A flow cytometry solution for fluorescent protein analysis and beyond

Maximizing resolution with the BD FACSCelesta™ BVYG configuration

Features

- Yellow-green laser optimally excites mCherry and other red fluorescent protein (RFP) variants
- Maximizes detection and resolution of dimly fluorescent cell populations
- Enables researchers to combine fluorescent protein (FP) analysis with immunophenotyping and cell function analysis
- Ideal for research using transgenic mouse and other species models incorporating FPs

Fluorescent proteins (FPs) are used as reporters in many kinds of experiments, from cell and molecular biology to stem cell and cancer research to microbiology. Flow cytometry, specifically designed to detect and measure fluorescence, is an excellent methodology for research using FPs. With up to three lasers and twelve fluorescence parameters, the BD FACSCelesta™ flow cytometer is well suited for FP analysis, while leaving additional channels available for immunophenotyping or cell function analysis.

Some popular FPs, such as green and yellow fluorescent proteins (GFP and YFP), are excited by a blue (488-nm) laser, which is standard in most flow cytometers. However, RFP and its variants, such as mCherry, are only weakly excited by blue and other standard lasers. When the red FP signal is dim, it can be difficult to detect and resolve the FP^+ cells.

A yellow-green (561-nm) laser excites these FPs much more strongly, which results in a brighter emission signal. This laser has become essential for many applications using red FPs, including the fruit series of fluorescent proteins (such as Living Colors® mCherry, mPlum, and DsRed dyes). The BD FACSCelesta Blue/Violet/Yellow-Green (BVYG) configuration is ideal for this research, since it can enable research using all of these RFP variants, as well as GFP and YFP.

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Figure 1 shows human embryonic kidney cells transfected with GFP or mCherry, excited by the blue or yellow-green laser of the BVYG configuration, respectively. In each case, separation between the positive and negative peaks is more than two orders of magnitude. For GFP, an intermediate peak is also resolved clearly.

The development of transgenic mouse models in which key genes are tagged with a fluorescent protein has opened up new approaches to research. Figure 2 shows an experiment using a mouse model in which a reporter expressing an RFP (specifically, monomeric red fluorescent protein or mRFP) has been knocked into the endogenous locus for FoxP3, the transcription factor that is the master regulator for regulatory T cells (Tregs).[1] As an intracellular marker, FoxP3 detection requires fixation and permeabilization of cells, and no stable and reliable surface-marker correlate has yet been found. The development of this mouse model has streamlined and facilitated Treg research, since Tregs can be definitively identified without fixation or permeabilization. Not only does this simplify and streamline the experimental protocols, but the cells also remain viable for use in downstream applications.

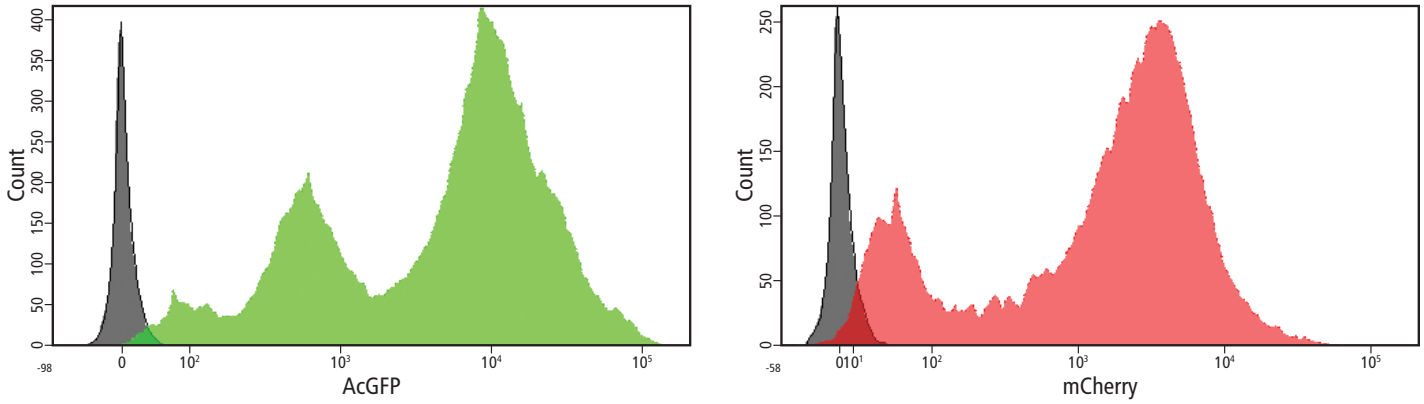

Figure 1. Expression of fluorescent proteins in transfected human embryonic kidney cells

HEK-293 cells were transfected over 24 hours with AcGFP (left) or mCherry (right), fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), and cryopreserved for one week. After thawing, cells were washed and then analyzed with the BD FACSCelesta BVYG configuration. Transfected cells (green or red) were compared to the wild type (black) for expression of AcGFP (excited by the blue laser) or mCherry (excited by the yellow-green laser), respectively.

In Figure 2, after gating on lymphocytes and then on T cells, $CD4^+CD25^+$ Tregs were identified. Ninety percent expressed FoxP3, as indicated by mRFP fluorescence. The clear resolution of this population, without the need to fix or permeabilize the cells, attests to the value of this transgenic mouse model.

As a low-density antigen, CD25 often stains dimly, so CD25 expression is not always easy to detect. That's where the violet laser facilitates discovery. In this experiment, CD25 was paired with BD Horizon Brilliant™ Violet 421, one of a family of advanced polymer fluorochromes for the violet laser. The brightness of BV421, and the violet laser that is standard in all configurations of the BD FACSCelesta, make it easier to detect and resolve populations that express low-density antigens.

With the BD FACSCelesta BVYG configuration, you don't have to give up the violet laser to get the yellow-green laser, which you can dedicate to detecting the tagged cells from your transgenic mice or transfected cell line. That leaves the blue and violet laser channels open for other purposes. For example, you can use them to immunophenotype the cells, allowing you to determine which subpopulations are expressing the FP. You can also use them for measures of cell function, such as apoptosis, viability, cell cycle, DNA damage, and/or proliferation.

As an added bonus, the yellow-green laser also maximizes resolution of other fluorochromes conventionally excited by the blue laser, including phycoerythrin (PE) and PE tandem dyes such as PE-CF594 and PE-Cy™7.

Combining innovations in instrumentation with optimization for bright new reagents and BD's unparalleled service and support, the BD FACSCelesta flow cytometer is designed to help you extract a deeper level of biological information from your cell types of interest. By enhancing FP detection while simultaneously enabling immunophenotyping and cell function analysis, the BD FACSCelesta BVYG configuration helps you gain new understanding and insights about your transgenic model or transfected cells.

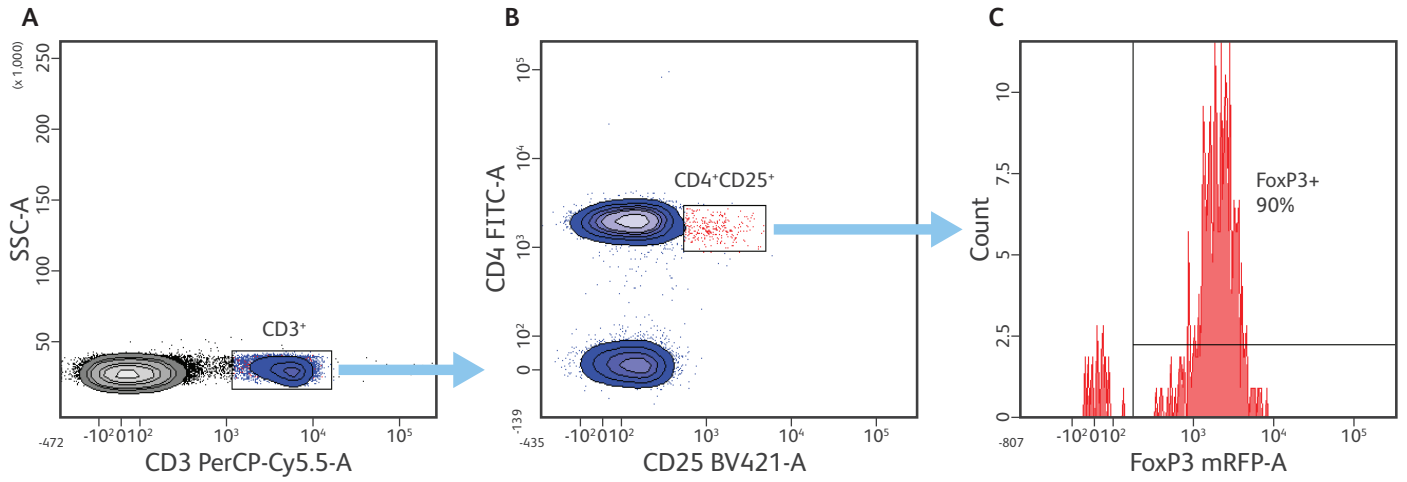

Figure 2. Treg analysis of FoxP3 transgenic mouse splenocytes

Splenocytes from FoxP3-IRES-mRFP mice (Jackson Laboratories) were isolated and stained with a cocktail of surface-marker antibodies, including CD3 PerCP-Cy™5.5, CD4 FITC and CD25 BV421. Lymphocytes were first gated based on light scatter properties (not shown), followed by gating of $CD3^+$ T cells **(A)**. From the gated T cells, $CD4^+CD25^+$ Tregs were identified **(B)** and analyzed for the expression of the signature transcription factor FoxP3, which was expressed by 90% of the cells **(C)**. The yellow-green laser optimally excites mRFP, resulting in clear resolution of FoxP3.

Ordering information	
Description	Cat. No.
BD FACSCelesta™ Flow Cytometer, BVYG Configuration	**660345**
BD FACSCelesta™ Flow Cytometer, BVR Configuration	660344
BD FACSCelesta™ Flow Cytometer, BVUV Configuration	660346
BD FACSCelesta™ Flow Cytometer, BV Configuration	660343

References

1. Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. *PNAS.* 2005;102:5126-5131.

Class 1 Laser Product.

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