

BD FACSaria™ Fusion

Description

Fluorescence activated cell sorter (FACS) with a quartz cuvette flow cell that is in fixed alignment with the laser, and is gel coupled to the collection optics. BD FACSaria™ Fusion is equipped with 5 spatially separated lasers, 18 fluorescent detectors and 2 light scatter detectors for fast multicolour analysis and cell sorting with accurate event data acquisition at up to 70,000 events per second. BD FACSaria™ Fusion is placed in the biosafety cabinet (BSC) Class II Type A2, designed in collaboration with The Baker Company, a leader in biosafety solutions.

Applications

Flow cytometry analysis and fluorescence activated cell sorting.

Excitation Optics

Fixed optical alignment of all Class IIIb lasers with the cuvette flow cell. All lasers are solid state and elliptical:

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

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

Power Out of the Laser Head

355 nm: >20 mW

405 nm: >85 mW

488 nm: >50 mW

561 nm: >50 mW

640 nm: >100 mW

Emission Optics

Forward Scatter Detector: 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:

UV Laser 355			YellGreen laser 561		
Indo-1 blue	BP530/30	505LP	PE-Cy7	BP780/60	750LP
INDO-1	BP450/50		PE-Cy5	BP690/20	670LP
			PE-Cy5.5	BP670/14	630LP
Violet laser 405			PE-Texas Red	BP610/20	600LP
			PE	BP585/15	
BV786	BP780/60	750LP			
BV711	BP710/50	690LP	Red laser 640		
Qdot605	BP610/20	595LP			
QdotT585	BP586/20	550LP	APC-Cy7	BP780/60	735LP
BV510	BP510/30	495LP	Alexa Fluor 700	BP705/20	685LP
DAPI	BP450/50		APC	BP670/30	
Blue laser 488					
PerCP-Cy5.5	BP695/40	655LP			
FITC	BP525/20	505LP			
SSC	BP488/10				

Fluidics

Sheath pressure is adjustable from 5 to 75 psi.

Sample Flow Rates adjustable dynamic range.

Nozzles are removable and can be sonicated; available in 3 sizes: 70, 85 and 100- μm

Temperature control of sample input: 4°C, 20°C, 37°C, and 42°C (adjusted in the software); sample output: from +5°C to +42°C

Collection devices: 15ml- and 5ml-tubes, eppendorfs, 6, 24, 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 SPHEROTM Rainbow Calibration Particles (RCP-30-5A).*

*MESF, can vary lot-to-lot.

- FITC <87 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 G0/G1 peak for PI-stained chicken erythrocyte nuclei (CEN)
- Hoechst: Area, <3.5%, full G0/G1 peak for Hoechst-stained chicken erythrocyte nuclei (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- μ m beads.

Forward and Side Scatter Resolution

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

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, mDCs and pDCs, respectively) were isolated from the peripheral blood mononuclear cells of three donors and sorted on the BD FACS Aria III system (one sort per donor) which uses the same cuvettebased flow cell design as the BD FACS Aria 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- α , TNF- α , 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

Single cell sort into multiwall plates: 6-; 24-; 96-; 384-well plates

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

Pulse Processing: height, area, and width measurements available for any parameter. Ratio measurements are also available.

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

Link to the manufacturer [BD Biosciences](#)

Recommended tool for multicolour experiment design: [SpectrumViewer](#)