

# Detecting Red Fluorescent Proteins on the BD Accuri™ C6

## Special Order BD Accuri™ C6 Research System Improves Detection and Resolution

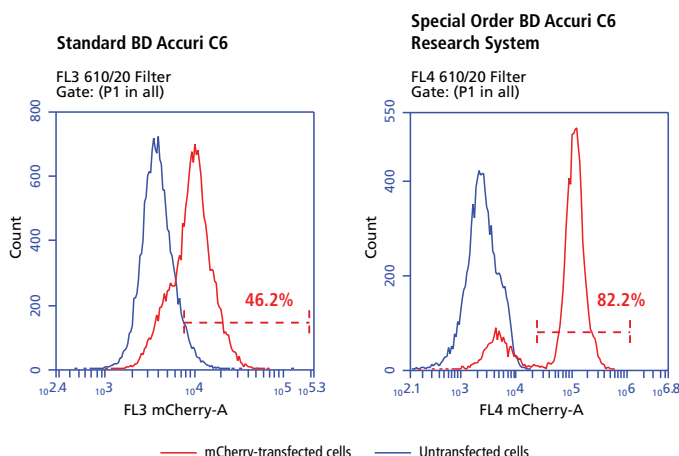
### Features

Yellow-green laser optimally excites mCherry and other red fluorescent protein variants

Improves detection and resolution of rare and dimly fluorescent cell populations

Offers optical flexibility while retaining portability and ease of use

Preconfigured and custom special order BD Accuri C6 systems maximize choice and flexibility to meet individual research requirements



**Figure 1. mCherry resolution for standard vs special order systems**

CHO cells were stably transfected with mCherry (red histogram) and, along with untransfected cells used as a control (blue histogram), were acquired on a standard BD Accuri C6 (left plot) and a special order BD Accuri C6 research system equipped with a yellow-green laser (right plot), and analyzed with BD Accuri™ C6 software. Histogram overlays show that mCherry resolution of the special order system exceeded the standard BD Accuri C6, as shown by the higher percentage of mCherry<sup>+</sup> cells and the clear separation between transfected and untransfected cells. The signal-to-noise ratio (MFIs of transfected/untransfected cells) was also higher for the special order system (36) than for the standard system (2.9). Finally, the bimodal distribution observed using the special order system demonstrates the ability to distinguish successfully vs unsuccessfully transfected cells.

The standard configuration of the BD Accuri™ C6 personal flow cytometer can detect and analyze many fluorescent proteins (FPs), including red FP variants such as mCherry. When the FP signal is bright, the BD Accuri C6 will resolve all of the FP<sup>+</sup> cells, even with the standard 670LP filter. Using an optional 610/20 filter can further improve resolution, as explained in the BD technical bulletin, *Optical Filter Guidelines for Fluorescent Protein Analysis with the BD Accuri™ C6 Flow Cytometer*.

When the FP signal is dim, however, it can be difficult to detect and resolve certain FP<sup>+</sup> cells on the standard BD Accuri C6, even with optional filters. A special order BD Accuri C6 research system equipped with a yellow-green (552-nm) laser can provide the needed resolution. This laser excites mCherry more efficiently than the blue (488-nm) laser in the standard system, which results in a brighter emission signal. The table shows the optical characteristics of both systems. The standard system detects the mCherry signal in the FL3 channel, while the special order system does so in FL4.

#### Standard BD Accuri C6

Laser	Detector	Filter
Blue (488 nm)	FL1	533/30
	FL2	585/40
	FL3	670LP (standard) 610/20 (optional)
Red (640 nm)	FL4	675/25

#### Special Order BD Accuri C6 Research System

Laser	Detector	Filter
Blue (473 nm)	FL1	520/35
	FL2	575/25
Yellow-Green (552 nm)	FL3	670/14
	FL4	610/20

Figure 1 shows histograms of Chinese hamster ovary (CHO) cells stably transfected with mCherry. The standard BD Accuri C6 (left plot) detected the mCherry<sup>+</sup> cells, but did not clearly separate them from negative (untransfected) cells, even using the optional filter. Only 46.2% of the transfected cells were clearly identified as mCherry<sup>+</sup>, and the signal-to-noise ratio—the ratio between the mean fluorescence intensity (MFI) of transfected vs untransfected cells—was only 2.9.

In contrast, the special order system better resolved the two populations. It characterized 82.2% of the cells as mCherry<sup>+</sup>, with a signal-to-noise ratio of 36. In addition, the histogram showed a bimodal distribution of transfected cells, clearly separating the mCherry<sup>+</sup> cells from those that were unsuccessfully transfected.

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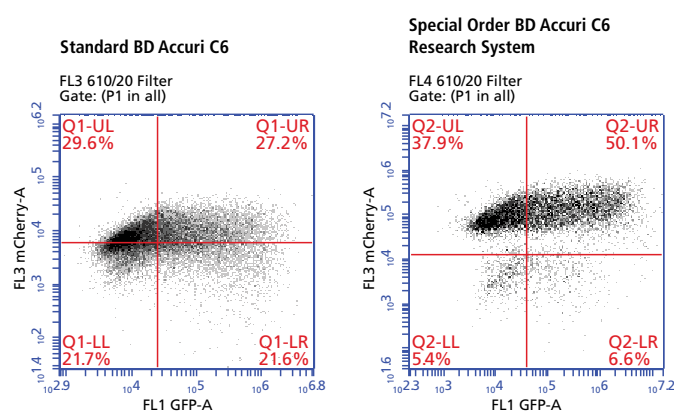
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The special order system can also adeptly resolve multiple fluorescent proteins. In Figure 2, CHO cells were transfected with both Green Fluorescent Protein (GFP) and mCherry. Both systems detect GFP in FL1, and both identified about 50% of the cells as successfully transfected (GFP<sup>+</sup>). But again, the standard system detected only 57% of the cells as mCherry<sup>+</sup>, compared to about 88% for the special order system. The special order system was able to clearly discriminate four subpopulations of cells (the four quadrants in the right plot), while the standard system did not show clear boundaries between them.

Special order BD Accuri C6 research systems offer researchers unparalleled choice in personal flow cytometry. A preconfigured special order system for gene expression, such as the one used in these experiments, can enable research using the fruit series

of fluorescent proteins (such as Living Colors® mCherry, mPlum, and DsRed dyes) as well as Green and Yellow Fluorescent Proteins. Other preconfigured special order systems are available or, for maximum flexibility, researchers can custom-design a 1- or 2-laser special order system with a choice of wavelengths and detectors.

Special order BD Accuri C6 systems fit a broad range of research application requirements, yet they retain the intuitive software and portability of the BD Accuri C6. Fixed alignment and preoptimized detector settings make them simple to use. A non-pressurized fluidics subsystem enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory offers automated sampling from 24-tube racks or multiwell plates.



**Figure 2.** Simultaneous analysis of GFP and mCherry

CHO cells were stably transfected with mCherry and transiently transfected with GFP, acquired on a standard BD Accuri C6 (left plot) or a special order BD Accuri C6 research system equipped with a yellow-green laser (right plot), and analyzed with BD Accuri C6 software. Gates were set based on untransfected controls. No difference in GFP resolution was found, since both systems identified approximately 50% of successfully transfected, GFP<sup>+</sup> cells. However, only the special order system was able to clearly discriminate four subpopulations of cells based on the differential expression of mCherry and GFP, represented here in the four Q2 quadrants.

## Ordering information

To discuss a special order BD Accuri C6 research system for your application, contact your BD Biosciences sales representative.

Description	Cat.No.
BD Accuri™ C6 Flow Cytometer System	653118
BD Accuri™ Optical Filter 610/20 nm	653186
BD CSampler™ Automated Sampling System (optional)	653124
Special Order BD Accuri C6 Research System (473/2 + 552/2)	656035

Class 1 Laser Product.

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