

# BD FACSCelesta™ Flow Cytometer Configuration Sheet

## Blue-Violet-Ultraviolet (BVUV) Laser Configuration



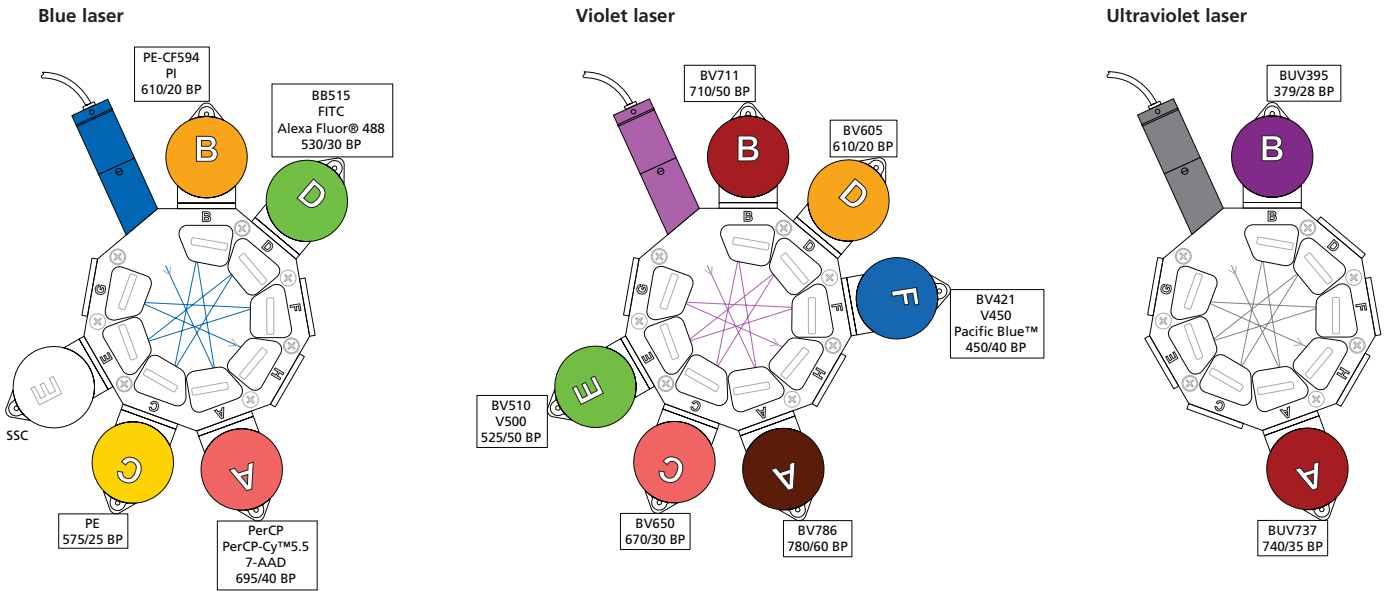
The BD FACSCelesta™ flow cytometer is designed to simplify the use of multicolor flow cytometry and allow researchers to benefit from new innovations in instrument and reagent technology. This platform offers multiple configurations to meet varied application needs.

The BVUV configuration of the BD FACSCelesta includes blue, violet, and ultraviolet lasers, along with mirrors, filters, and detectors optimized to get the most out of the legacy fluorochromes, viability stains, and BD Horizon Brilliant™ dyes shown in the optical path diagrams. While a blue laser has long been standard in flow cytometry, the violet laser is gaining popularity as more bright violet-excited fluorochromes, such as BD Horizon Brilliant™ Violet reagents, are introduced and enthusiastically adopted. The ultraviolet laser, which supports the use of BD Horizon Brilliant™ Ultraviolet reagents, allows markers to be spread across more lasers, further reducing the overall compensation needed for a multicolor panel.

Multicolor flow cytometry panel design has presented challenges for researchers, such as varying marker expression, varying dye brightness, and significant emission spillover between fluorescence channels. The combination of bright, narrow-spectrum, BD Horizon Brilliant fluorochromes and sensitive optics results in panels that can readily resolve even dim populations, yet are easy to use and compensate. The system operates with BD FACSDiva™ software, a collection of convenient and easy-to-use tools for flow cytometer and application setup, data acquisition, and data analysis.

### Optical path diagrams and fluorochrome support

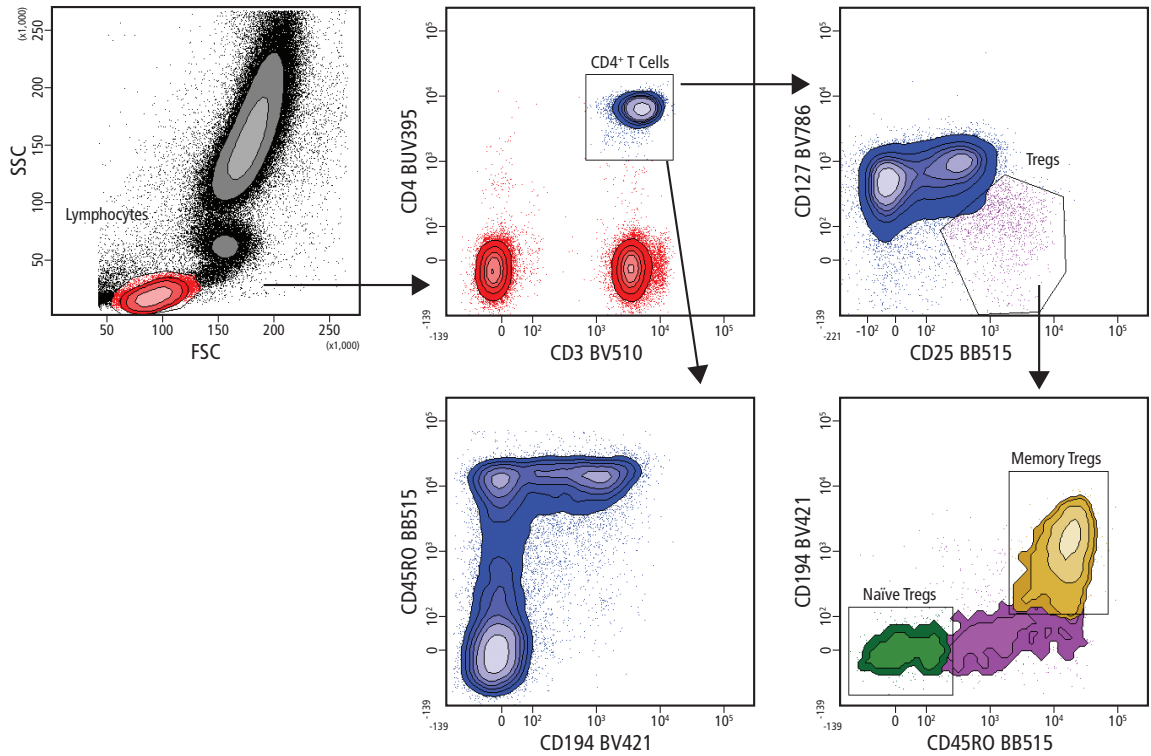
Laser polygons show fluorochromes, mirrors, filters, and optical paths for the BD FACSCelesta BVUV configuration.



Other fluorochromes supported:  
BD Horizon™ Fixable Viability Stain 520, 570, 620

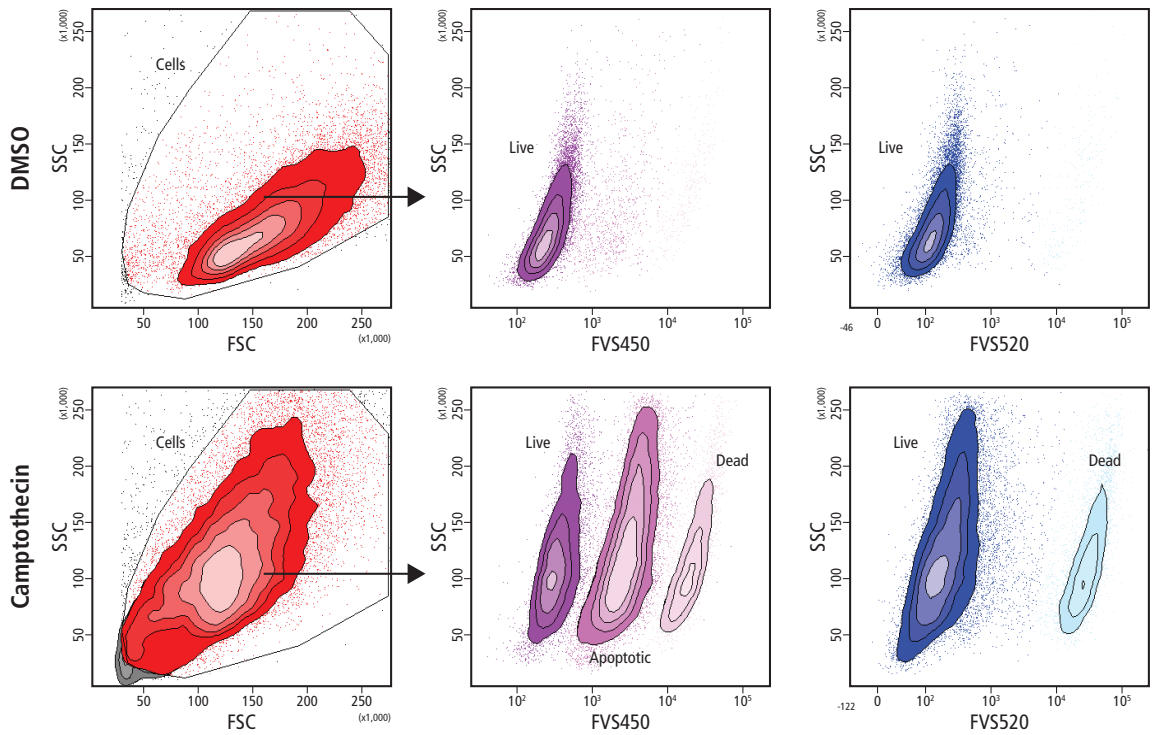
Other fluorochromes supported:  
BD Horizon™ Fixable Viability Stain 450, 510





**Six-color Treg analysis on the BD FACSCelesta BVUV configuration**

Normal human whole blood was washed, lysed, and stained to analyze Tregs and CD4<sup>+</sup> helper T-cell subsets. Cells were gated and lymphocytes were identified using light scatter. Lymphocytes were further gated on CD3<sup>+</sup>CD4<sup>+</sup> helper T cells and analyzed for rare T-cell subsets using CD45RO and CD194 surface markers or identification of Tregs (CD25<sup>+</sup>CD127<sup>-</sup>). Within the Treg subset, naïve (CD45RO<sup>-</sup>CD194<sup>-</sup>) and memory (CD45RO<sup>+</sup>CD194<sup>+</sup>) Tregs were identified.



**Viability analysis of fixed and cryopreserved Jurkat cells**

Jurkat cells were treated with 0.025% DMSO vehicle (top plots) or 5  $\mu$ M camptothecin (bottom plots) for 16 hours, harvested from culture, washed, and stained with BD Horizon™ Fixable Viability Stain 450 (Cat. No. 562247) or 520 (Cat. No. 564407) for 15 minutes at room temperature. Samples were washed twice with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), fixed with BD™ Cytotfix Fixation Buffer (Cat. No. 554655) for 15 minutes at room temperature, and then cryopreserved in freezing medium for one week. Cryopreserved samples were thawed, washed with BD Pharmingen Stain Buffer (FBS), and acquired by flow cytometry on a BD FACSCelesta system. Camptothecin-treated cells show an increase in Fixable Viability Stain-bright cells, indicating an increase in the number of dead cells after camptothecin treatment. Fixable Viability Stain 450 also resolves apoptotic Jurkat cells with an intermediate staining intensity.

Excitation Laser	Fluorochrome	Ex <sub>max</sub>	Em <sub>max</sub>	Relative Brightness
Ultraviolet (355 nm)	BD Horizon Brilliant™ Ultraviolet 737 (BUV737)	348 nm	737 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Ultraviolet 395 (BUV395)	348 nm	395 nm	■ ■ ■ ■ □
Violet (405 nm)	BD Horizon Brilliant™ Violet 786 (BV786)	407 nm	786 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Violet 711 (BV711)	407 nm	711 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Violet 650 (BV650)	407 nm	650 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Violet 605 (BV605)	407 nm	602 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Violet 510 (BV510)	405 nm	510 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Violet 421 (BV421)	407 nm	421 nm	■ ■ ■ ■ □
Blue (488 nm)	PerCP	482 nm	678 nm	■ ■ ■ ■ □
	PerCP-Cy™5.5	482 nm	695 nm	■ ■ ■ ■ □
	BD Horizon™ PE-CF594	496 nm	612 nm	■ ■ ■ ■ □
	PE	496 nm	578 nm	■ ■ ■ ■ □
	BD Horizon Brilliant™ Blue 515 (BB515)	490 nm	515 nm	■ ■ ■ ■ □
	FITC	494 nm	520 nm	■ ■ ■ ■ □
	Alexa Fluor® 488	495 nm	519 nm	■ ■ ■ ■ □

**Relative Brightness Key:**

- ■ ■ ■ □ Dim
- ■ ■ ■ □ Moderate
- ■ ■ ■ □ Bright
- ■ ■ ■ □ Brightest

**Ordering Information**

Description	Cat. No.
BD FACSCelesta™ Flow Cytometer, BVUV Configuration	660346
BD FACSCelesta™ High Throughput Sampler (HTS) Option	658946
BD FACFlow™ Supply System	649908
BD FACSCelesta™ Standard Workstation Bundle	660472

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