

# Immunophenotyping and functional analysis of rare cell populations

Analyze dendritic cell markers and functional properties with the BD FACSCelesta™ flow cytometer

## Features

- Identify and distinguish rare subsets of antigen-presenting cells
- Use deep immunophenotyping for a more comprehensive analysis of populations of interest
- Use BD OptiBuild™ custom reagents to “drop” new markers into an established panel
- Identify cytokine-producing cell subsets in a heterogeneous sample

The biology of rare cell types, such as dendritic cells (DCs) and regulatory T cells (Tregs), can be difficult to study because it's not easy to isolate component subsets for investigation, and their markers often overlap with other cell types. Flow cytometry is well suited for this analysis since, by assessing the expression of multiple intracellular and cell surface markers, it can rapidly analyze thousands of individual cells to provide both phenotypic and functional data in the same assay. In contrast, conventional techniques such as ELISA provide only the total level of cytokines secreted, without identifying the source cell types.

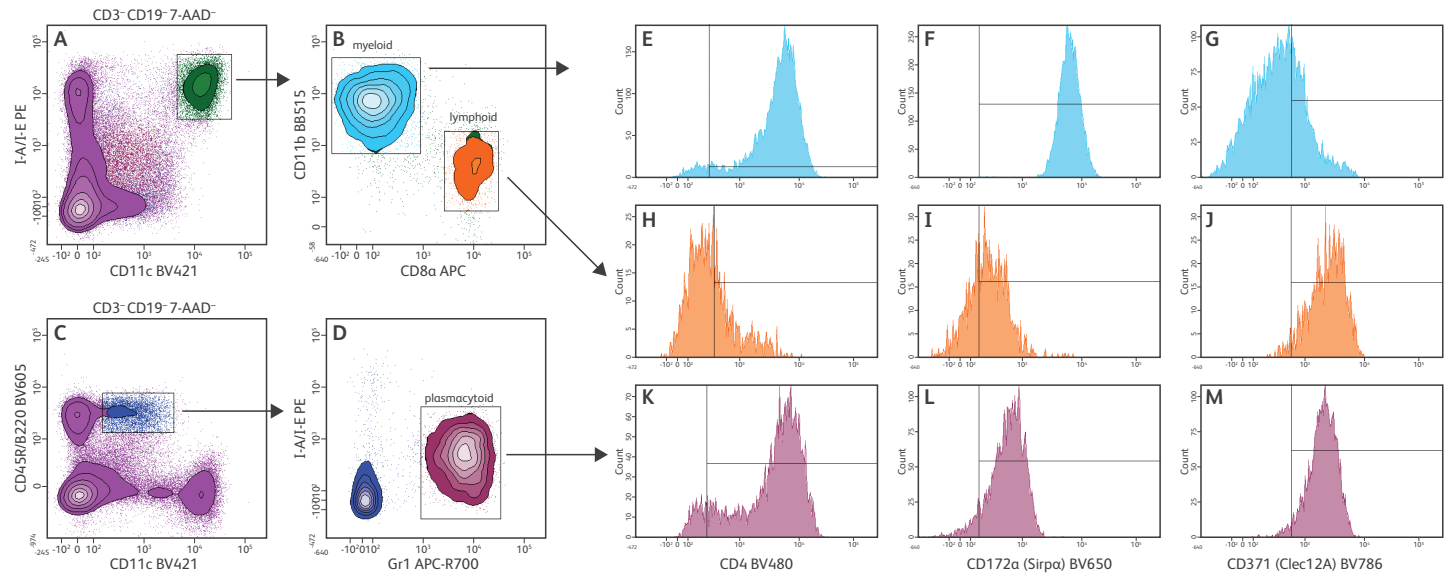
With up to three lasers and twelve fluorescence parameters, the BD FACSCelesta™ flow cytometer is particularly well suited for investigating rare cell populations. Even when cell subsets of interest comprise less than 0.1% of a heterogeneous sample, “deep immunophenotyping” on the BD FACSCelesta can resolve them clearly.



Figure 1 (left side) shows a standard immunophenotyping panel for mouse dendritic cells on the BD FACSCelesta Blue/Violet/Red (BVR) laser configuration. Two blue channels (FITC/BB515 and PE), two violet channels (BV421 and BV605), and two red channels (APC and APC-R700) were used to discriminate myeloid, lymphoid, and plasmacytoid (mDC, IDC, and pDC) subsets, based on their expression of I-A/I-E (MHC-II), CD11b, CD11c, CD8 $\alpha$  and B220.

On the right, three additional discriminatory markers were added for a deeper immunophenotypic characterization of the three DC subsets. Signal Regulatory Protein Alpha (Sirp $\alpha$ /CD172a) was used to characterize CD11c<sup>+</sup>CD11b<sup>+</sup>CD8 $\alpha$ <sup>-</sup> mDCs, while Clec12A (CD371) was used to characterize CD8 $\alpha$ <sup>+</sup> IDCs and pDCs. The expression of CD4 was also analyzed in all three subsets of DCs.

This panel used BD OptiBuild custom reagents to assess expression of Sirp $\alpha$  and Clec12A paired with BD Horizon Brilliant™ violet fluorochromes. These relatively new markers are otherwise available paired only with FITC, PE, and APC, which were already being used in the standard DC panel. BD OptiBuild reagents, which come in convenient 50- $\mu$ g sizes and ship in 72 hours or less, allowed us to “drop in” two additional fluorochromes without having to design and optimize a new panel. The BD FACSCelesta, with its multiple violet channels and optimization for BD Horizon Brilliant™ dyes, is an ideal instrument to take advantage of BD OptiBuild reagents.



**Figure 1.** Deep immunophenotyping of mouse dendritic cell subsets

Ten-color immunophenotypic characterization of the three main subsets of mouse DCs (myeloid, lymphoid and plasmacytoid) in the mouse spleen. BALB/c mouse spleen was enzymatically digested, stained with a cocktail of antibodies (including BD OptiBuild versions of Sirp $\alpha$  BV650 and Clec12A BV786) and analyzed on a BD FACSCelesta Blue/Violet/Red (BVR) laser configuration. Cells were initially gated on CD3, CD19 and 7-AAD negative cells (not shown). **A-D.** Gating strategy: I-A/I-E<sup>high</sup>CD11c<sup>high</sup> conventional dendritic cells (DCs) were further discriminated into CD11b<sup>+</sup> mDCs and CD8<sup>+</sup> IDCs. I-A/I-E<sup>low</sup>CD11c<sup>low</sup> B220<sup>+</sup>Gr1<sup>+</sup> cells were recognized as pDCs. **Results:** Differential expression of the additional markers CD4, CD172 $\alpha$  (Sirp $\alpha$ ) and CD371 (Clec12A) was further analyzed within the mDC (**E, F, G**), IDC (**H, I, J**) and pDC (**K, L, M**) subsets, respectively. Gates were drawn based on fluorescence minus one (FMO) controls.

In Figure 2, the functional responses of human dendritic cell subsets to different stimuli were assessed using the BD FACSCelesta Blue/Violet/Ultraviolet (BVUV) laser configuration. The cells were stimulated with lipopolysaccharide (LPS, a TLR4 ligand), R837 (a TLR7 ligand), or R848 (a TLR7/8 ligand), and analyzed for production of TNF- $\alpha$  and IFN- $\alpha$  using intracellular cytokine staining (ICS).

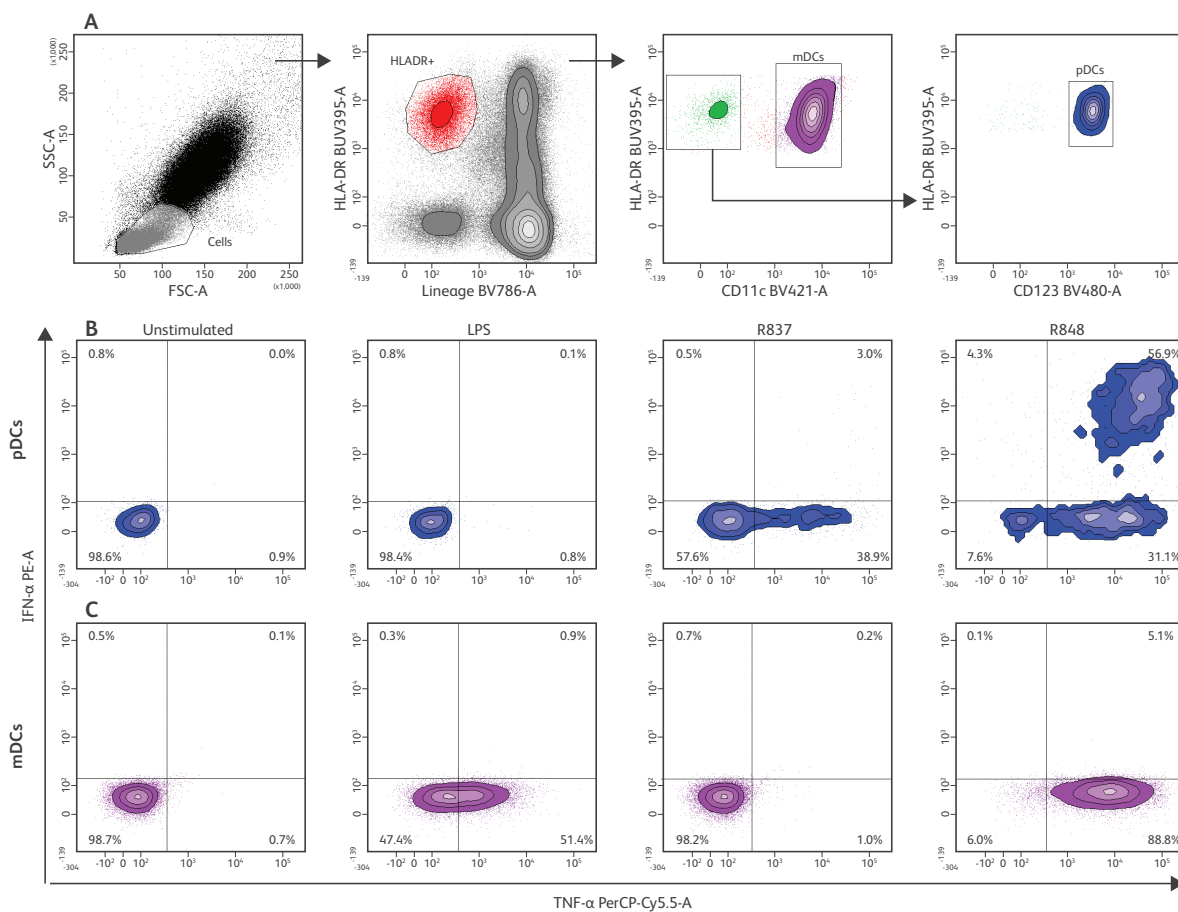
Figure 2A shows the identification of mDCs and pDCs. Cells were gated using light scatter, HLA-DR expression and a lineage-negative cocktail. HLA-DR was assessed using the UV laser to minimize spillover, both into and back from other fluorescence channels. Minimal spillover is key in detecting rare cell populations.

ICS (Figures 2B and 2C) showed that each dendritic subset exhibited a unique cytokine response depending on the stimulus (activating ligand) used. As expected, pDCs (blue), which express TLR7, secreted TNF- $\alpha$  when stimulated with R837 and R848,

both of which activate that receptor. Also, as the predominant cell type that produces type I interferons, pDCs expressed IFN- $\alpha$ , but only when stimulated with R848. mDCs (pink), which express TLR4 and TLR8, produced TNF- $\alpha$  when stimulated with LPS and R848, ligands specific to those receptors, but not with the TLR7 ligand R837.

These experiments demonstrate the ability of flow cytometry to clearly resolve and identify distinct DC subsets in both mouse and human models, and to assess cytokine secretion elicited by different experimental stimuli.

Combining innovations in instrumentation with optimization for bright new reagents, the BD FACSCelesta flow cytometer is designed to help you extract a deeper level of biological information from your cell types of interest. By enabling both deep and broad immunophenotyping, it allows you to design more complex biological panels and address important questions in biology.



**Figure 2.** Intracellular cytokine response in TLR ligand-activated human dendritic cell subsets

Six-color immunophenotypic and functional characterization of the two main subsets of human DCs (plasmacytoid and myeloid). Peripheral blood from a normal human donor was treated with lipopolysaccharide (LPS, a TLR4 ligand), Imiquimod (R837, a TLR7 ligand), or Resiquimod (R848, a TLR7/8 ligand) or left unstimulated for four hours in the presence of BD GolgiPlug™ protein transport inhibitor. Samples were stained with a cocktail of antibodies for detection of surface markers, lysed, fixed and permeabilized using BD Cytofix/Cytoperm™ buffers prior to staining with intracellular antibodies for detection of cytokines TNF- $\alpha$  and IFN- $\alpha$ . Cells were analyzed on the BD FACSCelesta Blue/Violet/UV (BVUV) laser configuration. **Results: A.** Cells were first gated based on light scatter, and then on HLA-DR expression and absence of lineage markers (HLA-DR<sup>+</sup>CD3<sup>-</sup>CD19<sup>-</sup>CD56<sup>-</sup>CD14<sup>-</sup>). DC subsets were then identified as myeloid (HLA-DR<sup>+</sup>CD11c<sup>+</sup>) or plasmacytoid (HLA-DR<sup>+</sup>CD11c<sup>-</sup>, with further confirmation as CD123<sup>+</sup>). **B-C.** Plots show the differential expression of TNF- $\alpha$  and IFN- $\alpha$  (x- and y-axis respectively) within the pDC (blue) and mDC (pink) subsets in unstimulated cells (left column) as well as cells stimulated with LPS, R837 and R848. Unstimulated cells expressed neither cytokine, while mDCs expressed TNF- $\alpha$  when stimulated with LPS or R848, and pDCs expressed TNF- $\alpha$  when stimulated with R837 or R848. Only pDCs stimulated with R848 expressed IFN- $\alpha$ .

## Ordering information

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

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