

The Immunophenotyping Core

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The UAB Heersink School of Medicine Immunology Institute and the O'Neal Comprehensive Cancer Center (OCCC), working in close partnership with the Flow Cytometry and Single Cell (FCSC) core, has established human IMMUNOPHENOTYPING services to all members of the Immunology Institute and the UAB community.

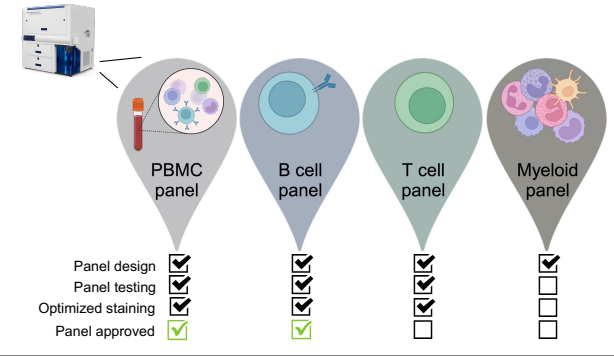
What is immunophenotyping?

Immunophenotyping is the identification and quantitation of heterogeneous populations of cells by multi-parameter flow cytometry using a panel of fluorescently-labelled antibodies that recognize specific antigens on a cell surface, known as cell markers.

Why use immunophenotyping in your work?

Immunophenotyping is used to monitor the molecular, metabolic, phenotypic and functional attributes of cells that circulate through the blood. Alterations in these cellular attributes, particularly over time, may be closely associated with specific disease manifestations, disease progression and responsiveness to treatment. Thus, immunophenotyping is a powerful approach to identify easily monitored biomarkers and cellular signatures that may be useful as diagnostic and prognostic indicators of disease. Immunophenotyping is particularly helpful when monitoring patients who are exposed to immunotherapies, such as checkpoint inhibitors and targeted immune modulators that are increasingly used in chronic disease settings.

Which immunophenotyping panels are available?



Composition of our validated PBMC and B cell panels and the T cell panel currently under development

PBMC FLOW CYTOMETRY PANEL (36-PARAMETER, 28-COLOR)

Parameters	
CD71/CD19 ⁺	CD11b
CD19 ⁺	CD19 ⁻
CD34	CD38
CD4	CD45
HLA-DR	CD45RA
CD8	CD56
CD138	CD138
CD14	CD14
CD141	CD141
CD333	CD333
CD37	CD37
CD33	CD33
CD393	CD393
CD27	CD27
CD8	CD8
CD45	CD45RA
CD56	CD56
CD7	CD7
PD-1/CD274	LIVE/DEAD
Side Scatter (SSC)	Side Scatter (SSC)

B CELL FLOW CYTOMETRY PANEL (24-PARAMETER, 22-COLOR)

Parameters	
CD11c	CD138
CD16	CD16
CD19	CD19
CD21	CD21
CD24	CD24
CD36	CD36
CD62L	CD62L
CD11	CD11
CD38	CD38
CD45	CD45
CD56	CD56
CD62L	CD62L
CD11	CD11
CD38	CD38
CD45	CD45RA
CD56	CD56
CD7	CD7
CD138	CD138
CD14	CD14
CD141	CD141
CD333	CD333
CD37	CD37
CD33	CD33
CD393	CD393
CD27	CD27
CD8	CD8
CD45	CD45RA
CD56	CD56
CD7	CD7
PD-1/CD274	LIVE/DEAD
Side Scatter (SSC)	Side Scatter (SSC)

T CELL FLOW CYTOMETRY PANEL (35-PARAMETER, 33-COLOR) – under development

Parameters	
CD103	CD127
CD14	CD14
CD19	CD19
CD22	CD22
CD27	CD27
CD38	CD38
CD45	CD45RA
CD56	CD56
CD7	CD7
CD138	CD138
CD14	CD14
CD141	CD141
CD333	CD333
CD37	CD37
CD33	CD33
CD393	CD393
CD27	CD27
CD8	CD8
CD45	CD45RA
CD56	CD56
CD7	CD7
PD-1/CD274	LIVE/DEAD
Side Scatter (SSC)	Side Scatter (SSC)

Identification of 44 human immune cell subsets using the PBMC panel

Cell Population	Cell Subset Name	Gating	
B Cells	Non-Progenitor Hematopoietic Cell	CD45+CD34-	
	B Cell	CD3+CD14-/CD16-/CD56-/CD19+	
	Transitional B Cell (T)	CD3-/CD14-/CD16-/CD56-/CD19+/CD24+/CD38+/IgM+	
	Naive B Cell	CD3-/CD14-/CD16-/CD56-/CD19+/IgD+/CD27-/IgM+	
	Unswitched Memory B Cell	CD3-/CD14-/CD16-/CD56-/CD19+/IgD+/CD27+/IgM+	
	Switched Memory B Cell	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+	
	IgG+ Switched Memory B Cells	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/IgG+	
	True IgM+ Memory B Cells	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/IgM+	
	Double Negative	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-	
	IgG+ Double Negative	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/IgG+	
Monocytes	IgM+ Double Negative	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/IgM+	
	Double Negative 2	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/CD11c+	
	Plasmablast	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD38+/CD24-/CD138-	
	Plasma Cell	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD38+/CD24-/CD138+	
	Classical Monocyte	CD3-/CD19-/CD56-/CD8-/HLA-DR+/CD14+/CD16-	
	Intermediate Monocyte	CD3-/CD19-/CD56-/CD8-/HLA-DR+/CD14+/CD16+	
	Non-Classical Monocyte	CD3-/CD19-/CD56-/CD8-/HLA-DR+/CD14+/CD16+	
	Dendritic Cells (DCs)	DC	CD3-/CD14-/CD16-/CD19-/CD56-/HLA-DR+
	CD1c+ Conventional DC	CD3-/CD14-/CD16-/CD19-/CD56-/CD11b-/HLA-DR+/CD11c+/CD141-/CD141+	
	CD141+ Conventional DC	CD3-/CD14-/CD16-/CD19-/CD56-/CD11b-/HLA-DR+/CD11c+/CD141+/CD141+	
Natural Killer (NK) Cells	Plasmacytoid DC	CD3-/CD14-/CD16-/CD19-/CD56-/CD11b-/HLA-DR+/CD11c-/CD123+/CD333+	
	NKT cell	CD19-/CD14-/CD3+/CD56+	
	Natural Killer (NK) Cells	CD19-/CD14-/CD3-/CD56bright/CD16-	
	CD56-dim CD16+ NK Cell	CD19-/CD14-/CD3-/CD56-dim/CD16+	
	CD56- CD16+ NK Cell	CD19-/CD14-/CD3-/CD56-CD16+	
	CD3+ T Cell	CD19-/CD14-/CD56-/CD3+	
	CD4+ T Cell	CD19-/CD14-/CD56-/CD3+/CD4+	
	CD4 Naive T cell (Nav)	CD19-/CD14-/CD56-/CD3+/CD4+/CD45RA+/CCR7+	
	CD4 Central Memory T Cell (CM)	CD19-/CD14-/CD56-/CD3+/CD4+/CD45RA-/CCR7+	
	CD4 Effector Memory T Cell (EM)	CD19-/CD14-/CD56-/CD3+/CD4+/CD45RA-/CCR7-	
CD8+ T Cells	CD4 Effector Memory RA+ T Cell (EMRA)	CD19-/CD14-/CD56-/CD3+/CD4+/CD45RA+/CCR7-	
	CD4 Anergic	CD19-/CD14-/CD56-/CD3+/CD4+/PD1+/CD57+	
	CD4 Senescent	CD19-/CD14-/CD56-/CD3+/CD4+/PD1-/CD57+	
	CD4 No-Expression	CD19-/CD14-/CD56-/CD3+/CD4+/PD1-/CD57-	
	CD4 Exhausted	CD19-/CD14-/CD56-/CD3+/CD4+/PD1+/CD57-	
	CD8+ T Cell	CD19-/CD14-/CD56-/CD3+/CD8+	
	CD8 Naive T Cell (Nav)	CD19-/CD14-/CD56-/CD3+/CD8+/CD45RA+/CCR7+	
	CD8 Central Memory T Cell (CM)	CD19-/CD14-/CD56-/CD3+/CD8+/CD45RA-/CCR7+	
	CD8 Effector Memory T Cell (EM)	CD19-/CD14-/CD56-/CD3+/CD8+/CD45RA-/CCR7-	
	CD8 Effector Memory RA+ T Cell (EMRA)	CD19-/CD14-/CD56-/CD3+/CD8+/CD45RA+/CCR7-	

Identification of 23+ human B cell subpopulations using the B cell panel

Cell Population	Cell Subset Name	Gating
B Cells	Non-Progenitor Hematopoietic Cell	CD45+
	B Cell	CD3-/CD14-/CD16-/CD56-/CD19+
	Transitional B Cell (T)	CD3-/CD14-/CD16-/CD56-/CD19+/CD24+/CD38+/IgM+
	Plasmablast (PB) + Plasma Cell (PC) Pool	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD38+/CD24-
	Proliferating Plasmablast	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD38+/CD24-/CD138-/CD71+
	Plasma Cell	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD38+/CD24-/CD138+/CD71+
	Naive B Cell (Nav)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD+/CD27-/IgM+
	Resting Naive (rNav)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD+/CD27-/IgM+/CXCR5+/CD11c-
	Activated Naive (aNav)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD+/CD27-/IgM+/CXCR5+/CD11c+
	Unswitched Memory B Cell (UnSw)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD+/CD27+/IgM+
B Cells	Switched Memory B Cell (Sw)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+
	Resting Sw (rSw)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CXCR5+/CD11c-
	Effectors Sw (eSw)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CXCR5+/CD11c+
	IgG+ Switched Memory B Cells	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/IgG+
	True IgM+ Memory B Cells	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/IgM+
	Resting & Lymphoid Tissue Homing Switched Memory B Cells	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD62L+/CD71-
	Activated Sw Memory B Cells	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27+/CD62L-/CD71+
	Double Negative B Cell (DN)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-
	Double Negative 1 (DN1)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/CXCR5+/CD11c-
	Double Negative 2 (DN2)	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/CXCR5+/CD11c+
	FCRL5+ DN2	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/CXCR5-/CD11c+/FCRL5+
	IgG+ Double Negative	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/IgG+
	IgM+ Double Negative	CD3-/CD14-/CD16-/CD56-/CD19+/IgD-/CD27-/IgM+

- Two channels are made available for additional surface markers and/or B cell tetramers.
- B cell tetramers can be used to identify antigen-specific populations of B cells.
- B cell tetramers are currently available from the Immunology Institute's Antibody Characterization and Serology (ACS) recharge facility. The facility offers 66 tetramers capable of identifying specificity to 17 different influenza antigens and 15 different COVID antigens.

Identification of T cell subpopulations using the T cell panel

The T cell panel will enable the identification of:

- Effector CD4+ and CD8+ T cells
- Exhausted CD4+ and CD8+ T cells
- Memory CD4+ and CD8+ T cells
- Regulatory T cells
- Follicular helper T cells

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