Immunophenotyping
Identification of 8 immune cell subsets from mouse spleen using 14-color panel

Background
Flow cytometry has become the method of choice for immunophenotyping and analysis of specific cellular subsets. Using antibodies against selective markers, it provides a quick overview of cell types that constitute a sample. At the same time it allows a thorough analysis on single-cell level for in-depth characterization. Using multiple markers simultaneously increases the number of parameters that can be analyzed per run and decreases the amount of starting material required to perform an assay. This can be critical for precious sample material and long-term immune monitoring studies. In this application note, we demonstrate a 14-color immunophenotyping of mouse splenocytes allowing for the simultaneous identification and analysis of 8 different immune cell subsets, including viability and activation state of the cells. The data was acquired using the MACSQuant® Analyzer 16, a compact and reliable benchtop flow cytometer equipped with three lasers for high content analysis.

Materials and methods
Splenocytes were labeled with CD11c-VioBlue®, MHCII-VioGreen™, CD11b-BV570™, CD64-BV605™, NK1.1-BV650™, CD172a-Vio® Bright 515, XCR1-PE, CD19-PE-Vio 770, Siglec-H-PE-Vio 770, CD86-APC, BD Horizon™ Fixable Viability Stain (FVS) 700 and CD45-APC-Vio 770. Data was acquired on the MACSQuant Analyzer 16 using MACSQuantify™ Software. The markers used to identify different immune cell populations are described in table 1.

Cell staining protocol
• Resuspend 2×10^6 splenocytes in 100 μL of PE Buffer (phosphate buffered saline, pH 7.2, 2 mM EDTA, 0.5% BSA).
• Centrifuge at 300xg for 10 minutes, aspirate supernatant completely and resuspend cells in 100 μL of PBS. Repeat washing step once.

Table 1: Identification of different immune cell subsets in murine splenocytes using flow cytometry. Surface markers for characterization compiled from references 1, 2 and 3.
Results

Figure 1 depicts the gating strategy applied to identify the immune cell subsets of interest. By utilizing the expanded fluorescence capability of the MACSQuant Analyzer 16, it is possible to simultaneously detect the presence of T cell, B cells, NK cells, NK-T cells, macrophages, conventional dendritic cells (cDCs) lineage 1, cDCs lineage 2 and plasmacytoid DCs (pDCs), as well as to screen for cell viability.

Figure 1: Immunophenotyping of mouse splenocytes. After doublet and dead cell exclusion, further gating was used to identify macrophages, T cells, NK cell, NKT cells, B cells, cDCs1, dDCs2 and pDCs.
Figure 2 shows the evaluation of the expression of the activation marker CD86 on all the above-identified subsets. The data clearly showed that the MACSQuant Analyzer 16 enables high quality flow cytometry data acquisition with a 14-color panel, opening new possibilities for deeper phenotyping and immune monitoring studies.

**Conclusion**

This application note demonstrates the utility of flow cytometry for the detection and analysis of 8 immune cell subsets simultaneously including viability and activation status of the identified cells using a 14-color panel. It highlights:

- The possibility to design flow panels with up to 14 colors for the identification of different cellular subsets and for in-depth characterization of cells
- The advantage of high-content flow cytometric analyses when dealing with precious sample material
- The MACSQuant Analyzer 16 as a compact benchtop flow cytometer applicable for advanced immunophenotyping panels of murine cells

**References**

3. Takagi et al. (2011) Immunity 35, 958-971

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**Others**

- Brilliant Violet 570 anti-mouse/human CD11b Antibody
  - M1/70 -
- Brilliant Violet 605 anti-mouse CD64 Antibody
  - X54-5/71 -
- Brilliant Violet 650 anti-mouse NK1.1 Antibody
  - PK136 -
- PerCP anti-mouse CD3e Antibody
  - 145-2C11 -
- BD Horizon™ Fixable Viability Stain (FVS) 700
  - N/A -

**Figure 2: Expression of CD86 on different immune cell subsets.** Staining for the cell activation marker CD86 (green) or appropriate control (Fluorescence minus one (FMO), red) on the different subsets identified in figure 1. Cave: staining performed on freshly isolated, unstimulated splenocytes.