

BD FACSAria Cell Sorter

New 375-nm Near UV Laser Option

Stem cell research is a rapidly expanding area of investigation. However, isolating true precursor stem cells from their differentiated counterparts for analysis and further manipulation is challenging. The new BD FACSAria™ cell sorter 375-nm Near UV laser option was designed to sort and analyze mouse and human hematopoietic stem cells by supporting the side population (SP) application stem cell enrichment protocol.

Features

- Supports side population (SP) application for mouse and human hematopoietic stem cell enumeration and enrichment
- Supports a variety of dyes including Hoechst, PI, DAPI, Pacific Blue™, Cascade Blue®
- Includes a filter set for side population analysis

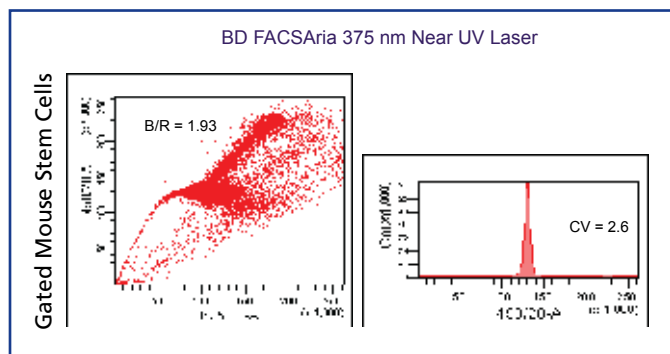
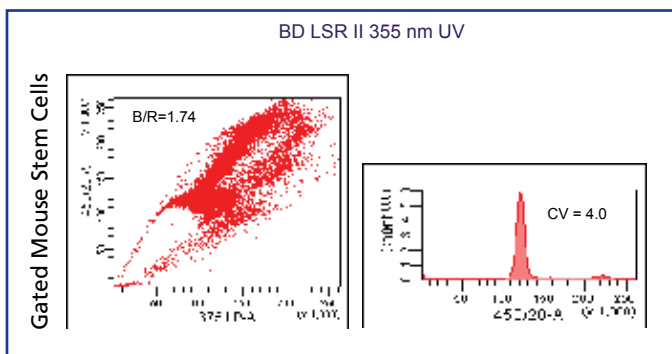
Side Population (SP) Application

The side population (SP) application is a specific stem cell enumeration and enrichment protocol using flow cytometry. Goodell PhD and co-workers were the first to describe the method.¹⁻³ It relies on the selective efflux of the dye Hoechst 33342 (bis-benzimide) by primitive progenitor cells, as compared to their differentiated counterparts. The same phenomenon occurs in mouse, primate, and human bone marrow, as well as a variety of tissues such as skeletal muscle and epidermis.⁴⁻⁶ The actual mechanism responsible for this finding is actively being studied, but not completely confirmed.^{7,8} Of particular interest is that these cells are low to negative expressors of CD34, an indication held by some to suggest that the SP cells may be the most primitive progenitor cells identified to date.¹

Traditionally the SP procedure is performed by first labeling cells with the Hoechst dye, and then analyzing them with the 350-nm line of a UV laser. Simultaneous measurement of emitted fluorescence using the blue (450 nm) and far red (> 675 nm) detectors allows the SP cells to be distinguished and separated from the other cells.

SP Just Got Easier – Upgrade the BD FACSAria Cell Sorter

Experiments performed in 2003 by Telford and Frolova at the National Institute of Health (NIH) determined that SP cells could be analyzed by exciting the Hoechst dye with a 375-nm Near UV laser.⁹ This laser excitation line, along with the novel BD “Top Hat” optical design, allows for a compact laser configuration. The laser option seamlessly integrates on the standard BD FACSAria cell sorter without any changes to the optical bench. The laser is available as a production upgrade to current systems and does not require changes to the BD FACSAria software.



SP comparison using the 355-nm laser on the BD™ LSR II flow cytometer and the 375-nm laser on the standard BD FACSAria cell sorter. The Top Hat technology almost triples the amount of light at the sample intercept. As a consequence of the even sample illumination, cells have a greatly improved CV (coefficient of variation).

BD FACSAria 375-nm Near UV Laser Option

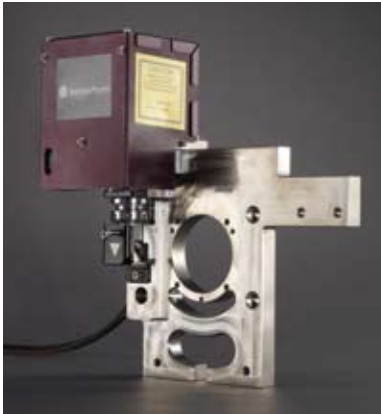
The 375-nm Near UV Laser is Small and Installation is Fast

Measuring only 19 × 7 × 7 cm, the fully-housed 375-nm laser fits in the small space immediately left of the flow cell on the BD FACSAria cell sorter. The laser produces a 10-mW focused beam to illuminate the cells in the flow cell. Due to its small size the laser is available as an upgrade to the standard BD FACSAria cell sorting system.

Installation of the 375-nm Near UV laser does not require reworking of the optical bench. Service installation and alignment typically take less than one working day to complete.

Simultaneous Laser Use

The 375-nm laser can be operated together with the red and blue lasers on

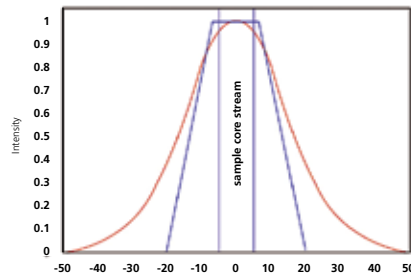


375-nm Near UV laser for the BD FACSAria cell sorter

the BD FACSAria cell sorter. If the unit is equipped with a violet laser, the 375-nm laser and the violet laser share the same beam spot at the point of interception within the cuvette. The user must select to run either the 375-nm laser, the violet laser, or neither.

Top Hat Optics Design

The Top Hat optics design is an innovation of BD Biosciences engineers. It refers to the patent-pending approach of modifying the usual Gaussian beam, which illuminates a sample at higher intensity in the middle of the beam and lower intensity at the edges, with a composite “square” beam that illuminates the middle and edges of the sample core stream at the same intensity.



Gaussian (red line) vs. Top Hat (blue line) illumination

References

1. Goodell MA, Rosenzweig M, Kim H, et al., Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med.* 1997 3(12):1337-45.
2. Storms RW, Goodell MA, Fisher A, et al., Hoechst dye efflux reveals a novel CD7(+)/CD34(-) lymphoid progenitor in human umbilical cord blood. *Blood.* 2000 96(6):2125-33.
3. Wulf GG, Wang RY, Kuehnle I, et al. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. *Blood.* 2001 98(4):1166-73.
4. Dunnwald M, Tomanek-Chalkley A, Alexandrunas D, et al. Isolating a pure population of epidermal stem cells for use in tissue engineering. *Exp Dermatol.* 2001 10(1):45-54.
5. Gussoni E, Soneoka Y, Strickland CD, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature.* 1999 401(6751):390-4.
6. Jackson KA, Mi T, and Goodell MA. Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci U S A.* 1999 96(25):14482-6.
7. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side - population phenotype. *Nat Med.* 2001 7(9):1028-34.
8. Masaki Mogi, Jiang Yang, Jean-Francois Lambert, et al. Akt Signaling Regulates Side Population Cell Phenotype via Bcrp1 Translocation. *J. Biol. Chem.* 2003 278 (40):39068-39075.
9. Telford WG and Frolova EG. Discrimination of the Hoechst side population in mouse bone marrow with violet and near-ultraviolet laser diodes. *Cytometry Part A.* 2004 57(1):45-52.

Ordering Information

Each Near UV Upgrade Kit provides the following filters: 450/20 BP, 670 LP, and a 610 DM LP.

Description	Cat. No.
2 Laser Near UV Upgrade Kit (for 2 Laser Configuration Only: includes a trigon)	641190
2 Laser Near UV Upgrade Kit (for 2 Laser Configuration with PMT upgrade: includes a trigon)	641191
3 Laser Near UV Upgrade Kit (for 3 Laser Configuration Only)	641192

Please consult your BD Sales Representative or call BD Customer Support to upgrade your current BD FACSAria cell sorting system with the 375-nm Near UV laser.



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