

Natural Killer (NK) and Natural Killer T (NKT) Cells

Tools for Research and Results

Topics

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Ly-49, KIR and NCR Families

NK Cell Activation and Function

Activate, Stimulate and Proliferate
Intracellular Flow Cytometry Staining
Immunoassay Quantitation

NK Cell Transcriptional Control

NK T Cells

NK Cell Marker Guide



eBioscience (US) Tel: +1-888-999-1371 ■ Tel: +1-858-642-2058 ■ eBioscience (EU) Tel: +43 1 796 40 40 304 ■ info@ebioscience.com
Affymetrix, Inc. Tel: +1-888-362-2447 ■ Affymetrix UK Ltd. Tel: +44-(0)1628-552550 ■ Affymetrix Japan K.K. Tel: +81-(0)3-6430-4020
Panomics Solutions Tel: +1-877-726-6642 panomics.affymetrix.com ■ USB Products Tel: +1-800-321-9322 usb.affymetrix.com

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IMM02507-1 NK and NKT Reagents PLF 0913
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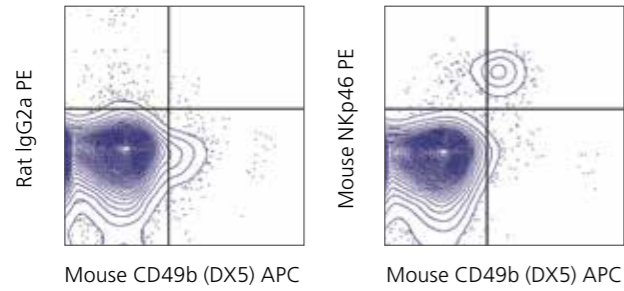
Natural killer (NK) cells are lymphoid cells poised and ready to assist in the destruction of virally infected cells and tumor cells from the body. NK cells are part of the innate immune system and mediate their effect in an antigen-independent manner that, in general, does not give rise to immunological memory or long-term protective immunity. NK cells are characterized by the expression of CD56 (both high and low levels) and the KIR family receptors in humans, and CD49b (DX5) and Ly-49 family members in mice.

NK Cell Receptors

Ly-49, KIR and NCR Families

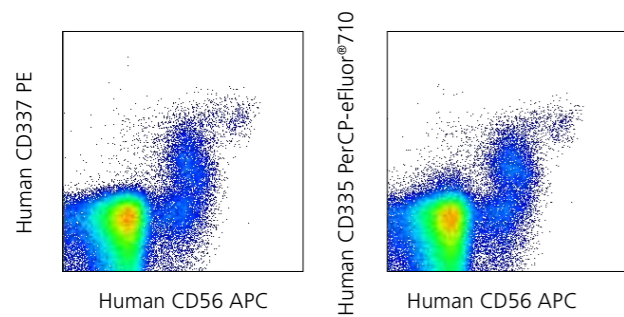
NK cells, unlike Natural Killer T (NKT) cells, do not express an antigen-specific receptor. Regulation of the cytotoxic activity of NK cells is mediated by activating and inhibiting receptors expressed on the cell surface including natural cytotoxicity receptors (NCR), lectin-like receptors and CD158 family of Killer Immunoglobulin-like Receptors (KIRs). These bind to specific components present on the surface of bacteria, virally-infected cells, stressed cells or cancer cells. Rodents lack KIRs, and instead express functionally equivalent lectin-like Ly-49 receptors. These receptors can be activating or inhibiting depending upon

semi-conserved motifs (ITAM and ITIM) found in the receptor's intracellular domain which allows a unique and controlled response by the NK cell. One important family of NK mediators is the activating Natural Cytotoxicity Receptors which include NKp30, NKp44 and NKp46. Upon stimulation, the receptors deliver potent signals to NK cells in order to kill target cells and produce inflammatory cytokines such as IFN γ . NKp46 is found on both mouse and human NK cells, while mice lack a homolog for NKp44 and contain only a pseudogene for NKp30.



Mouse NKp46

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.



Human NKp30 vs NKp46

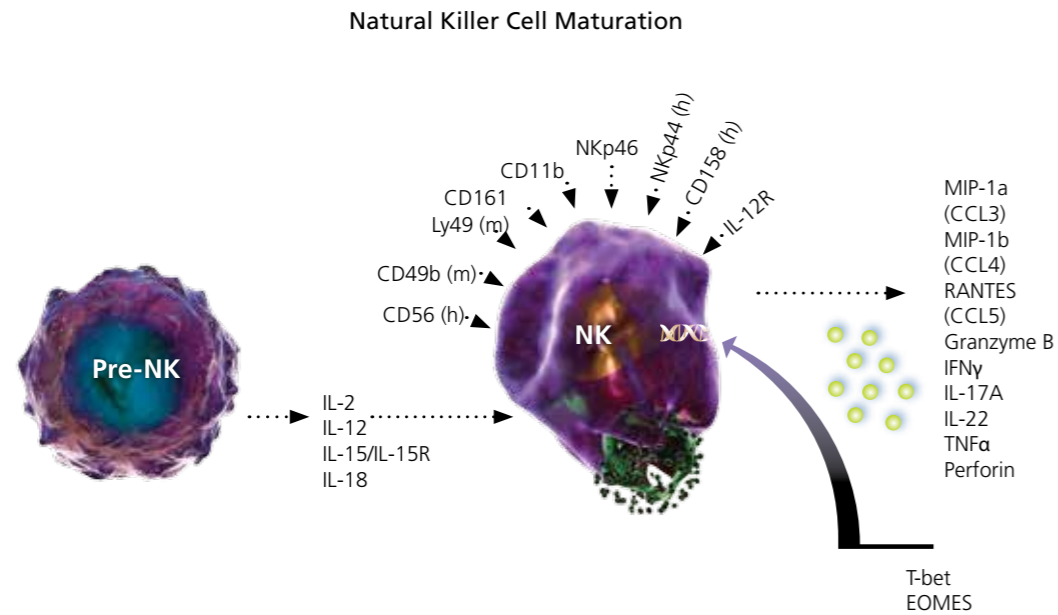
Staining of normal human peripheral blood cells with Anti-Human CD56 (NCAM) APC (cat. no. 17-0567) and Anti-Human CD337 (NKp30) PE (cat. no. 12-3379) (left) or Anti-Human CD335 (NKp46) PerCP-eFluor 710 (cat. no. 46-3359) (right).

Human NK Cell Receptor Antibodies													
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser			Green, Yellow-Green Lasers		Red Laser	
						eFluor [®] 450	FITC	PerCP-Cyanine5.5	PerCP-eFluor [®] 710	PE	PE-Cyanine7	APC	eFluor [®] 660
CD94 (NKG2)	DX22 HP-3D9	0949 5094	■				■			■		■	
CD158a (KIR2DL1/S1)	HP-MA4	1589				■	■	■		■	■	■	
CD158f (KIR2DL5A)	UP-R1	1588								■		■	
CD314 (NKG2D)	1D11 5C6	5878 5879	■	■	■		■		■	■		■	
CD335 (NKp46)	9E2	3359			■	■			■			■	
CD336 (NKp44)	44.189	3369			■				■			■	
CD337 (NKp30)	AF29-4D12	3379				■				■	■		
Mouse NK Cell Receptor Antibodies													
CD94 (NKG2)	18d3	0941	■	■		■	■			■			
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//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	■	■	■					■		■	
NKG2A/C/E	20d5	5896	■	■	■		■		■				
NKG2AB6	16a11	5897	■	■						■			

NK Cell Activation and Function

NK cells are activated in response to IL-2, IL-12, IL-15, IL-15/IL-15RA complex and IL-18, and produce and secrete a variety of cytokines, chemokines (including IFN γ , TNF α , IL-17, and IL-22) and death-eliciting proteins (perforin and granzymes). Similar to cytotoxic CD8+ T cells, activated NK cells contain cytoplasmic granules that contain proteins

such as perforin and granzymes to create pores in the cell membrane and initiate apoptosis via a caspase cascade in target cells. Of the granzyme family, granzyme B is the best-characterized, but granzymes A through M are also involved and have been shown to have unique ligand specificity.



Activate, Stimulate and Proliferate NK Cells

eBioscience provides high purity, low endotoxin bioactive recombinant proteins that can be used to activate, stimulate and proliferate NK cells. Our recombinant proteins boasts the industry's most demanding quality control and

performance criteria including endotoxin levels <0.01 ng/ μ g (tenfold lower than other suppliers) and functional testing to ensure consistent bioactivity with every lot.

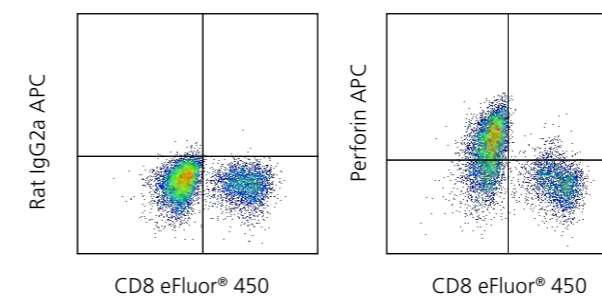
Natural Killer (NK) Differentiation Profile			
Cytokine	Human Cat. No.	Mouse Cat. No.	Role
IL-2	14-8029 34-8029	14-8021 34-8021	Augments NK cell activity and boosts cytolytic activity by activating various kinase pathways
IL-12 p70	14-8129 34-8129	14-8121 34-8121	Induces activation, stimulates cytotoxicity and production of IFN γ and TNF
IL-15/IL-15R	--	14-8152 34-8152	Involved in proliferation, accumulation and survival
IL-15	14-8159 34-8159	14-8153 34-8153	Involved in proliferation, accumulation and survival
IL-18	--	--	Upregulates NK cell cytotoxicity

Intracellular Flow Cytometry Staining

Cytokine detection requires the appropriate stimulation conditions and kinetics of cytokine production that will vary depending on the cell type and the particular cytokine being assayed. For *in vitro* stimulation of cells, it is necessary to block secretion of cytokines with protein transport inhibitors, such as Monensin or Brefeldin A Solution, during the final hours of the stimulation protocol. Simple buffer modifications allow

simultaneous analysis of surface molecules and intracellular antigens at the single-cell level by flow cytometry. Typically, cells are fixed with formaldehyde to stabilize the cell membrane, and then permeabilized with detergent or alcohol to create pores in the cell membrane that require the continuous presence of the permeabilization buffer during all subsequent steps to allow antibodies to have access to the cytoplasm of the cell.

Human NK Intracellular Flow Cytometry Antibodies																	
Antigen	Clone	Cat. No.	Purified	Functional Grade	Violet Laser	Blue Laser			Green, Yellow-Green Lasers		Red Laser						
					eFluor [®] 450	FITC	AlexaFluor [®] 488	PerCP-Cyanine5.5	PerCP-eFluor [®] 710	PE	PE-Cyanine7	APC	eFluor [®] 660	APC-eFluor [®] 780	AlexaFluor [®] 700	Cy5	
CCL3 (MIP-1 α)	PFFM3	7539			■	■				■							
CCL4 (MIP-1 β)	FL34Z3L	7540											■				
CCL5 (RANTES)	VL1	9905												■			
Granulysin	DH2	8828															
Granzyme B	GB11	8899															
Granzyme K	G3H69	8897															
IL-10	JES3-9D7	7108		■	■		■										
IL-22	22URT1	7229			■												
IFN γ	4S.B3	7319	■		■	■	■	■	■	■	■	■	■	■	■	■	
LAP	FNLAP	9829															
Perforin	dG9	9994	■		■	■	■	■	■	■	■	■	■	■	■	■	
TNF α	MAB11	7349	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
Mouse NK Intracellular Flow Cytometry Antibodies																	
Granzyme A	GzA-3G8.5	5831			■												
Granzyme B	NGZB	8898			■	■											
IL-10	JES5-16E3	7101		■	■	■	■	■	■	■	■	■	■	■	■		
IL-22	IL22JOP	7222		■	■												
IFN γ	XMG1.2	7311		■	■	■	■	■	■	■	■	■	■	■	■	■	■
LAP	TW7-16B4	9821															
Perforin	eBioOMAK-D	9392	■		■	■	■	■	■	■	■	■	■	■	■	■	
TNF α	MP6-XT22	7321	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■



Mouse Perforin

BALB/c splenocytes were stimulated for 4 days with Mouse IL-2 Recombinant Protein (cat. no. 14-8021). Cells were harvested and stained with Anti-Mouse CD8a eFluor[®] 450 (cat. no. 48-0081) followed by fixation and permeabilization using the Intracellular Fixation & Permeabilization Buffer Set and protocol (cat. no. 88-8824). Cells were subsequently stained intracellularly with Rat IgG2a K Isotype Control APC (cat. no. 17-4321) (left) or Anti-Mouse Perforin APC (cat. no. 17-9392) (right). Cells in the lymphocyte gate were analyzed.

Immunoassay Quantitation

Immunoassay is a simple, effective assay platform used to quantitatively measure either secreted or intracellular protein biomarkers in biological samples such as serum or cell lysate. Research to gain greater insight into these biomarker profiles will ultimately produce better understandings 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	ELISA Ready-Set-Go!®	High Sensitivity ELISA	Instant ELISA	ELISPOT Ready Set Go!®	ProcartaPlex™ Simplex
Granzyme A	BMS2026					
Granzyme B	BMS2027					
Granzyme K		88-8379				
IFN γ	BMS228	88-7316	BMS228HS		88-7386	EXP010-10288
IL-10	BMS215/2	88-7106	BMS215HS	BMS215INST	88-7805	EXP010-10215
IL-15		88-7158				
IL-22	BMS2047	88-7522				
MIP-1 α (CCL3)		88-7035		BMS2029INST		EXP010-12029
MIP-1 β (CCL4)		88-7034		BMS2030INST		EXP010-12030
RANTES (CCL5)				BMS287/2INST		EXP010-10287
TGF β 1	BMS249/4	88-8350				
TNF α	BMS223/4	88-7346	BMS223HS	BMS223INST		
Mouse Immunoassays						
Granzyme B	BMS6029	88-8022				
IFN γ	BMS606	88-7314	BMS609	BMS606INST	88-7384	EXP010-20606
IL-10	BMS614/2	88-7104		BMS614INST	88-7804	EXP010-20614
IL-15/IL-15R	BMS6023	88-7215				
IL-22	BMS6022	88-7422				
RANTES (CCL5)				BMS6009INST		EXP010-26009
TGF β 1	BMS608/4	88-8350				
TNF α	BMS607/3	88-7324	BMS607HS	BMS607/2INST		EXP010-20607



Ready-Set-Go!® ELISA – Keep costs low. Each affordable, coat-it-yourself ELISA kit includes the reagents required to prepare and run the ELISA, including: ELISA-optimized antibody matched pairs, standards, detection reagents, wash and coating buffers. Plates are optional.



Platinum ELISA – Quantitate with confidence. Pre-coated ELISA kits provide lower inter- and intra-assay variability with ready-to-use reagents that ensure consistent data throughout your research.



Instant ELISA® – Reduce steps and hands-on time to increase productivity. The 1-wash ELISA introduces fewer handling steps, leaving more time for your research.



ProcartaPlex™ Multiplex Immunoassays – Quantitate more with less sample. ProcartaPlex Multiplex Immunoassays utilize Luminex® xMAP® technology for the biomarker profiling of up to 50 analytes in a single 25 μ L sample. Gain a greater understanding of biology and disease in the same time it takes to perform an ELISA.

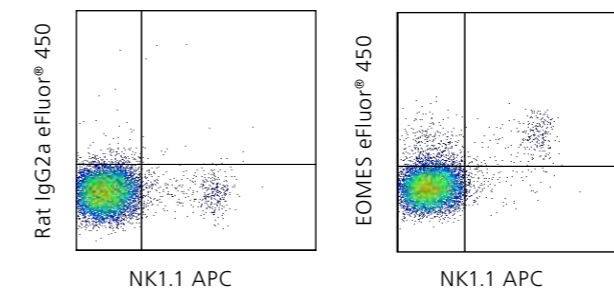
Visit eBioscience.com/ProcartaPlex for information regarding characteristic assay details and a **complete listing of multiplex panels and individual analytes**.

NK Cell Transcriptional Control

NK cells are the prototypical type I Innate Lymphoid Cells (ILC). All ILC are developmentally dependent on the transcription factor inhibitor of DNA-binding 2 (Id2). NK cells express the transcription factor E4BP4 upstream of Id2. Additionally, the T-box transcription factors, Eomesodermin

(EOMES) and T-bet direct the fate and function of cytotoxic cell lineages including NK cells and CD8+ T cells. Furthermore, T-bet controls the developmental stability of immature NK cells, while EOMES regulates NK maturation.

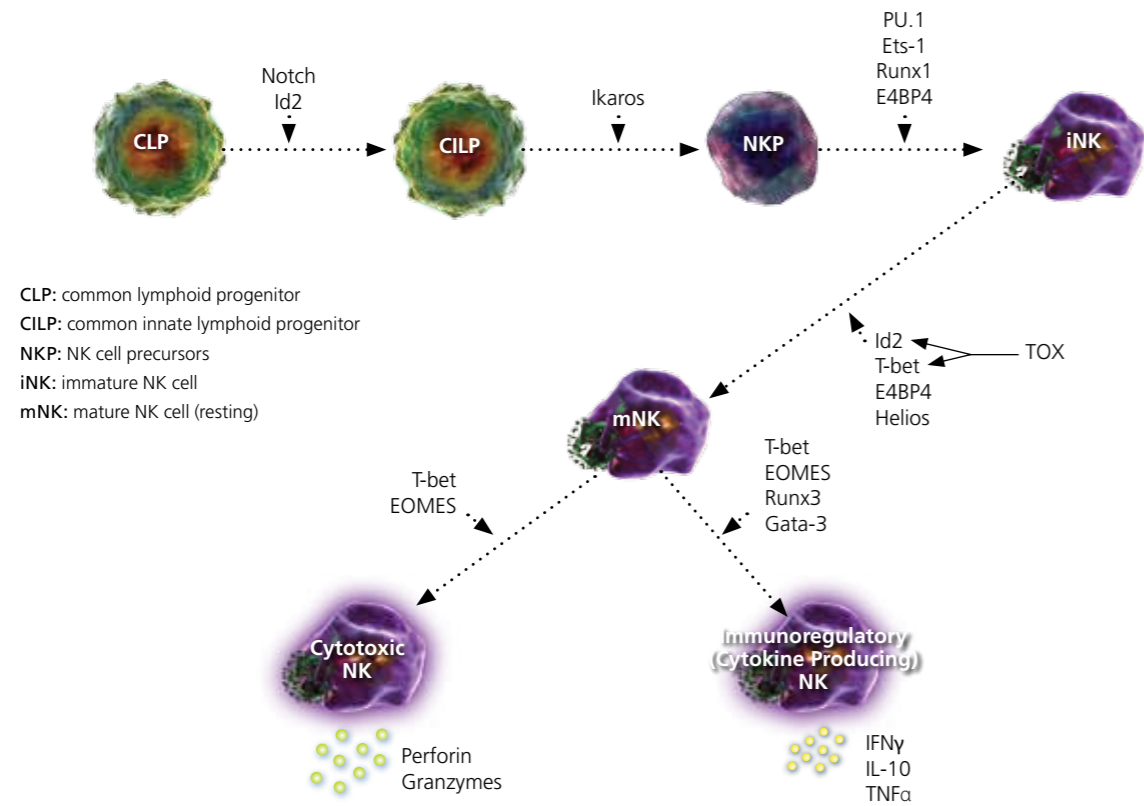
Human Transcription Factor Antibodies														
Antigen	Clone	Cat. No.	Purified	Biotin	Violet Laser	Blue Laser				Green, Yellow-Green Lasers		Red Laser		
					eFluor®450	FITC	AlexaFluor® 488	PerCP-Cyanine5.5	PerCP-eFluor®710	PE	PE-Cyanine7	APC	eFluor®660	
E4BP4	MABA223	9812									■			
EOMES	21Mags8 WD1928	4876 4877	■			■				■				■
GATA-3	TWAJ	9966	■							■				■
Helios	22F6	9883			■	■				■			■	
PU.1	phpu13	9819	■											
Runx1	RXDMC	9816								■				
Runx3	R3-5G4	9817	■	■										■
T-bet	4B10	5825	■					■		■	■			■
TOX	TXRX10	6502	■							■				■
Mouse Transcription Factor Antibodies														
E4BP4	S2M-E19	5927									■			
EOMES	21Mags8 Dan11mag	4876 4875	■		■		■			■	■			■
GATA-3	TWAJ	9966	■							■				■
Helios	22F6	9883			■	■				■			■	
Ikaros	2A9-mlkaros	5780	■											
PU.1	phpu13	9819	■											
Runx1	RXDMC	9816								■				
T-bet	4B10	5825	■					■		■	■			■
TOX	TXRX10	6502	■							■				■



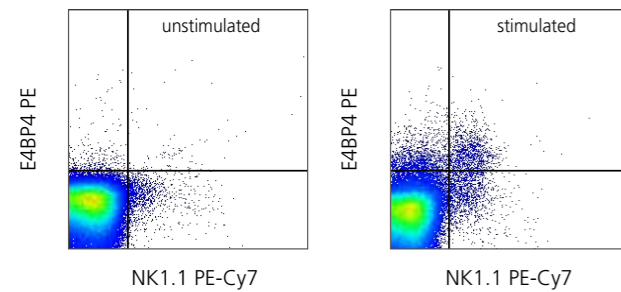
Mouse EOMES

C57Bl/6 splenocytes were surface stained with Anti-Mouse NK1.1 APC (cat. no. 17-5941) followed by fixation and permeabilization using the Foxp3 Buffer Set (cat. no. 00-5523) and protocol. Cells were intracellularly stained with Rat IgG2a K Isotype Control eFluor® 450 (cat. no. 48-4321) (left) or Anti-Mouse EOMES eFluor® 450 (cat. no. 48-4875) (right). Cells in the lymphocyte gate were analyzed.

NK Cell Development and Maturation Transcriptional Control

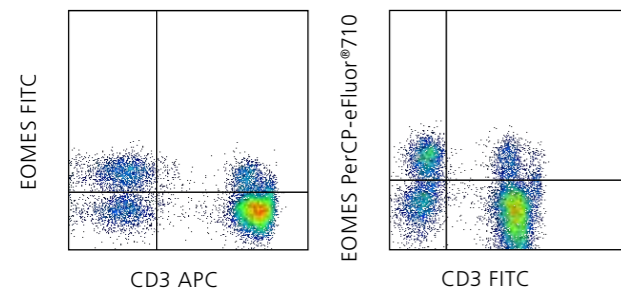


Transcription factors, Notch and Id2, promote commitment to type I innate lymphoid cells. Commitment to the NK cell lineage is dependent upon E4BP4 and PU.1. Multiple transcription factors, Id2, T-bet and E4BP4, ensures development from immature NK to mature NK. Finally Runx3, GATA-3, T-bet and EOMES regulate mature NK cell response.



Mouse E4BP4 (NFIL3)

Mouse splenocytes unstimulated (left) or stimulated overnight with Mouse IL-15/IL-15R Complex Recombinant Protein Carrier-Free (cat. no. 34-8152) (right) were stained with Anti-Mouse NK1.1 PE-Cy7 (cat. no. 25-5941) and Anti-Mouse E4BP4 (NFIL3) PE (cat. no. 12-5927). Intracellular staining for E4BP4 was performed using the Foxp3 Staining Buffer Set (cat. no. 00-5523) and protocol. Cells in the lymphocyte gate were analyzed.



Human EOMES

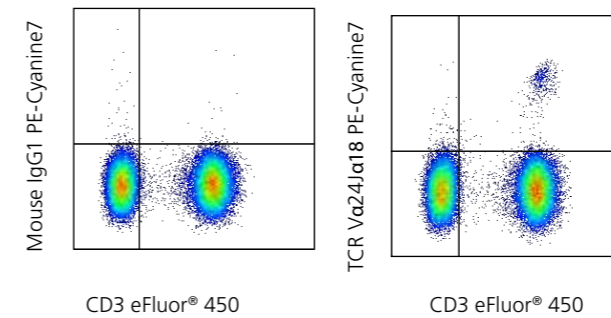
Staining of normal human peripheral blood cells with Anti-Human CD3 APC (cat. no. 17-0037) (left) or FITC (cat. no. 11-0038) (right) followed by fixation and permeabilization using the Foxp3/Transcription Factor Buffer Set (cat. no. 00-5523). Cells were then intracellularly stained with Anti-Human EOMES FITC (cat. no. 11-4877) (left) or PerCP-eFluor® 710 (cat. no. 46-4877) (right). Cells in the lymphocyte gate were analyzed.

NK T Cells

NKT cells represent a distinct lineage of T cells that express an invariant T Cell Receptor (TCR) and share a number of cell surface markers in common with NK cells. NKT cells are non-polymorphic CD1d molecule-restricted T cells and are activated by glycolipid antigens presented by CD1d. Expression of CD160 and Va24Ja18 TCR can be used to identify mouse and human NKT cells, respectively. Expression of transcriptional repressor, Promyelocytic Leukemia Zinc Finger (PLZF), in immune cells differs between mice and humans.

In mice, PLZF is highly expressed in immature CD1d-restricted invariant NKT cells and a subset of gamma delta (Vg1.1+Vd6.3+) T cells. In humans, PLZF is expressed in NK cells, gamma delta T cells, as well as CD4+ and CD8+ T cells. PLZF exists as a homodimer or in complex with PLZP, known to be involved in the development of NKT cells, NK cell function, cellular quiescence and growth suppression. PLZP has also been shown to inhibit gene expression induced by retinoic acid receptor.

Human NKT Cell Antibodies and Formats												
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser		Green, Yellow-Green Lasers		Red Laser	
						eFluor® 450	FITC	AlexaFluor® 488	PerCP-eFluor® 710	PE	PE-Cyanine7	APC
CD1d	51.1	0016	■							■		
CD160	BY55	1609						■		■		■
PLZF	Mags.21F7	9320								■		
TCR Va24Ja18	6B11	5806	■	■		■	■		■	■	■	
Mouse NKT Cell Antibodies and Formats												
α GalCer:CD1d Complex	L363	2019			■					■		
CD1d	1B1	0011	■	■	■			■	■	■		
	WTH-2	0013									■	
CD160	CNX46-3	1601	■	■	■					■		■
PLZF	Mags.21F7	9320								■		



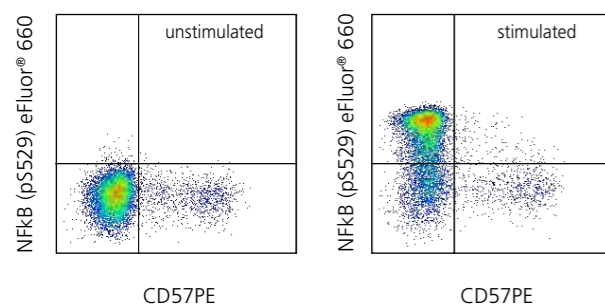
Human TCR Va24Ja18

Staining of normal human peripheral blood cells with Anti-Human CD3 eFluor® 450 (cat. no. 48-0037) and Mouse IgG1 K Isotype Control PE-Cyanine7 (cat. no. 25-4714) (left) or Anti-Human Va24Ja18 TCR PE-Cyanine7 (cat. no. 25-5806) (right). Cells in the lymphocyte gate were used for analysis.

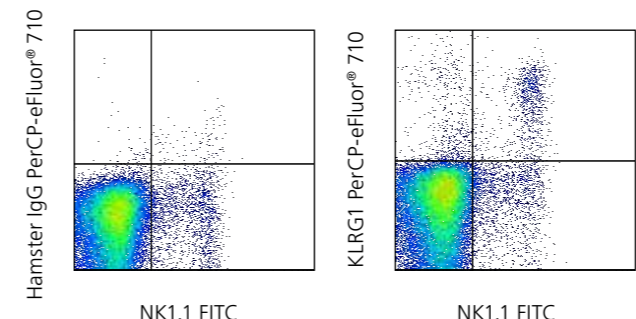
NK Cell Marker Guide

Human NK Cell Antibodies and Formats																	
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers				Red Laser		
						eFluor® 450	FITC	AlexaFluor® 488	PerCP-Cyanine5.5	PerCP-eFluor® 710	PE	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	■	■		■		■			■				■		
CD27	LG.7F9	0271	■	■	■		■				■				■		
	O323	0279	■	■		■					■	■			■		■
CD56	CMSSB	0567	■	■							■	■	■		■		
CD57	TB01	0577			■	■	■				■						
CD62L (L-Selectin)	DREG56	0629	■		■	■	■				■	■			■		
CD96 (TACTILE)	NK92.39	0969									■						
CD127	eBioRDR5	1278	■	■	■	■	■				■	■			■		
CD161	HP-3G10	1619				■		■			■				■		
CD181 (CXCR1)	8F1-1-4	1819	■				■				■						
CD184 (CXCR4)	12F5	9999	■	■	■						■	■			■		
CD195 (CCR5)	T21/8	1957	■	■							■						
CD197 (CCR7)	3D12	1979	■	■			■				■						■
CD218a (IL-18Ra)	H44	7183	■	■			■				■				■		
CD244	eBioDM244	5837					■										
	C1.7	5838	■	■	■						■						
CD253 (TRAIL)	RIK-2	9927	■	■	■												
CD319 (CRACC)	162	2229									■						
CD328 (Siglec7)	QA79	5759	■	■							■						
CX3CR1	2A9-1	6099		■							■						
IL-15R	eBioJM7A4	7159	■				■				■						
phospho NF kappa B p65 (S529)	B33B4WP	9863													■		

Mouse NK Cell Antibodies and Formats																	
Antigen	Clone	Cat. No.	Purified	Biotin	Functional Grade	Violet Laser	Blue Laser				Green, Yellow-Green Lasers				Red Laser		
						eFluor® 450	FITC	AlexaFluor® 488	PerCP-Cyanine5.5	PerCP-eFluor® 710	PE	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	APC	eFluor® 660	APC-eFluor® 780
Asialo GM1	poly	6507			■			■									
CD11b	M1/70	0112	■	■	■	■	■	■	■		■	■			■		■
CD16/32	93	0161	■	■	■	■	■	■	■		■	■	■		■		■
CD25	PC61.5	0251	■	■	■						■	■	■		■		■
CD27	7D4	0252		■												■	
	3C7	0253				■											
CD27	LG.7F9	0271	■	■	■		■				■				■		
	DX5	5971	■	■		■					■	■			■		
CD49b (DX5)	HMa2	0491	■	■	■		■				■	■			■		
CD62L (L-Selectin)	MEL-14	0621	■	■	■	■	■				■	■			■		■
CD96 (TACTILE)	6A6	0960			■						■						
CD127	A7R34	1271	■	■	■	■	■				■	■			■		■
CD183 (CXCR3)	CXCR3-173	1831		■	■		■				■				■		
CD184 (CXCR4)	2B11	9991	■	■	■	■					■				■		
CD195 (CCR5)	HM-CCR5 (7A4)	1951	■	■							■						
CD197 (CCR7)	4B12	1971	■	■	■	■					■				■		■
CD218a (IL-18Ra)	P3TUNYA	5183									■						
CD244.1	C9.1	2440									■						
CD244.2	eBio244F4	2441	■				■				■				■		
CD253 (TRAIL)	N2B2	5951	■	■	■						■						
IL-15Ra	DNT15Ra	7149									■				■		
KLRG1	2F1	5893	■	■	■	■	■				■				■		
NK1.1	PK136	5941	■	■	■	■	■				■				■		■



NF-kB p65 Signaling
 Normal human peripheral blood cells were left untreated (left) or stimulated for 15 min with 50 nM PMA (right). Cells were permeabilized with Intracellular Fixation & Permeabilization Buffer Set (cat. no. 88-8824), and then intracellularly stained with Anti-Human CD57-PE (cat. no. 12-0577-42) and Anti-Human phospho-NF-kB (Ser529) eFluor® 660 (cat. no. 50-9863).



Mouse KLRG1
 Staining of C57Bl/6 splenocytes with Anti-Mouse NK1.1 APC (cat. no. 17-5941) and Golden Syrian Hamster IgG Isotype Control PerCP-eFluor® 710 (cat. no. 46-4914) (left) or Anti-Mouse KLRG1 PerCP-eFluor® 710 (cat. no. 46-5893) (right). Total viable cells were used for analysis.