Miltenyi Biotec

13-color immunophenotyping of mouse splenocytes

Background

Flow cytometry has become the method of choice for immunophenotyping and identifying specific cellular subsets. Within seconds, it provides a thorough overview of the cell types that constitute a sample. 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 by up to 40%. This can be critical for precious sample material and long-term immune monitoring studies. In this application note, we demonstrate 13-color immunophenotyping of mouse splenocytes using the MACSQuant® Analyzer 16, a compact and reliable benchtop flow cytometer equipped with three lasers that combines the ease of use of previous MACSQuant Analyzer instruments with the option for high content analysis. The markers selected allow for the simultaneous identification and analysis of 11 different cell subsets.

Materials and methods

Mouse splenocytes were labeled with Viobility™ Dye, CD4-VioBlue®, Anti-MHCII-VioGreen™, CD8-BV570™, Anti-Ly-6c-BV605™, Anti-NK1.1-BV650™, CD3ε-FITC, CD19-PE-Vio 615, Anti-Ly-6G-PerCP-Vio 700, CD11c-APC, CD11b-PE, Anti-XCR1-PE-Vio 770, and Anti-SiglecF-APC-R700. Data was acquired on the MACSQuant Analyzer 16 using MACSQuantify™ Software for acquisition and Flowlogic™ Software for analysis.

Cell staining protocol

1. Resuspend 2×10^6 splenocytes in 100 µL of PEB Buffer (phosphate buffered saline, pH 7.2, 2 mM EDTA, 0.5% BSA).
2. Centrifuge at 300×g for 5 minutes and aspirate supernatant completely. Resuspend cells in 100 µL of PBS.
3. Add 1 µL of Viobility Dye and incubate for 15 minutes in the dark at room temperature.
4. Add 1 mL of PEB to wash cells.
5. Centrifuge at 300×g for 5 minutes and aspirate supernatant.
6. Resuspend cells in 100 µL PEB buffer.
7. Add conjugated antibodies at vendor recommended concentdrations.
8. Incubate for 10–15 minutes at 4 °C.
9. Add 1 mL of PEB Buffer to wash cells. Centrifuge at 300×g for 5 minutes and aspirate supernatant.
10. Resuspend pellet in 500 µL of PEB Buffer.

Cell type	Function	Phenotype
Pan T cells	Adaptive immunity	$CD3^+$ $CD19^-$
Cytotoxic T cells	Killing of virally infected cells	$CD3^+$ $CD19^-$ $CD8^+$
T helper cells	Immune regulation	$CD3^+$ $CD19^-$ $CD4^+$
B cells	Adaptive immunity	$CD3^-$ $CD19^+$
Dendritic cells	Antigen presentation	$CD3^-$ $CD19^-$ $CD11c^+$ $MHCII^+$
Conventional DC lineage 1	Adaptive immunity; activation of $CD8^+$ T cells	$CD3^-$ $CD19^-$ $CD11c^+$ $MHCII^+$ $XCR1^+$ $CD11b^{-/low}$
Conventional DC lineage 2	Humoral immunity; activation of $CD4^+$ T cells	$CD3^-$ $CD19^-$ $CD11c^+$ $MHCII^+$ $XCR1^-$ $CD11b^+$
Natural killer cells	Viral and cancer clearance	$CD3^-$ $CD19^-$ $CD11b^+$ Ly-$6g^-$ $NK1.1^+$
Monocytes	Phagocytosis of pathogens and dead cells	$CD3^-$ $CD19^-$ $CD11b^+$ Ly-$6g^-$ Ly-$6c^+$
Neutrophils	Innate immunity	$CD3^-$ $CD19^-$ $CD11b^{+/-}$ Ly-$6g^+$
Eosinophils	Innate immunity	$CD3^-$ $CD19^-$ $CD11b^+$ Ly-$6g^-$ $SinglecF^+$

Table 1: Identification and analysis of PBMC subsets. The table shows a selection of surface markers that can be used for a characterization of immune cell subtypes by flow cytometry. Compiled from references 1 and 2.

Results

Figure 1 depicts the gating strategy used to identify cell populations of interest using the MACSQuant Analyzer 16. By utilizing the expanded fluorescence capability of the MACSQuant Analyzer 16, it is possible to simultaneously detect the presence of T cells, cytotoxic T cells, T helper cells, B cells, dendritic cells (DCs), conventional dendritic cells (cDCs) lineage 1, cDCs lineage 2, natural killer (NK) cells, monocytes, eosinophils and neutrophils. The data clearly show that the MACSQuant Analyzer 16 enables high quality flow cytometry data acquisition with a 13-color panel, opening new possibilities for deeper phenotyping and immune monitoring studies.

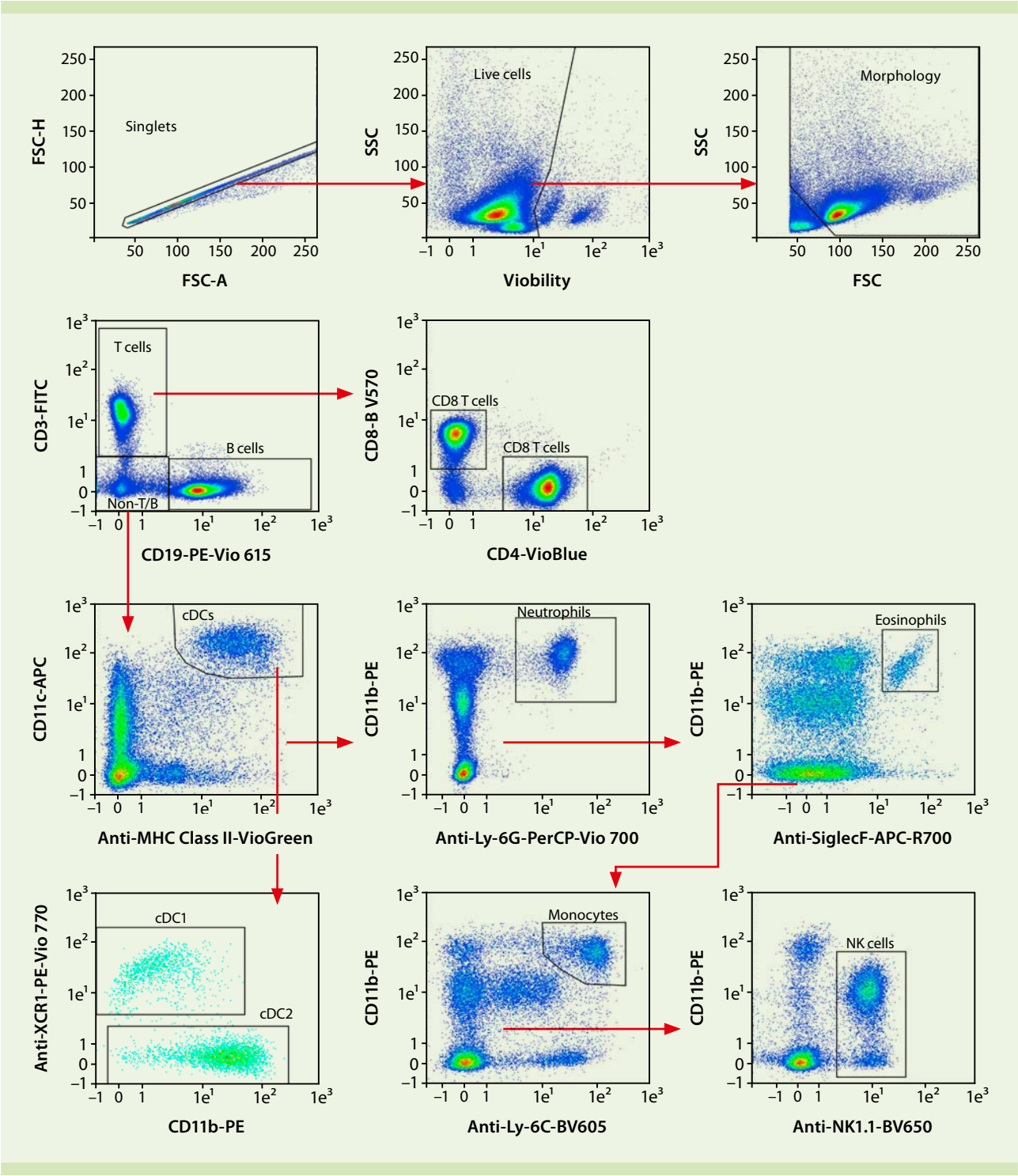

Figure 1: Immunophenotyping of mouse splenocytes. After doublet and dead cell exclusion, further gating was used to identify T cells, cytotoxic T cells, T helper cells, B cells, cDCs, cDCs 1, cDCs 2, NK cells, monocytes, eosinophils, and neutrophils.

Conclusions

This application note demonstrates the utility of flow cytometry for the detection and enumeration of 11 cellular populations including secondary subsets using a 13-color panel. It highlights:

- the MACSQuant® Analyzer 16 as a novel, compact, benchtop flow cytometer, applicable for advanced immunophenotyping panels of murine cells
- the possibility to design flow panels with up to 14 colors for the analysis of complex cellular subsets
- the usefulness of high-content flow cytometric analyses when dealing with precious sample material

References

1. Merad, M. *et al.* (2013) Annu. Rev. Immunol. 31: 563–604.
2. Olingy, C.E. *et al.* (2017) Sci. Rep. 7: 447.

Product	Clone	Order no.
Miltenyi Biotec products		
CD3ε-FITC	17A2	130-118-958
CD4-VioBlue	REA604	130-118-568
CD11b-PE	REA592	130-113-806
CD11c-APC	REA754	130-110-702
CD19-PE-Vio 615	REA749	130-111-890
Anti-MHC Class II-VioGreen	REA813	130-112-238
Anti-Ly-6G-PerCP-Vio 700	REA526	130-117-500
Anti-XCR1-PE-Vio 770	REA707	130-111-374
Viobility™ Dye	N/A	Coming soon
Others		
Brilliant Violet 570™ anti-mouse CD8a	53-6.7	
Brilliant Violet 605™ anti-mouse Ly-6C	HK1.4	
Brilliant Violet 650™ anti-mouse NK-1.1	PK136	
Anti-SiglecF-APC-R700	E50-2440	

Miltenyi Biotec

Miltenyi Biotec GmbH | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

V.01