- 510/80-nm, AmCyan fluorescent protein, CFP

##### Wavelengths Detected from the 375-nm Laser

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

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

##### Steering Optics

488, 640, 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

- Sheath and cleaning fluid tanks, as well as waste collection, are located in an easy-to-reach drawer at the base of the system.
- An auxiliary air input is designed to connect the cytometer to a lab's air filtration and drying system. If needed, a third-party compressor is available as an option.

- Sheath pressure is adjustable from 5 to 75 psi.

#### Fluidic Reservoirs

Autoclavable 10-L sheath and waste containers and 5-L cleaning reservoirs are 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- $\mu\text{m}$  nozzles are removable and can be sonicated.

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

#### 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, to avoid air bubbles from reaching the nozzle. The bubbles are then purged when unloading the sample tube.

#### Sample Collection Cooling and Heating

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

#### Automatic Cell Deposition Unit

Sorts into slides and 6, 24, 48, 96, and 384-well plates. Index sorting can be enabled when sorting single cells. This capability indexes the cell surface phenotype to the well containing that cell.

### Performance

#### Fluorescence Sensitivity

Measurements performed at 70 psi and 90 kHz using SPHERO™ Rainbow Calibration Particles (RCP-30-5A).\*

\*MESF can vary lot-to-lot.

- FITC<sup>†</sup>: <87 molecules of equivalent soluble fluorochrome (MESF-FITC)
- PE<sup>†</sup>: <29 molecules of equivalent soluble fluorochrome (MESF-PE)

<sup>†</sup>Average MESF from four instruments.

#### Qr<sup>‡</sup> (x1000)

Measurements performed at 70 psi and 90 kHz. 488-nm laser excitation.

FITC<sup>§</sup> (BD FACSAria Fusion): 97

(BD FACSAria™ III): 40

PE<sup>§</sup> (BD FACSAria Fusion): 669

(BD FACSAria III): 333

<sup>‡</sup>Qr is the relative fluorescence detection efficiency, used for describing the light collection efficiency of a detector, measured in assigned BD units (ABD units). One ABD unit, for a given fluorochrome, is defined as the fluorescence of one antibody bound to a CD4<sup>+</sup> cell. The higher the Qr value, the better the relative fluorescence detection efficiency.

<sup>§</sup>Qr values from the same lot of beads were taken from one BD FACSAria Fusion and one BD FACSAria III. Qr values can vary between instruments and instrument configurations. The BD FACSAria III was used for comparison.

# Technical Specifications

## Fluorescence Resolution

Coefficient of variation (CV)

- PI: Area, <3.0%, full G<sub>0</sub>/G<sub>1</sub> peak for PI-stained chicken erythrocyte nuclei (CEN)
- Hoechst: Area, <3.5%, full G<sub>0</sub>/G<sub>1</sub> 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 87 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's statistics.

### Functionality

Dendritic cells (myeloid and plasmacytoid, mDs and pDCs, respectively) were isolated from the peripheral blood mononuclear cells of three donors and sorted on the BD FACSAria III system (one sort per donor) which uses the same cuvette-based flow cell design as the BD FACSAria Fusion. Post-sort cell viability was assessed using a live/dead exclusion marker, and functionality was assessed by intracellular cytokine staining after 6 or 18 hours of stimulation with the TLR 7 & 8 agonist R848. Post-sort viability at 6–18 hours was >90% for all three donors. Both pDCs and mDCs for all three donors produced IFN- $\alpha$ , TNF- $\alpha$ , and IL-12 stimulation, demonstrating post-sort functionality.

## Sort Collection Devices

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

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

## Sort Collection Cooling

Water recirculator for refrigeration or heating (optional)

## BD FACSTM 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 the 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 fluorescence and 2 scatter)

### Fluorescence 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 laser, with the ability to use multiple thresholds from different lasers simultaneously.

## Loading

### Sample Input Sizes

Microtubes, 12 x 75-mm and 15-mL tubes

Polystyrene or polypropylene tubes can be used.

### Sample Input Agitation

Adjustable through the software to keep the sample constantly suspended

### Temperature Control

Sample input: 4°C, 20°C, 37°C, and 42°C (adjusted in the software)

Sample output: water recirculation unit (optional)

## Data Management

### Workstation

PC workstation with at least an Intel® Xeon® processor, 3.0 GHz or faster

### Memory

>4 GB of RAM

### Data Storage

- 250-GB and 500-GB hard drives
- 16x DVD +/- RW, dual layer
- Floppy drive

### Networking

- 10/100/1000 Ethernet
- FireWire® serial bus

### Monitor

- Two 19-inch LCDs, 2,560x1,024 resolution (standard)
- One 20-inch LCD, 1,600x1,200 resolution (optional)
- One 23-inch LCD, 1,920x1,200 resolution (optional)

### Printer

Color network laser printer

### Data File Structure

Flow Cytometry Standard (FCS) 3.0 or 2.0

### Software

BD FACSDiva™ software v8.0 or later

### Operating System

Microsoft® Windows® 7

## Technical Specifications

### Installation Requirements

#### Dimensions (H x W x D)

BD FACSAria Fusion without BSC:  
142 x 128 x 87 cm  
(56 x 50 x 34 in.)  
Weight: 475 kg (1,047 lb)

BD FACSAria Fusion with BSC:  
220 x 127 x 89 cm  
(87 x 50 x 35 in.)  
Weight: 765 kg (1,687 lb)

#### Temperature Operating Range

Without BSC: 17.5°C–27.5°C  
(63.5°F–81.5°F)

With BSC: 17.5°C–22.5°C  
(63.5°F–72.5°F)

#### Workspace Table (Optional)

94 x 71.1 x 66 cm  
(37 x 28 x 26 in.)

#### Heat Dissipation

Without BSC: 1,965 BTU/hour  
(maximum, depends on the choice and number of lasers)

With BSC: 4,422 BTU/hour (5-laser system)

#### Power

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

Maximum power: 1,800 watts

#### Water Supply

None required

#### Air Supply

95–100 psi, regulated air, clean (<5 ppm) and free of oil

Approximately 0.52 standard cubic meters per hour

### Options

#### Integrated Biosafety Cabinet (BSC) Option

The BD FACSAria Fusion can be equipped either at the time of purchase or as a field upgrade with a fully integrated custom-tailored biosafety cabinet designed in collaboration with The Baker Company.

The BSC has been verified by The Baker Company to meet personnel and product protection standards: for a Class II Type A2 biosafety cabinet, the National Sanitation Foundation International Standard 49, the European Standard 12469, and the Australian Standard AS 2252.2–2009. No verification to other aspects of this or other standards has been made.

While the biosafety cabinet protects the operator from aerosol exposure during a sort, the built-in aerosol management system also evacuates aerosols and operates independently of the cabinet for an added measure of safety.

The system can be easily field upgraded with the cabinet at a later date.

#### Aerosol Management Option (AMO) for Systems Without the BSC Option

The BD FACSAria Fusion 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.

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).

#### Temperature Control Option

This option can be used for both sort tube and plate temperature regulation during a sort and includes:

- Recirculating water bath
- Specially designed collection tube holders with ports for recirculating water:
  - Two-way 15 mL
  - Four-way 12 x 75 mm
  - Four-way 1.5-mL Eppendorf style

#### Lasers Available for Upgrade

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

A 355-nm laser is available through the special order program.

### Compliance with Safety Standards

- UL 61010 (US)
- IEC 61010 and IEC 60825 (Europe)
- CAN/CSA - C22.2 No. 61010 (Canada)
- Class 1 Laser Product per CDRH regulations and EN/IEC 60825

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

APC-Cy7: US Patent 5,714,386

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