

Innate Lymphoid Cells

Innate Effector Subsets

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Innate lymphoid cells (ILCs) are a group of innate effectors with transcriptional, functional, and phenotypic similarities to various T helper (Th) subsets. Like Th subsets, ILCs are found at mucosal surfaces and play important roles in immune response and protective immunity, and their deregulation can lead to pathological roles in allergic and autoimmune diseases. However, unlike Th cells that are dependent upon a cognate antigen for proliferation, ILCs constitutively express specific fate-determining transcription factors and do not respond in an antigen-specific manner.

Introduction

ILCs include lineage marker negative (lin⁻) ILCs, natural killer (NK) cells, and lymphoid tissue-inducer (LTI) cells. Three distinct groups of ILCs have been classified based upon their immune response of Type 1, 2, and 3. Group 1 ILCs consists of IFN- γ -producing ILC1s or NK cells. Group

2 ILCs contain IL-5 and IL-13, producing ILC2s, while Group 3 is comprised of ILC3s that are either IL-22-producing natural cytotoxicity receptor (NCR) positive, IL-17-producing NCR negative, or lymphotoxin-expressing Lymphoid Tissue-inducer (LTI) cells.

Role in mucosal immunology

In the human body, cells of microbial origin outnumber human cells ten to one, and bacterial genes outnumber human genes one hundred-fold. This awareness has increased appreciation of the role microbes play in promoting human health and the effect perturbations in the symbiotic relationship between human cells and microbiota may have on diseases such as rheumatoid arthritis, diabetes, multiple sclerosis, and obesity.

While epithelial cells are often viewed as the first line of defense against pathogenic insult, the commensal microbiota that thrive on epithelial surfaces serve as an invisible barrier to invading pathogens. Symbiosis of host cells with non-pathogenic microbes prevents disease in a number of ways, including competition for adherence to the epithelia and mucosal surfaces, as well as acidification of the microenvironment to prevent attachment competition for nutrients, secretion of anti-microbial peptides, and development of gut-associated lymphoid tissues (GALT).

When the commensal microbiota is disturbed, as in the case of broad-spectrum antibiotic use, the resulting imbalance can lead to infection by opportunistic bacteria and perturbations in the immunosystem. Additionally, abnormal microbiota has been described in many inflammatory and autoimmune diseases (Crohn's disease, ulcerative colitis, inflammatory arthritis, type-1 diabetes, multiple sclerosis, and the like.)

Unlike Th effector subsets, ILC groups continuously express lineage-specific transcription factors, creating a group of effector cells ready to respond at mucosal surfaces. The various ILC cell types play different but important mucosal immune roles:

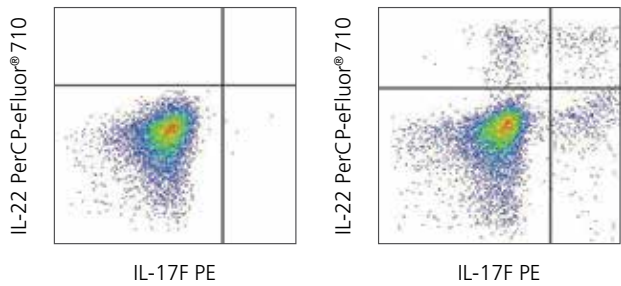
- LTI cells express lymphotoxins (LT α /TNF β and LT β) to mediate the expression of epithelium adhesion molecules and the growth of Peyer's Patches.
- ILC2s are contained within Peyer's Patches and express B cell-stimulating cytokines, IL-5, IL-6, and IL-13. Furthermore, during worm infections, ILC2s play an important role by proliferating in response to the release of IL-25 (IL-17E) and IL-33 by epithelial cells to promote a Type 2 effector response.
- NCR+ ILC3s secrete IL-22 in response to bacterial pathogens. IL-22 is critical in controlling the secretion of anti-microbial peptides from epithelial cells and preserving cell integrity and homeostasis with commensal microbiota. Although ILC3s have been shown to be the major source of IL-22 in early phase infections, Th1 and Th17 cell response is still required to eliminate most infections.

Intracellular cytokine and chemokine staining

ILCs, like Th cells, are defined and influence a variety of immune cells by their effector cytokine expression pattern. To measure this response, cytoplasmic staining requires fixation to crosslink proteins and to stabilize the cell membrane, followed by permeabilization to allow antibodies access to intracellular antigens. Intracellular Fixation and Permeabilization Buffer (cat. no. 88-8824) is designed for optimal detection of cytoplasmic proteins and secreted proteins, such as cytokines and chemokines. In this regard,

expression of most cytokines requires an induction protocol to reach detectable levels by flow cytometry. The appropriate stimulation conditions and kinetics of cytokine production will vary depending upon the cell type and the particular cytokine being assayed. It is necessary to block secretion of cytokines with protein transport inhibitors, such as Monensin (cat. no. 00-4505) or Brefeldin A (cat. no. 00-4506). Investigators should evaluate the use and efficacy of different protein transport inhibitors in their specific assay system.

Human Intracellular Antibodies																			
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers					Red Laser			FlowRNA Probe
						eFluor® 450	FITC	Alexa Fluor® 488	PerCP-Cyanine 5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	APC	eFluor® 660	APC-eFluor® 780	
ILC Group 1																			
CCL3 (MIP-1α)	PFFM3	7539				■	■			■	■								■
CCL4 (MIP-1β)	FL34Z3L	7540																	■
CCL5 (RANTES)	VL1	9905																	■
Granzysin	DH2	8828																	■
Granzyme B	GB11	8899																	■
Granzyme K	G3H69	8897																	■
Granzyme M	4B2G4	9774																	■
IFN _γ	4S.B3	7319	■			■	■	■	■										■
LAP	FNLAP	9829								■	■								■
Perforin	dG9	9994	■				■			■	■								■
TNFα	MAb11	7349	■			■	■	■	■	■	■								■
ILC Group 2																			
IL-4	8D4-8	7049	■																■
IL-5	TRFK5	7052	■	■															■
IL-6	MQ2-13A5	7069	■			■	■												■
IL-9	MH9D1	7098																	■
IL-10	JES3-9D7	7108	■			■	■												■
IL-13	PVM13-1	7139	■			■	■												■
GM-CSF	GM2F3	7356																	■
ILC Group 3																			
IL-17A	eBio64DEC17	7179	■	■			■	■	■	■									■
IL-17AF	20LJS09	9179					■	■	■	■									N/A
IL-17F	SHLR17	7169																	■
IL-22	22URTI	7229																	■
TNFβ (LTα)	LTX-21	BMS105	■																■
Mouse Intracellular Antibodies																			
ILC Group 1																			
CCL3 (MIP-1α)	DNT3CC	7532																	■
Granzyme A	GzA-3G8.5	5831																	■
Granzyme B	NGZB	8898					■	■	■	■									■
IFN _γ	XMG1.2	7311				■	■	■	■	■									■
LAP	TW7-16B4	9821																	■
Perforin	eBioOMAK-D	9392	■																■
TNFα	MP6-XT22	7321																	■
ILC Group 2																			
IL-4	11B11	7041	■																■
IL-5	TRFK5	7052	■	■															■
IL-6	MP5-20F3	7061	■																■
IL-9	RM9A4	8091																	■
IL-10	JES5-16E3	7101	■																■
IL-13	eBio13A	7135	■																■
GM-CSF	MP1-22E9	7331																	■
ILC Group 3																			
IL-17A	eBio17B7	7177		■			■	■	■	■									■
IL-17AF	B8KN8R	9171																	N/A
IL-17F	eBio18F10	7471	■																■
IL-22	IL22JOP	7222																	■



IL-22 and IL-17F staining in Th17-polarized PBMCs

Intracellular staining of Th17-polarized normal human peripheral blood cells treated with Brefeldin A (cat. no. 00-4506) (left) or restimulated with PMA/Ionomycin in the presence of Brefeldin A (right) with Anti-Human IL-17F PE (cat. no. 12-7169) and Anti-Human IL-22 PerCP-eFluor® 710 (cat. no. 46-7229). Cells in the lymphocyte gate were used for analysis.

Immunoassay biomarker detection

To obtain a high-level understanding of ILC effector cytokine response and overall cytokine environment, immunoassays are a simple, effective assay platform used to quantitatively measure either secreted or intracellular protein biomarkers in biological samples, such as serum or cell culture. Research to gain greater insight into these biomarker profiles will ultimately produce a

better understanding of disease and cell biology. eBioscience produces multiple platforms for analyte assessment and biomarker profiling—from coat-it-yourself Ready-SET-Go![®] ELISAs and traditional Platinum ELISAs to ProcartaPlex[™] Multiplex Immunoassays, using Luminex[®] xMAP[®] technology.

Human Immunoassays						
Antigen	Platinum ELISA	Ready-SET-Go! [®] ELISA	High Sensitivity ELISA	Instant ELISA [®]	ELISPOT Ready-SET-Go! [®]	ProcartaPlex [™] Simplex
ILC Group 1						
IFN γ	BMS228	88-7316	BMS228HS		88-7386	EPX010-10228
IL-15		88-7158				EPX010-12089
MIP-1 α (CCL3)		88-7035		BMS2029INST		EPX010-12029
MIP-1 β (CCL4)		88-7034		BMS2030INST		EPX010-12030
RANTES (CCL5)				BMS287/2INST		EPX010-10287
TGF β 1 (LAP)	BMS2065; BMS249/4	88-50390				EPX010-10249
Granzyme B	BMS2027					EPX010-12027
Granzyme K		88-8379				
TNF α	BMS2034; BMS223/4	88-7346	BMS223HS	BMS223INST		EPX010-10223
ILC Group 2						
IL-4	BMS225/2	88-7046	BMS225HS	BMS225INST		EPX010-10225
IL-5	BMS278	88-7056		BMS278INST		EPX010-10278
IL-6	BMS213/2	88-7066	BMS213HS	BMS213INST		EPX010-10213
IL-9	BMS2081	88-7958				EPX010-12081
IL-10	BMS215/2	88-7106	BMS215HS	BMS215INST	88-7805	EPX010-10215
IL-13	BMS231/3	88-7439		BMS231INST		EPX010-10231
GM-CSF	BMS283	88-8337		BMS283INST		EPX010-10283
ILC Group 3						
IL-17A	BMS2017	88-7176	BMS2017HS		88-7876	EPX010-12017
IL-17AF	BMS2082	88-7117				
IL-17F	BMS2037/2	88-7478				EPX010-12160
IL-22	BMS2047	88-7522				EPX010-12047
TNF β (LT α)	BMS202			BMS202INST		EPX010-10202

Mouse Immunoassays						
ILC Group 1						
IFN γ	BMS606/609	88-7314		BMS606INST	88-7384	EPX010-20606
IL-15/IL-15R	BMS6023	88-7215				EPX010-26023
MIP-1 α (CCL3)		88-56013				EPX010-26013
MIP-1 β (CCL4)						EPX010-26014
RANTES (CCL5)				BMS6009INST		EPX010-26009
TGF β 1	BMS608/4	88-8350				EPX010-20608
Granzyme B	BMS6029	88-8022				
TNF α	BMS607/3	88-7324	BMS607HS	BMS607/2INST		EPX010-20607
ILC Group 2						
IL-4	BMS613	88-7044	BMS613HS	BMS613INST		EPX010-20613
IL-5	BMS610	88-7054			88-7825	EPX010-20610
IL-6	BMS603/2	88-7064	BMS603HS			EPX010-20603
IL-9		88-8092				EPX010-26041
IL-10	BMS614/2	88-7105		BMS614INST	88-7804	EPX010-20614
IL-13	BMS6015	88-7137				EPX010-26015
GM-CSF	BMS612	88-7334				EPX010-20612
ILC Group 3						
IL-17A	BMS6001	88-7371			88-7370	EPX010-26001
IL-17AF	BMS6026	88-8711				
IL-17F	BMS6020	88-7472				EPX010-26020
IL-22	BMS6022	88-7422				EPX010-26022

ProcartaPlex™ Multiplex Immunoassay Panels & Bead Sets

Human ProcartaPlex™ Panels

A	Th1/Th2 Cytokine Panel (11 plex) cat. no. EPX110-10810-901			
	GM-CSF	IL-2	IL-6	IL-18
	IFN γ	IL-4	IL-12 p70	TNF α
	IL-1 β	IL-5	IL-13	
B	Th9/Th17/Th22/Treg Cytokine Panel (7 plex) cat. no. EPX070-10817-901			
	IL-9	IL-17A	IL-22	IL-27
	IL-10	IL-21	IL-23	
C	Cytokine Panel 1C (7 plex) cat. no. EPX070-10010-901			
	IFN α	IL-1RA	IL-15	TNF β (LT α)
	IL-1 α	IL-7	IL-31	
D	Chemokine Panel 1 (9 plex) cat. no. EPX090-12187-901			
	Eotaxin (CCL11)	MIP-1 α (CCL3)		
	GRO α (CXCL1)	MIP-1 β (CCL4)		
	IL-8 (CXCL8)	SDF-1 α (CXCL12)		
	IP-10 (CXCL10)	RANTES (CCL5) is included in all kits, not only 34 plex & 45 plex		
	MCP-1 (CCL2)			
Essential Th1/Th2 Cytokine Panel (6 plex) cat. no. EPX060-10009-901				
IFN γ	IL-5	IL-12 p70		
IL-4	IL-6	TNF α		
Essential Panel 2 (4 plex) cat. no. EPX040-10008-901				
IL-8 (CXCL8)	IL-1 β	IL-10		
IL-17				
Cytokine & Chemokine Panel 1D (9 plex) cat. no. EPX090-12177-901				
IL-1 α	IL-10	MCP-1 (CCL2)		
IL-1 RA	IL-17A	MIP-1 α (CCL3)		
IL-8 (CXCL8)	IP-10 (CXCL10)	MIP-1 β (CCL4)		

Mouse ProcartaPlex™ Panels

A	Th1/Th2 Cytokine Panel (11 plex) cat. no. EPX110-20820-901		
	GM-CSF	IL-4	IL-13
	IFN γ	IL-5	IL-18
	IL-1 β	IL-6	TNF α
	IL-2	IL-12 p70	
B	Th9/Th17/Th22/Treg Cytokine Panel (6 plex) cat. no. EPX060-20822-901		
	IL-9	IL-17A	IL-23
	IL-10	IL-22	IL-27
C	Cytokine Panel 1B (10 plex) cat. no. EPX100-26091-901		
	G-CSF (CSF-3)	IL-31	ENA-78 (CXCL5)
	IFN α	IL-1 α	M-CSF (CSF-1)
	IL-15 (IL-15R)	IL-3	
	IL-28	LIF	
D	Chemokine Panel 1 (9 plex) cat. no. EPX090-20821-901		
	Eotaxin (CCL11)	MCP-1 (CCL2)	MIP-1 β (CCL4)
	GRO α (CXCL1)	MCP-3 (CCL7)	MIP-2 (CXCL2)
	IP-10 (CXCL10)	MIP-1 α (CCL3)	RANTES (CCL5)
Essential Th1/Th2 Cytokine Panel (6 plex) cat. no. EPX060-20831-901			
IFN γ	IL-5	IL-12 p70	
IL-4	IL-6	TNF α	
Essential Panel 2 (8 plex) cat. no. EPX080-20832-901			
IL-1 β	IL-10	IL-17	
GRO α (CXCL1)	MCP-1 (CCL2)	MIP-2 (CXCL2)	
MIP-1 α (CCL3)	MIP-1 β (CCL4)		

Human ProcartaPlex™ Panel Combinations

B	C	Th9/Th17/Th22/Treg & Cytokine Panel 1C (14 plex) cat. no. EPX140-12174-901		
C	D	Cytokine 1C & Chemokine Panel 1 (16 plex) cat. no. EPX160-12176-901		
B	D	Th9/Th17/Th22/Treg & Chemokine Panel 1 (16 plex) cat. no. EPX160-12175-901		
A	C	Th1/Th2 & Cytokine Panel 1C (18 plex) cat. no. EPX180-12172-901		
A	B	Th1/Th2/Th9/Th17/Th22/Treg Cytokine Panel (18 plex) cat. no. EPX180-12165-901		
A	D	Th1/Th2 & Chemokine Panel 1 (20 plex) cat. no. EPX200-12173-901		
A	B	C	Cytokine Panel 1B (25 plex) cat. no. EPX250-12166-901	
A	B	C	D	Cytokine & Chemokine Panel 1A (34 plex) cat. no. EPX340-12167-901

Mouse ProcartaPlex™ Panel Combinations

B	D	Th9/Th17/Th22/Treg & Chemokine Panel (15 plex) cat. no. EPX150-26089-901		
A	B	Th1/Th2/Th9/Th17/Th22/Treg Cytokine Panel (17 plex) cat. no. EPX170-26087-901		
A	D	Th1/Th2 & Chemokine Panel I (20 plex) cat. no. EPX200-26090-901		
A	B	D	Cytokine & Chemokine Panel 1 (26 plex) cat. no. EPX260-26088-901	
A	B	C	D	Cytokine & Chemokine Panel 1A (36 plex) cat. no. EPX360-26092-901

*Bead sets for the detection of individual analytes can be added to ProcartaPlex™ panels for increased customization. Alternatively, multiple Simplex Bead Sets can be combined and run using a ProcartaPlex™ Basic Kit (Human EPX010-10420-901; Mouse EPX010-20440-901). All analytes included in panels are available as Simplex Bead Sets.

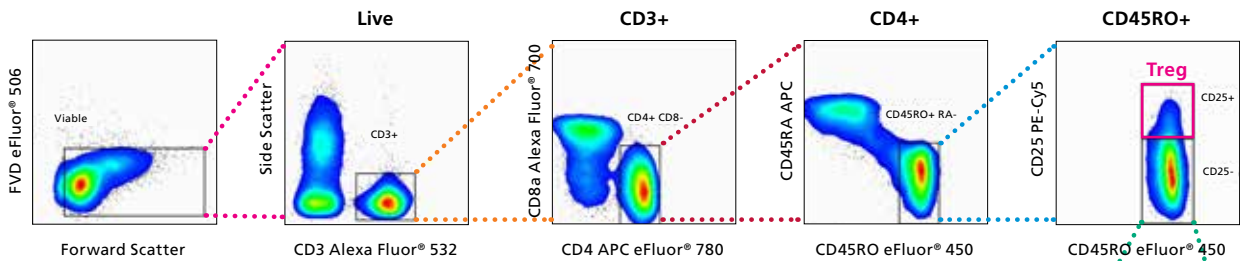
ProcartaPlex™ Multiplex Immunoassays are available in multiple formats across six species: human, mouse, rat, porcine, canine, and non-human primate. For a current product listing, visit www.ebioscience.com/procartaplex

Chemokine receptors

ILC groups express characteristic chemokine receptors that help control the homeostatic distribution to specific locations.

Human Antibodies																	
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers			Red Laser		FlowRNA Probe	
						eFluor® 450	FITC	Alexa Fluor® 488	PerCP-Cyanine 5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine7	APC		eFluor® 660
CXCR1 (CD181)	8F1-1-4	1819	■				■										■
CXCR2 (CD182)	5E8-C7-F10	1829	■														■
CXCR3 (CD183)	CEW33D	1839		■												■	■
CXCR4 (CD184)	12G5	9999	■	■	■												■
CXCR5 (CD185)	MU5UBEE	9185	■	■													■
CCR4 (CD194)	D8SEE	1949															■
CCR5 (CD195)	NP-6G4	1956															■
CCR6 (CD196)	R6H1	1969	■				■										■
CCR7 (CD197)	3D12	1979	■	■			■										■
CCR9 (CD199)	BBC3M4	1999														■	■
CX3CR1	2A9-1	6099		■													■

Mouse Antibodies																	
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers			Red Laser		FlowRNA Probe	
CXCR3 (CD183)	CXCR3-173	1831															■
CXCR4 (CD184)	2B11	9991	■	■	■												■
CXCR5 (CD185)	SPRCL5	7185															■
CCR5 (CD195)	HM-CCR5 (7A4)	1951	■														■
CCR6 (CD196)	sirx6	7196															■
CCR7 (CD197)	4B12	1971	■	■	■												■
CCR9 (CD199)	CW-1.2	1991	■														■

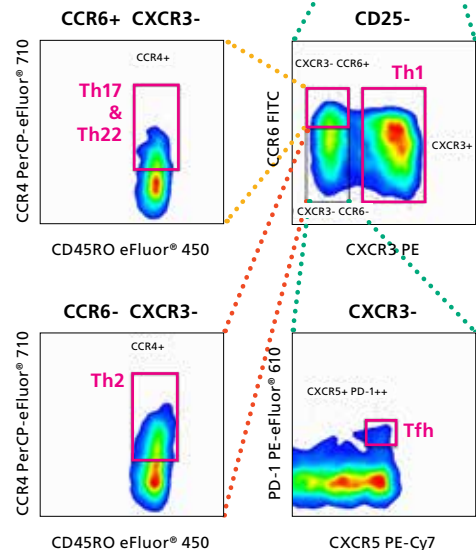


Live cell sorting of T helper subsets based upon chemokine receptor staining

Normal human peripheral blood cells were surface stained with the reagents indicated. Viable cells, as determined by Fixable Viability Dye eFluor® 506, were used for analysis. Samples were analyzed on an LSR II SORP equipped with Blue (488 nm), Red (640 nm), Green (532 nm), and Violet (405 nm) laser lines.

Panel:

- Anti-Human CCR6 FITC (clone R6H1)
- Anti-Human CCR4 PerCP-eFluor® 710 (clone D8SEE)
- Anti-Human CD45RA APC (clone HI100)
- Anti-Human CD8a Alexa Fluor® 700 (clone OKT8)
- Anti-Human CD4 APC-eFluor® 780 (clone RPA-T4)
- Anti-Human CD45RO eFluor® 450 (clone UCHL1)
- Anti-Human CD3 Alexa Fluor® 532 (clone UCHT1)
- Anti-Human CD183 (CXCR3) PE (clone CEW33D)
- Anti-Human CD279 (PD-1) PE-eFluor® 610 (clone J105)
- Anti-Human CD25 PE-Cyanine5 (clone BC96)
- Anti-Human CD185 (CXCR5) PE-Cyanine7 (clone MU5UBEE)
- Fixable Viability Dye eFluor® 506



Functional activity

eBioscience provides high-purity, low-endotoxin bioactive Recombinant Proteins and Functional Grade Antibodies for optimal results when used *in vitro* or *in vivo* for

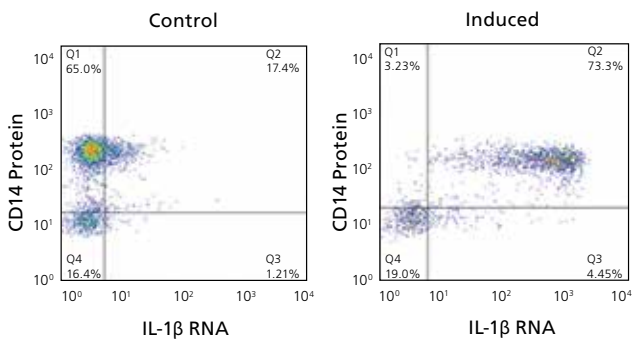
polarization, activation, blocking, or neutralization studies, enabling you to explore myriad biologic systems with consistent, reproducible results.

Functional Proteins and Antibodies				
Antigen	Recombinant Protein		Neutralizing Functional Grade Antibody	
	Mouse Cat. No.	Human Cat. No.	Mouse Cat. No.	Human Cat. No.
ILC Group 1				
IL-2	8021	8029	16-7021	16-7027
IL-7	8071	8079	16-1271	
IL-12	8121	8129	16-7123	16-7129
IL-15	8153	8159	16-7154	16-0157
IL-15/IL-15R	8152		16-8156	
Flt3-ligand	8001	8513		
ILC Group 2				
IL-2	8021	8029	16-7021	16-7027
IL-7	8071	8079	16-1271	
IL-25 (IL-17E)	9175	8736		
IL-33	8332	8338		
TSLP	8498	8497	16-5491	
ILC Group 3				
IL-2	8021	8029	16-7021	16-7027
IL-23	8231	8239	16-7123	16-7129

Gene expression by flow cytometry

FlowRNA enhances the understanding of gene transcription by allowing messenger RNA (mRNA) detection within a single cell using flow cytometry. This novel assay complements gene expression arrays by deepening the information gathered in heterogeneous cellular environments. Detection of RNA can be

supplemented to many existing flow cytometry workflows in order to recognize transcriptional events in specific cell subsets; to label cells expressing proteins for which there are limited or no commercially available antibodies; and to potentially identify viral or non-coding RNA molecules.



Cytokine RNA profiling of human monocytes

Human PBMCs treated with 1 µg/mL LPS and 2.5 µg/mL R-848 for four hours, and processed using the FlowRNA Assay. Expression of IL-1β in monocytes.

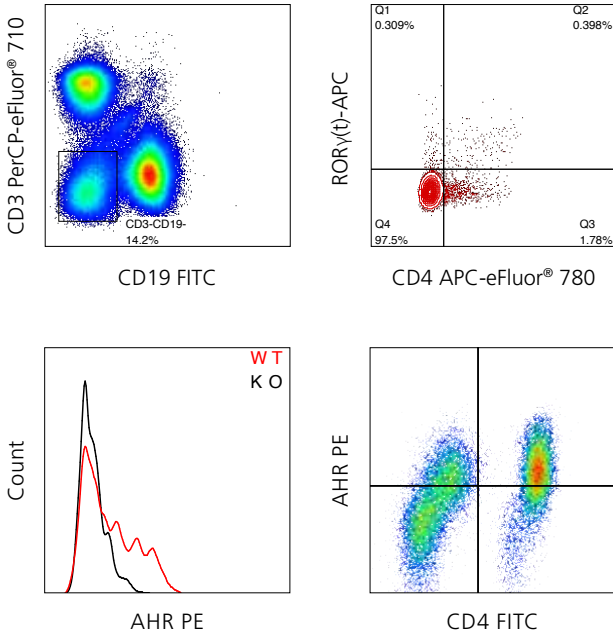
Assay features:

- Simultaneous detection of three RNA transcripts on a single-cell level
- Data analysis using a standard flow cytometer
- More than 4500 validated RNA *in situ* hybridization probes
- Compatible with surface and intracellular antibody labeling
- Custom probe set design and shipment in five days or less – from sequence submission to probe delivery

Fate-determining transcriptional control

All ILCs are developmentally dependent on the transcription factor inhibitor of DNA-binding 2 (Id2). Group 1 ILCs include ILC1, which are similar to Th1 and are regulated by T-bet. Thus far, ILC1s are thought to be ILC3s that have lost ROR γ (t) expression in response to a cytokine environment of IL-2, IL-12, and IL-18. NK cells are also considered a part of group 1 ILC and express transcription factor E4BP4 (NFIL3)

upstream of Id2. Additionally, T-bet and Eomes direct their fate and function. Similar to Th2 cells, group 2 ILC2s rely on ROR α and GATA3 for development. Finally, group 3 ILCs, which include natural cytotoxicity receptor NCR-positive IL-22-producing, NCR-negative IL-17-producing, and LTI cells, require ROR γ (t) and aryl hydrocarbon receptor (AHR) for development similar to that of Th17 and Th22 cells.



ROR γ (t) staining of CD3⁻/CD19⁻ Splenocytes

Intracellular staining of C57BL/6 splenocytes with Anti-Mouse CD4 APC-eFluor[®] 780 (cat. no. 47-0041) and of Anti-Mouse ROR γ (t) APC (cat. no. 17-6981) (*right*) using the Foxp3 Staining Buffer Set (cat. no. 00-5523). Cells were also stained with Fixable Viability Dye eFluor[®] 450 (cat. no. 65-0863), Anti-Mouse CD19 FITC (cat. no. 11-0193), and Anti-Mouse CD3 PerCP-eFluor[®] 710 (cat. no. 46-0032) (*left*). Viable, CD3⁻ CD19⁻ cells were used for analysis.

Mouse AHR under Th17 polarization

Left: Splenocytes from C57BL/6 mice (WT, red histogram) or AHR-knockout mice (KO, black histogram) were polarized under Th17 conditions for three days. Cells were stained with Fixable Viability Dye eFluor[®] 780 (cat. no. 65-0865), fixed and permeabilized with IC Fixation & Permeabilization Buffers (cat. no. 00-8222 and cat. no. 00-8333), and then intracellularly stained with Anti-Mouse CD4 PerCP-eFluor[®] 710 (cat. no. 46-0042) and 1.0 μ g of Anti-Mouse AHR PE. Viable CD4⁺ cells were used for analysis.

Right: C57BL/6 splenocytes were polarized under Th17 conditions for 3 days. Cells were stained with Fixable Viability Dye eFluor[®] 780 (cat. no. 65-0865), fixed and permeabilized with IC Fixation & Permeabilization Buffers (cat. no. 00-8222 and cat. no. 00-8333), and then intracellularly stained with Anti-Mouse CD4 FITC (cat. no. 11-0042) and Rat IgG2a K Isotype Control PE (cat. no. 12-4321) or Anti-Mouse AHR PE. Total viable lymphocytes were used for analysis, and quadrant lines were drawn based on isotype control.

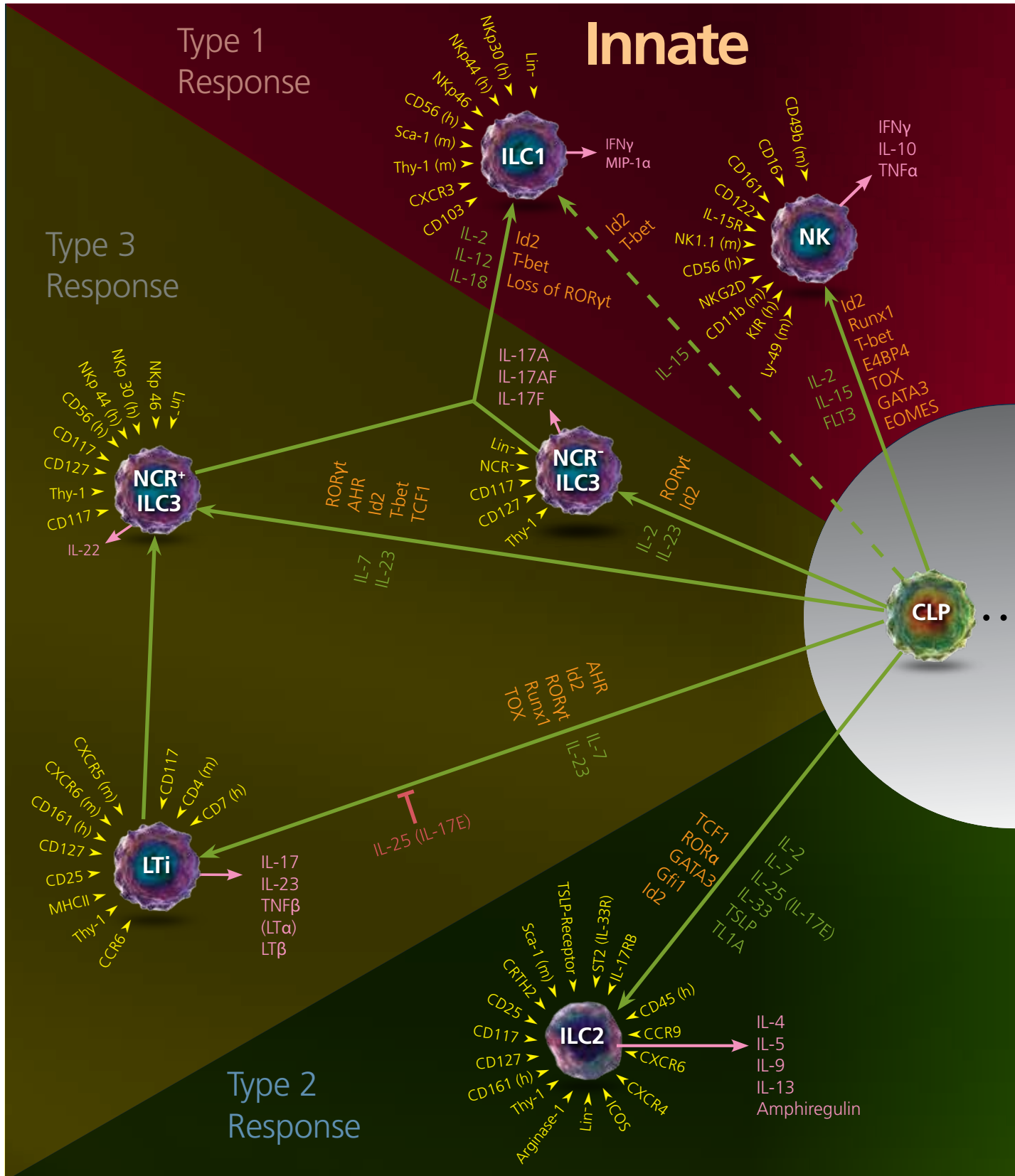
Notch signaling

Notch signaling plays important developmental roles in various ILC populations. The Notch family of transmembrane receptors controls cell-fate decisions during the development of many organs in a wide variety of species. While the extracellular domain contains numerous epidermal growth factor-like repeats for ligand binding, the intracellular domain is involved in cell signaling. Upon binding its membrane-

bound ligand (either Delta or Jagged), the Notch receptor undergoes proteolytic cleavage, first by ADAM-family metalloproteases and then by γ -secretase. The second cleavage event releases the Notch intracellular domain (NICD), which subsequently translocates into the nucleus, where it is able to suppress the expression of lineage-specific genes by interacting with transcriptional repressors.

Human Transcription Factor Antibodies															
Antigen	Clone	Cat. No.	Purified	Biotin	Violet Laser	Blue Laser			Green, Yellow-Green Lasers				Red Laser		FlowRNA Probe
					eFluor® 450	FITC	Alexa Fluor® 488	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5.5	PE-Cyanine7	APC	eFluor® 660	
ILC Group 1															
EOMES	WD1928	4877				■			■	■	■			■	■
GATA3	TWAJ	9966	■						■	■				■	■
Id2	N/A														■
PLZF	Mags.21F7	9320					■			■					■
Runx1	RXDMC	9816								■					■
Runx3	R3-5G4	9817	■	■										■	■
T-bet	4B10	5825	■							■		■	■	■	■
TOX	TXRX10	6502	■							■		■	■	■	■
ILC Group 2															
c-Maf	sym0F1	9855							■					■	■
GATA3	TWAJ	9966	■						■	■				■	■
Id2	N/A														■
p-STAT6	CHI2S4N	9013							■	■				■	N/A
RORα	N/A														■
TCF-1	N/A														■
ILC Group 3															
AHR	FF3399	9854	■						■	■				■	■
Id2	N/A														■
RORγ(t)	AFKJS9	6988	■							■			■	■	■
T-bet	4B10	5825	■							■	■	■	■	■	■
TCF-1	N/A														■
Notch Signaling															
Notch 1 (extracellular)	MHN1-519	9889												■	■
Notch 1 (intracellular)	mN1A	5785	■	■						■				■	■
Notch 2	16F11	5786								■					■
Notch 3	MHN3-21	5787												■	■
Mouse Transcription Factor Antibodies															
ILC Group 1															
EOMES	Dan11mag	4875	■		■			■	■		■			■	■
GATA3	TWAJ	9966	■						■	■				■	■
Id2	N/A														■
PLZF	Mags.21F7	9320								■					■
Runx1	RXDMC	9816								■					■
T-bet	4B10	5825	■							■		■	■	■	■
TOX	TXRX10	6502	■							■				■	■
ILC Group 2															
c-Maf	sym0F1	9855							■					■	■
GATA3	TWAJ	9966	■						■	■				■	■
Id2	N/A														■
p-STAT6	CHI2S4N	9013							■	■				■	N/A
RORα	N/A														■
TCF-1	N/A														■
ILC Group 3															
AHR	4MEJJ	5925						■		■				■	■
Id2	N/A														■
RORγ(t)	B2D	6981	■						■	■	■		■	■	■
T-bet	4B10	5825	■							■		■	■	■	■
TCF-1	N/A														■
Notch Signaling															
Notch 1 (extracellular)	22E5	5765								■				■	■
Notch 1 (intracellular)	mN1A	5785	■	■						■				■	■
Notch 2	16F11	5786								■					■
Notch 3	HMN3-133	5763								■				■	■
Notch 4	HMN4-14	5764								■					■

Comparative characteristics of ILC and T helper cell subsets

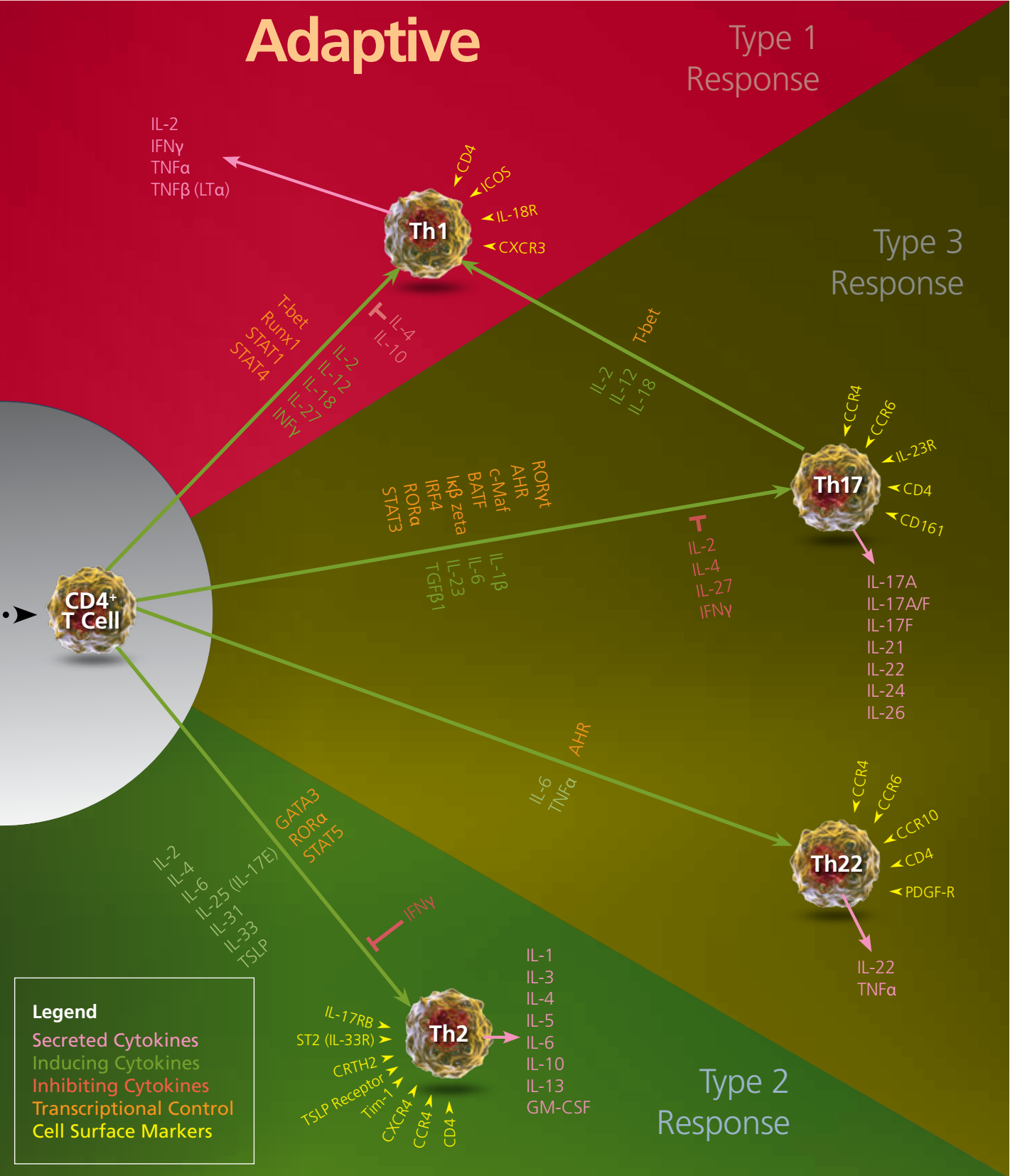


Adaptive

Type 1 Response

Type 3 Response

Type 2 Response



Cell surface markers

Hematopoietic lineage cocktail

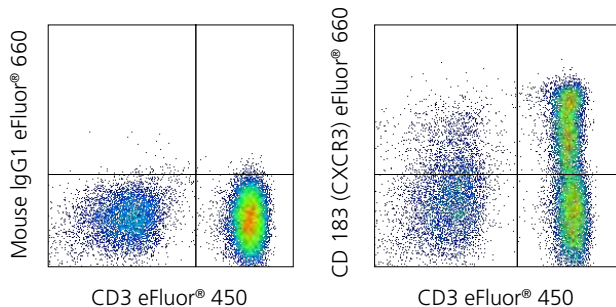
Mouse and Human Hematopoietic Lineage Cocktails contain antibodies that can identify, enrich, or deplete cells committed to the T, B, NK, myeloid and erythroid lineages based on the expression of cell-surface antigens.

Mouse cocktails contain antibodies against CD3, CD45R (B220), CD11b, TER-119, and Ly-G (Gr-1). Human cocktails include antibodies against CD2, CD3, CD14, CD16, CD19, CD56 and CD235a.

Hematopoietic Lineage Cocktails				
Species	Biotin	Violet Laser	Blue Laser	Red Laser
		eFluor® 450	FITC	APC
Human		▪	▪	▪
Mouse	▪	▪	▪	

ILC Group 1

Human ILC Group 1 Antibodies																			
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers			Red Laser			FlowRNA Probe		
						eFluor® 450	FITC	Alexa Fluor® 488	PerCP-Cyanine 5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	APC		eFluor® 660	APC-eFluor® 780
Asialo GM1	poly	6507			▪			▪											
CD11b	ICRF44	0118	▪	▪			▪			▪						▪		▪	▪
CD11b (activation epitope)	CBRM1/5	0113	▪	▪	▪		▪			▪								▪	▪
CD16	CB16	0168	▪	▪	▪		▪			▪						▪		▪	▪
CD25	BC96	0259	▪	▪			▪		▪							▪		▪	▪
	LG.7F9	0271	▪	▪	▪		▪			▪						▪		▪	▪
CD27	O323	0279	▪	▪			▪			▪						▪		▪	▪
CD56 (NCAM)	CMSSB	0567	▪	▪			▪			▪						▪		▪	▪
CD57	TB01	0577			▪		▪			▪							▪		▪
CD62L (L-Selectin)	DREG56	0629	▪		▪		▪			▪						▪		▪	▪
CD94 (NKG2)	DX22	0949	▪				▪			▪									▪
	HP-3D9	5094														▪			▪
CD96 (TACTILE)	NK92.39	0969								▪									▪
CD103	Ber-ACT8	1037					▪			▪						▪			▪
CD127	eBioRDR5	1278	▪	▪	▪		▪			▪									▪
CD158a/h/b2/f/g (KIR2D)	HP-MA4	1589					▪			▪						▪		▪	▪
CD158f (KIR2DL5A)	UP-R1	1588								▪									▪
CD183 (CXCR3)	CEW33D	1839		▪			▪			▪						▪	▪		▪
CD218a (IL-18R alpha)	H44	7183		▪			▪			▪						▪	▪		▪
CD244	eBioDM244	5837								▪									▪
	C1.7	5838	▪	▪	▪					▪									▪
CD253 (TRAIL)	RIK-2	9927	▪	▪	▪														▪
CD314 (NKG2D)	1D11	5878	▪	▪	▪					▪									▪
	5C6	5879	▪																▪
CD319 (CRACC)	162	2229								▪									▪
CD328 (Siglec7)	QA79	5759	▪	▪						▪									▪
CD335 (NKp46)	9E2	3359			▪	▪				▪									▪
CD336 (NKp44)	44.189	3369								▪									▪
CD337 (NKp30)	AF29-4D12	3379								▪									▪
IL-15R	eBioJM7A4	7159	▪				▪			▪									▪



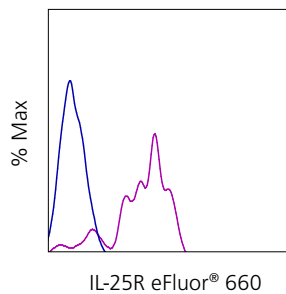
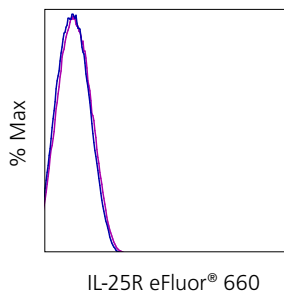
Staining of Human CXCR3 in PBMCs

Staining of normal human peripheral blood cells with Anti-Human CD3 eFluor® 450 (cat. no. 48-0037) and Mouse IgG1 K Isotype Control eFluor® 660 (cat. no. 50-4714) (left) or Anti-Human CD183 (CXCR3) eFluor® 660 (cat. no. 50-1839) (right). Cells in the lymphocyte gate were used for analysis.

Mouse ILC Group 1 Antibodies																		
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers				Red Laser			FlowRNA Probe
						eFluor® 450	FITC	Alexa Fluor® 488	PerCP-Cyanine 5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	APC	eFluor® 660	
Asialo GM1	poly	6507																
CD11b	M1/70	0112																
CD16/32	93	0161																
CD25	PC61.5	0251																
CD27	7D4	0252																
	3C7	0253																
	LG.7F9	0271																
CD49b (DX5)	DX5	5971																
	HMa2	0491																
CD62L (L-Selectin)	MEL-14	0621																
CD94 (NKG2)	18d3	0941																
CD96 (TACTILE)	6A6	0960																
CD103	2E7	1031																
CD127	A7R34	1271																
CD218a (IL-18Ra)	P3TUNYA	5183																
CD244.1	C9.1	2440																
CD244.2	eBio244F4	2441																
CD253 (TRAIL)	N2B2	5951																
CD314 (NKG2D)	A10	5872																
	C7	5873																
	MI-6	5880																
	CX5	5882																
CD335 (NKp46)	29A1.4	3351																
IL-15R alpha	DNT15Ra	7149																
Ly-49A	A1 (Ly49A)	5856																
Ly-49A/D	12A8	5783																
Ly-49D	4E5	5782																
Ly-49C//F/H	14B11	5991																
Ly-49E/F	CM4	5848																
Ly-49G	AT-8	5885																
Ly-49G2	4D11	5781																
Ly-49H	3D10	5886																
Ly-49I	YLI-90	5895																
Ly-108	13G3-19D	1508																
KLRG1	2F1	5893																
NK1.1	PK136	5941																
NKG2A/C/E	20d5	5896																
NKG2AB6	16a11	5897																

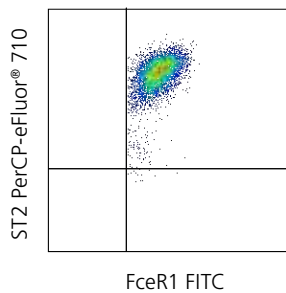
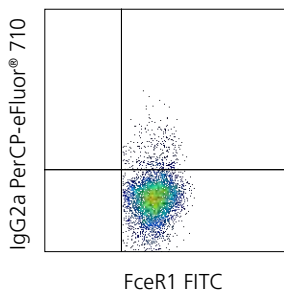
ILC Group 2

Human ILC Group 2 Antibodies																	
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers			Red Laser			FlowRNA Probe
						eFluor® 450	FITC	Alexa Fluor® 488	PerCP-Cyanine 5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	APC	
CD25	BC96	0259	■	■		■		■	■	■							
	LG.7F9	0271	■	■	■												
CD45	HI30	0459	■	■			■	■	■	■							
	2D1	9459				■											
CD90 (Thy1)	5E10	0909	■	■			■	■	■	■							
CD117 (c-kit)	104D2	1178				■	■										
	YB5.B8	1179	■	■													
CD127	eBioRDR5	1278	■	■	■	■											
CD161	HP-3G10	1619				■		■									
CD278 (ICOS)	ISA-3	9948	■	■	■				■								
CD294 (CRTH2)	BM16	2949		■													
Arginase-1	sl6arg	9779	■					■									
TSLP-R	eBio1A6 (1A6)	5499	■						■								
Mouse ILC Group 2 Antibodies																	
CD25	PC61.5	0251	■	■	■	■		■	■	■	■	■	■	■	■	■	■
CD90.1 (Thy1.1)	HIS51	0900	■	■	■	■											
CD90.2 (Thy1.2)	30-H12	0903	■	■	■				■								
	53-2.1	0902				■	■										
CD117 (c-kit)	2B8	1171	■	■	■	■											
	ACK2	1172	■	■	■												
CD127	A7R34	1271	■	■	■	■											
	HK5.3	5985	■	■	■												
CD278 (ICOS)	15F9	9940							■								
	7E.17G9	9942	■	■	■												
	C398.4A	9949	■	■	■												
IL-17RB (IL-25R)	MUNC33	7361															
Ly-6A/E (Sca-1)	D7	5981	■	■	■												
ST2 (IL-33R)	RMST2-2	9335		■					■								
	RMST2-33	9333		■	■												



Staining of Mouse IL-17RB in ILC2 and CD4+ T Cells

ILC2, but not CD4+ T cells, in the mesenteric lymph nodes express IL-17RB (IL-25R). CD4+ T cells (CD45+, CD3+, CD4+) (*left*) and ILC2 (CD45+, CD19-, CD5-, CD11b-, CD11c-, NK1.1-, CD4-, CD90.2+, CD25+, CD127+, IL-33R+) (*right*) were stained with Rat IgG2a K Isotype Control eFluor® 660 (cat. no. 50-4321) (blue histogram) or Anti-Mouse IL-25R (IL-17RB) eFluor® 660 (cat. no. 50-7361) (purple histogram). Single, viable cells were used for analysis. Data are courtesy of the Artis laboratory (Department of Microbiology and Institute for Immunology, Perelman School of Medicine, University of Pennsylvania).

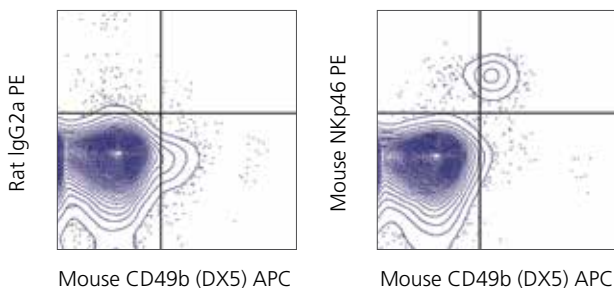


Staining of Mouse ST2 (IL-33R) in Mast Cells

Staining of bone marrow-derived mast cells with Anti-Mouse Fc epsilon Receptor 1 alpha (FcεR1) FITC (cat. no. 11-5898) and Rat IgG2a K Isotype Control PerCP-eFluor® 710 (cat. no. 46-4321) (*left*) or Anti-Mouse ST2 (IL-33R) PerCP-eFluor® 710 (*right*). Total viable cells were used for analysis.

ILC Group 3

Human ILC Group 3 Antibodies																			
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers				Red Laser			FlowRNA Probe	
						eFluor® 450	FITC	Alexa Fluor® 488	PerCP-Cyanine 5.5	PerCP-eFluor® 710	PE	PE-eFluor® 610	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	APC	eFluor® 660		APC-eFluor® 780
CD4	RPA-T4	0049	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD7	124-1D1	0079	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD25	BC96	0259	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	LG.7F9	0271	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD56 (NCAM)	CMSSB	0567	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD90 (Thy1)	5E10	0909	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD94 (NKG2)	DX22	0949	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	HP-3D9	5094	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD117 (c-kit)	104D2	1178	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	YB5.B8	1179	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD127	eBioRDR5	1278	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD314 (NKG2D)	1D11	5878	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	5C6	5879	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD335 (NKp46)	9E2	3359	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD336 (NKp44)	44.189	3369	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD337 (NKp30)	AF29-4D12	3379	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Mouse ILC Group 3 Antibodies																			
CD25	PC61.5	0251	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	DX5	5971	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD49b (DX5)	HMa2	0491	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD90.1 (Thy1.1)	HIS51	0900	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD90.2 (Thy1.2)	30-H12	0903	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	53-2.1	0902	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD94 (NKG2)	18d3	0941	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD117 (c-kit)	2B8	1171	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	ACK2	1172	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD314 (NKG2D)	A10	5872	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	C7	5873	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	MI-6	5880	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	CX5	5882	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
CD335 (NKp46)	29A1.4	3351	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49A	A1 (Ly49A)	5856	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49A/D	12A8	5783	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49D	4E5	5782	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49C//I/F/H	14B11	5991	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49E/F	CM4	5848	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49G	AT-8	5885	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49G2	4D11	5781	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49H	3D10	5886	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ly-49I	YLI-90	5895	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
NK1.1	PK136	5941	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
NKG2A/C/E	20d5	5896	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
NKG2AB6	16a11	5897	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
MHC Class II I-Ab	AF6-120.1	5320	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
MHC Class II (I-A)	NIMR-4	5323	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
MHC Class II (I-A/I-E)	M5/114.15.2	5321	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
MHC Class II (I-Ek)	14-4-45	5980	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■



Staining of Mouse NKp46 in Splenocytes

Staining of C57Bl/6 splenocytes with Anti-Mouse CD49b (DX5) APC (cat. no. 17-5971) and Rat IgG2a Isotype Control PE (cat. no. 12-4321) (*left*) or Anti-Mouse NKp46 (29A1.4) PE (cat. no. 12-3351) (*right*). Total viable cells were analyzed.

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