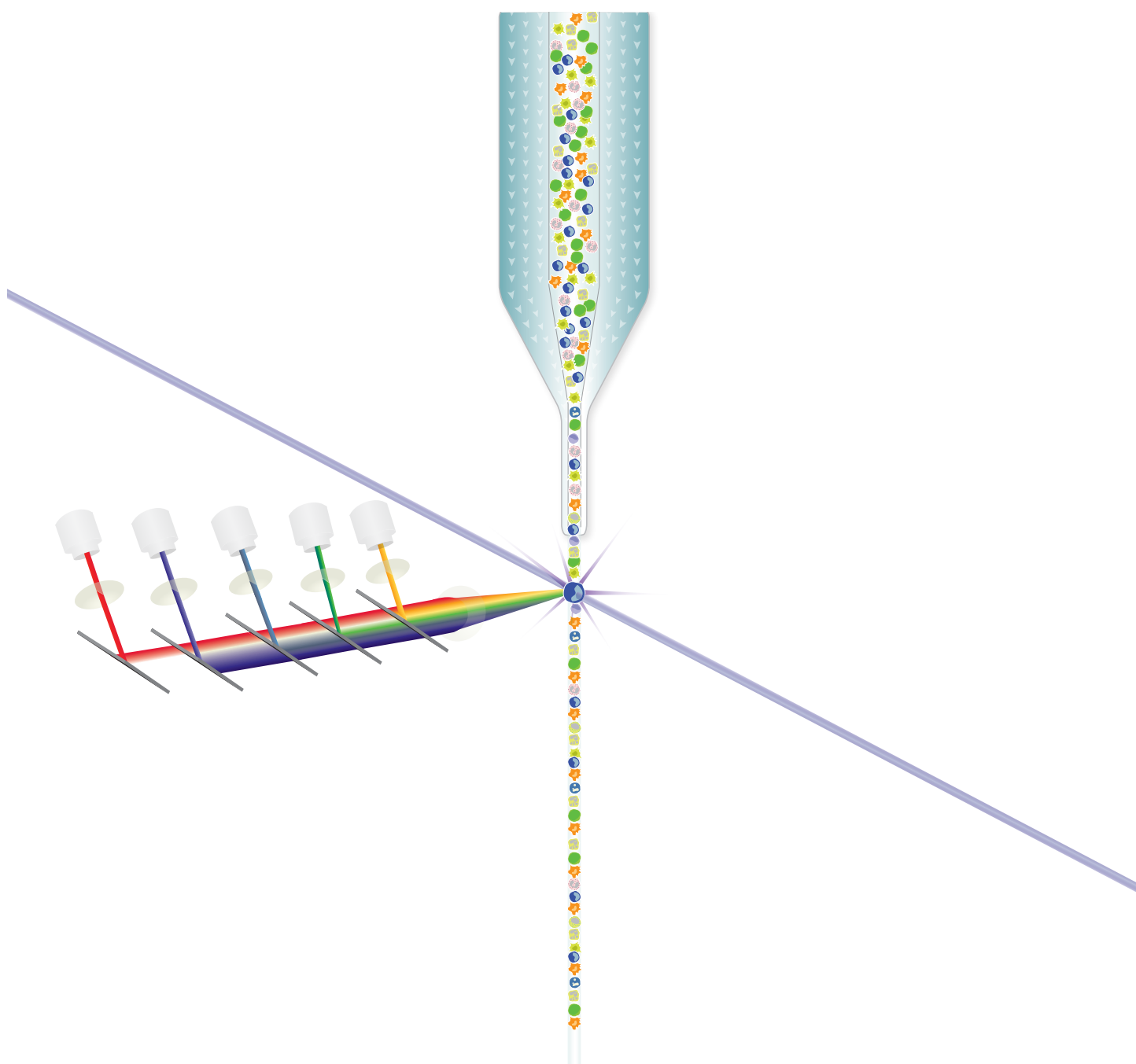




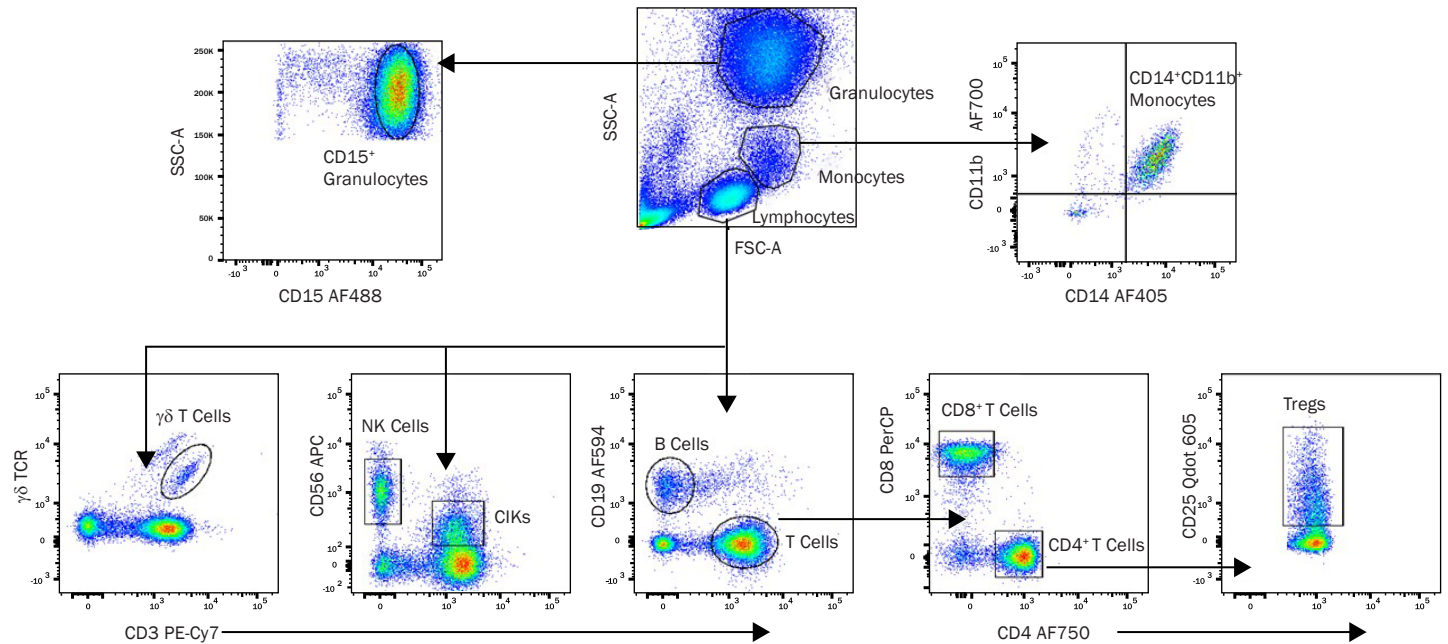
A Guide to Human Immune Cell Characterization by Flow Cytometry



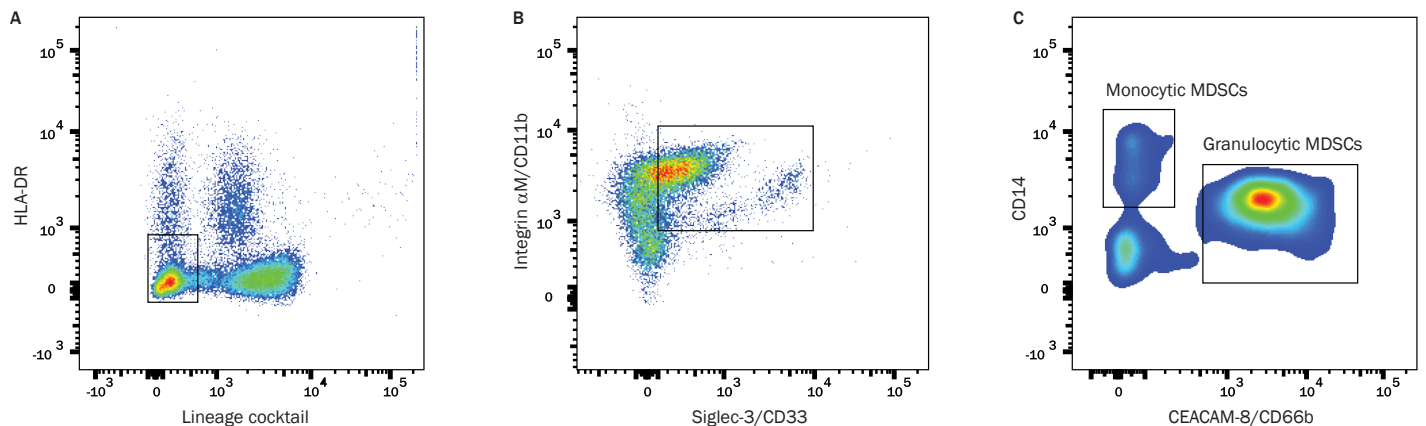
Immune Cell Characterization by Flow Cytometry

Flow cytometry is a powerful technique that is widely used to identify and characterize different immune cell types in heterogeneous samples. It primarily relies on the use of fluorochrome-conjugated antibodies to detect the expression of specific cell surface or intracellular antigens on single cells in suspension. Using a combination of antibodies against different targets that are conjugated to fluorochromes with different emission wavelengths, multiple antigens can be detected simultaneously. As a result, distinct cell populations can be identified, quantified, and gated for further analysis.

Bio-Techne offers an unparalleled selection of fluorochrome-conjugated R&D Systems and Novus Biologicals antibodies qualified for flow cytometry that can be used to detect markers on different immune cell types present in mixed population samples, such as peripheral blood or tissue samples. Hundreds of world-renowned unique clones are available from the R&D Systems brand, many of which have been used to establish CD nomenclature through HLDA workshops. Additionally, the Novus Biologicals brand includes an expansive collection of both proprietary antibodies and some of the most highly referenced antibody clones on the market.



10-Parameter Flow Cytometric Analysis of Immune Cells in Human Whole Blood. Human whole blood was stained with the following panel of monoclonal antibodies: Alexa Fluor® 700-conjugated Mouse Anti-Human CD11b/Integrin α M (R&D Systems, Catalog # FAB1699N), Alexa Fluor 405-conjugated Mouse Anti-Human CD14 (R&D Systems, Catalog # FAB3832V), Alexa Fluor 488-conjugated Mouse Anti-Human CD15/Lewis X (R&D Systems, Catalog # FAB7368G), PE-Cy7-conjugated Mouse Anti-Human CD3 (Novus Biologicals, Catalog # NB100-2726PECY7), Alexa Fluor 750-conjugated Mouse Anti-Human CD4 (R&D Systems, Catalog # FAB3791S), PerCP-conjugated Mouse Anti-Human CD8 α (R&D Systems, Catalog # FAB1509C), Alexa Fluor 594-conjugated Mouse Anti-Human CD19 (R&D Systems, Catalog # FAB4867T), AmCyan-conjugated Anti-Human $\gamma\delta$ TCR, BV605-conjugated Anti-Human CD25/IL-2 R α , and APC-conjugated Mouse Anti-Human CD56/NCAM-1 (R&D Systems, Catalog # FAB2408A). Different immune cell populations identified by the specific staining shown in each dot plot were gated and labeled or further stained as indicated to identify subsets of that particular cell type.



Identification of Human Granulocytic and Monocytic Myeloid-derived Suppressor Cells by Flow Cytometry. (A) Lin⁻/HLA-DR⁻ cells were detected in human peripheral blood mononuclear cells by staining with a lineage cocktail containing Alexa Fluor 700-conjugated Mouse Anti-Human CD3 ϵ and CD19 Monoclonal Antibodies (R&D Systems, Catalog # FAB100N and # FAB4867N, respectively) and an Alexa Fluor 750-conjugated Mouse Anti-Human HLA-DR Monoclonal Antibody (R&D Systems, Catalog # FAB4869S). Lin⁻/HLA-DR⁻ cells were gated. (B) CD11b⁺/CD33⁺ cells were detected in the Lin⁻/HLA-DR⁻ cell population by staining with an APC-conjugated Mouse Anti-Human Siglec-3/CD33 Monoclonal Antibody (R&D Systems, Catalog # FAB1137A) and an Alexa Fluor 405-conjugated Mouse Anti-Human Integrin α M/CD11b Monoclonal Antibody (R&D Systems, Catalog # FAB16991V). CD11b⁺/CD33⁺ cells were gated. (C) Human granulocytic myeloid-derived suppressor cells (Lin⁻/CD11b⁺/CD14⁺/CD33⁺/CEACAM-8⁺/HLA-DR⁻) and monocytic myeloid-derived suppressor cells (Lin⁻/CD11b⁺/CD14⁺/CD33⁺/CEACAM-8⁻/HLA-DR⁻) were detected in the Lin⁻/HLA-DR⁻/CD11b⁺/CD33⁺ cell population by staining with a PE-conjugated Mouse Anti-Human CEACAM-8/CD66b Monoclonal Antibody (R&D Systems, Catalog # FAB4246P) and a PerCP-conjugated Mouse Anti-Human CD14 Monoclonal Antibody (R&D Systems, Catalog # FAB3832C).

Flow Cytometry Antibodies for Characterizing Human Immune Cell Types

Cell Surface Markers	Clone	Fluorochrome-conjugated Antibodies (Catalog # - Fluorochrome)
CCR2	48607* ■	FAB150-R, S, T, U, V; FAB151-A, C, G, N, P
CCR3	61828*	FAB155-A, C, F, G, N, P, R, S, T, U, V
CCR4	205410* ■	FAB1567-A, C, F, G, N, P, R, S, T, U, V
CCR5	CTC5 ■	FAB1802-A, F, G, N, P, R, S, T, U, V
CCR6	53103* ■	FAB195-A, C, F, G, N, P, R, S, T, U, V
CCR7	150503*	FAB197-A, F, G, N, P, R, S, T, U, V
CCR10	314305 ■	FAB3478-A, G, N, P, R, S, T, U, V
CD1a	703217	FAB7076-A, G, N, P, R, S, T, U, V
CD3	UCHT1*	FAB100-A, C, F, G, N, P, R, S, T, U, V
	OKT3	NBP2-24867-A, C, G, N, P, S, T, U, V **
CD4	34930	FAB379-G, N, R, S, T, U, V
	RPA-T4	NBP2-27216-A, C, F, G, N, P, R, S, T, U, V **
CD8α	37006	FAB1509-A, C, F, G, N, P, R, S, T, U, V
	53-6.7	NBP1-49045-A, C, G, N, P, R, S, T, U, V **
	RPA-T8	NBP2-25195-A, C, F, G, N, P, R, S, T, U, V
CD11b/Integrin αM	ICRF44	FAB1699-G, N, R, S, T, U, V
	M1/70.15	NB600-1327-A, C, G, N, P, R, S, T, U, V **
CD11c	ICRF3.9	FAB1777-A, C, G, N, P, R, S, T, U, V
CD14	134620 ■	FAB3832-A, C, F, G, N, P, R, S, T, U, V
	M5E2	NB100-77758-A, C, F, G, N, R, S, T, U, V **
CD15	ICRF29-2	FAB7368-G, N, R, S, T, U, V
CD16/FcγRIII	245536	FAB2546-A, C, F, G, N, P, R, S, T, U, V
	3G8	NBP1-50056-A, C, F, G, N, P, R, S, T, U, V **
CD19	4G7-2E3 ■	FAB4867-A, C, F, G, N, P, R, S, T, U, V
	1D3	NBP2-24965-A, C, F, G, N, P, R, S, T, U, V **
CD20	396444	FAB4225-G, N, P, R, S, T, U, V
CD25/IL-2 Rα	24212	FAB1020-A, G, N, P, R, S, T, U, V
CD45	2D1	FAB1430-A, C, G, N, P, R, S, T, U, V
	30-F11	NB100-77417-A, C, F, G, N, P, R, S, T, U, V **
CD45RA	MEM-56	NB500-329-A, C, F, G, N, P, R, S, T, U, V **
CD45RO	UCHL-1	NBP2-33104-A, C, F, G, N, P, R, S, T, U, V **
CD56/NCAM-1	301040	FAB2408-A, G, N, P, R, S, T, U, V
CD62L/L-Selectin	4G8	FAB9787-G, R, T
CD63	460305	IC5048-G, N, P, R, S, T, U, V
	H5C6	NBP2-42225-A, C, G, N, P, R, S, T, U, V **
CD66b/CEACAM-8	913542 ■	FAB4246-A, G, N, P, R, S, T, U, V
CD68/SR-D1	298807	IC20401-A, F, G, N, P, R, S, T, U, V
	KP1	NB100-683-A, C, F, G, N, P, R, S, T, U, V **

Cell Surface Markers	Clone	Fluorochrome-conjugated Antibodies (Catalog # - Fluorochrome)
CD69	298614	FAB23591-A, G, N, P, R, S, T, U, V
CD80/B7-1	37711	FAB140-F, G, N, P, R, S, T, U, V
CD86/B7-2	37301	FAB141-A, C, F, G, N, P, R, S, T, U, V
CD115/M-CSF R	61708	FAB329-A, F, G, N, P, R, S, T, U, V
CD117/c-kit	47233	FAB332-A, G, N, P, R, S, T, U, V
	2B8	NB100-77477-A, C, F, G, N, P, R, S, T, U, V **
CD123/IL-3 Rα	32703	FAB301-A, C, G, N, P, R, S, T, U, V
CD127/IL-7 Rα	40131	FAB306-A, G, N, P, R, S, T, U, V
CD141/BDCA-3	501733	FAB3947-A, G, N, R, S, T, U, V
CD163	215927	FAB1607-A, C, G, N, R, S, T, U, V
CD172/SIRPα	602411	FAB4546-G, N, P, R, S, T, U, V
CD206/MMR	685641	FAB25342-A, G, N, P, R, S, T, U, V
CLEC9a	683409	FAB6049-A, G, N, R, S, T, U, V
CRTH-2	301109	FAB33381-A, G, N, P, R, S, T, U, V
CXCR3	49801 ■	FAB160-A, C, F, G, N, P, R, S, T, U, V
CX3CR1	528728	FAB5204-P
Fcc R1a	773704	FAB6678-C, G, N, R, S, T, U, V
	AER-37	NBP1-43278-A, C, F, P **
HLA-DR	L203	FAB4869-A, C, F, G, N, P, R, S, T, U, V
	L243	NB100-77855-A, C, F, G, N, P, R, S, T, U, V **
Neuropilin-1/BDCA-4	446921	FAB3870-A, F, G, N, P, R, S, T, U, V
NKp44/NCR2	253415	FAB22491-A, G, N, P, R, S, T, U, V
XCR1	1097A ■	FAB8571-G, N, P, R, S, T, U, V

Transcription Factors	Clone	Fluorochrome-conjugated Antibodies (Catalog # - Fluorochrome)
AHR	RPT9	NB300-515-A, G, N, P, R, S, T, U, V **
EOMES	644730	IC6166-A, G, N, R, S, T, U, V
FoxP3	1054C ■	IC8214-A, G, N, P, S, T, U, V
	376209R ■	IC8970-G, N, R, S, T, U, V
GATA-3	634919	IC63301-A, T
	291106	IC26051-P
PU.1	732322	IC5870-G, N, R, S, T, U, V
RORγt	1181A	IC9125-A, G, N, R, S, T, U, V
	600380	IC6006-A, C, P
T-bet	525803	IC5385-C, F, G, N, R, S, T, U, V
	525831 ■	IC53851-G, N, R, S, T, U, V
	4B10	NBP1-43298-A, C, G, N, P, R, S, T, U, V **

■ Indicates a Novus Biologicals Antibody

■ Indicates a R&D Systems Antibody

* Clone was used by HLDA to establish CD designation.

■ This antibody is either a recombinant monoclonal antibody or is available as a recombinant monoclonal antibody.

Fluorochrome Key:

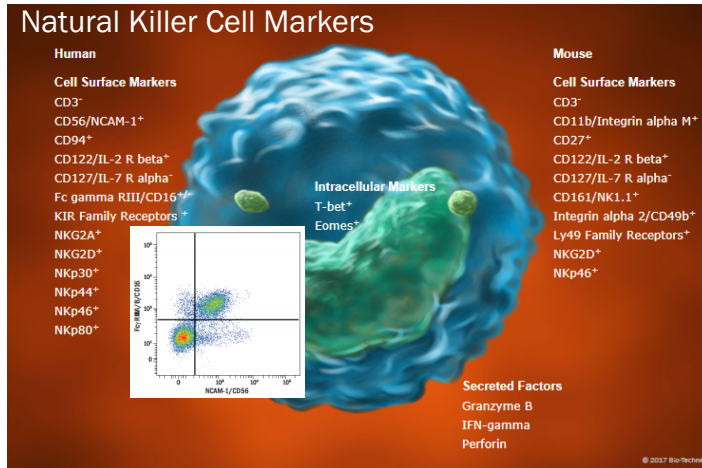
A Allophycocyanin, C PerCP, F Fluorescein, G Alexa Fluor®488, N Alexa Fluor 700, P Phycoerythrin, R Alexa Fluor 647, S Alexa Fluor 750, T Alexa Fluor 594,

U Alexa Fluor 350, V Alexa Fluor 405

** Janelia Fluor and/or tandem dye conjugates are also available for these antibodies. Please visit novusbio.com for a complete listing of available fluorochromes.

Explore Our New Resources for Flow Cytometry

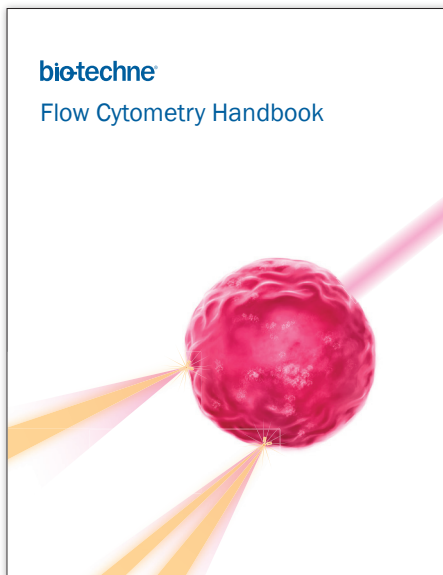
Cell Markers Interactive Resource Tool



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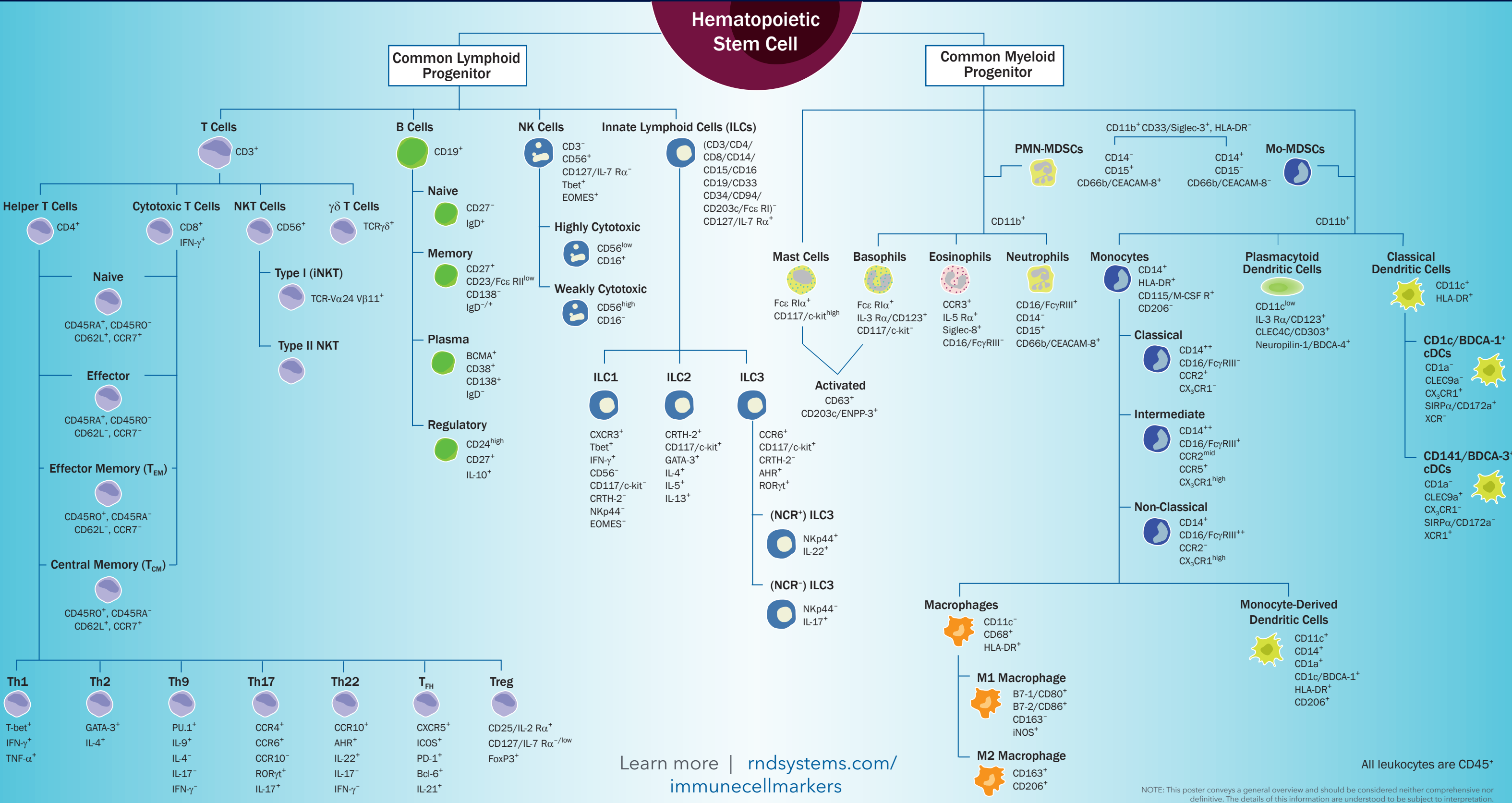
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NOTE: This poster conveys a general overview and should be considered neither comprehensive nor definitive. The details of this information are understood to be subject to interpretation.

All leukocytes are CD45⁺