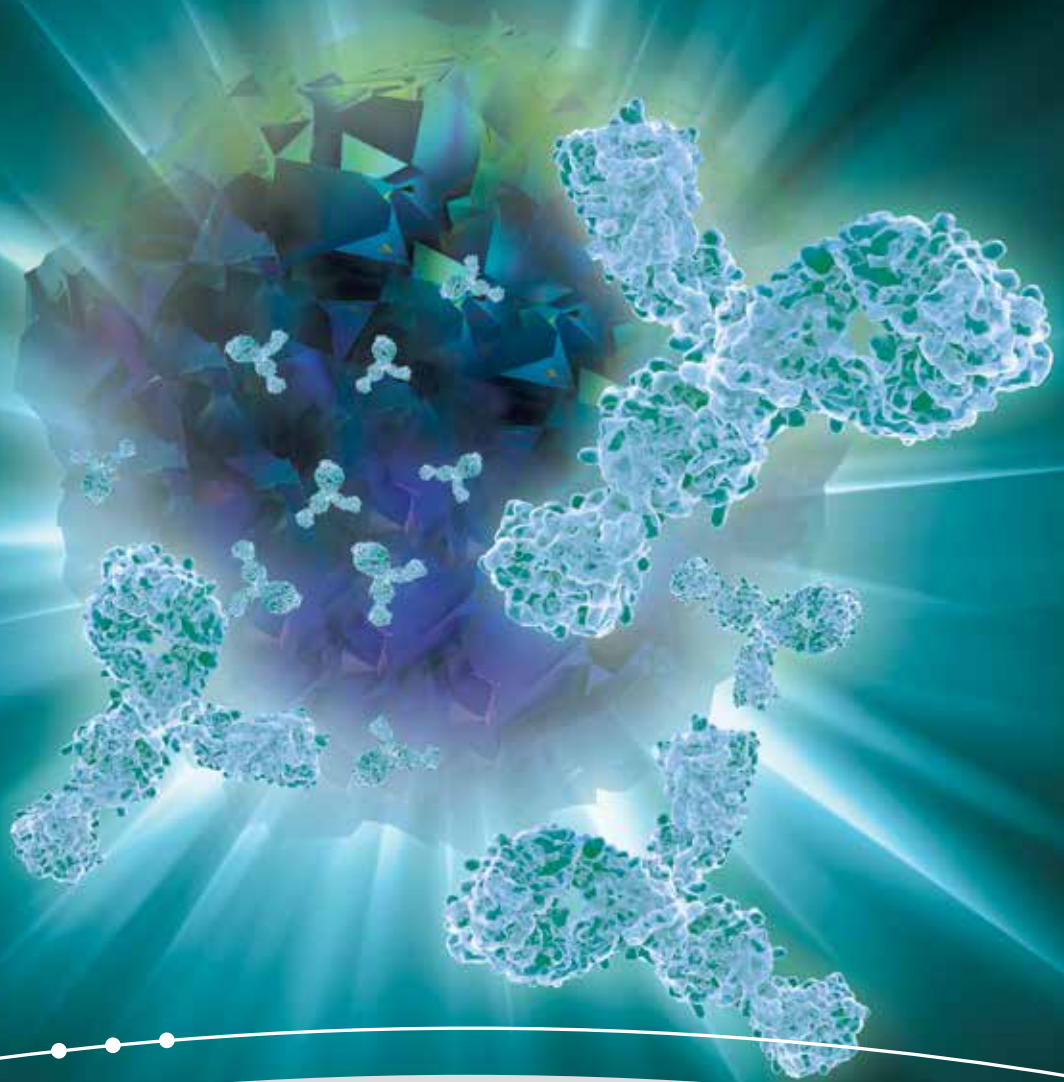


# Functional Activity

*In Vitro / In Vivo* Product Guide



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# Functional Activity

## Activation, Blocking and Neutralization

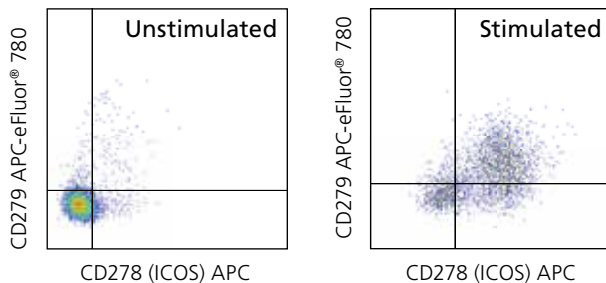
An essential part of evaluating any biological system is the ability to mimic and/or inhibit relevant pathways, both at the cellular and tissue level and in whole organism models using antibodies, proteins or small molecules. Use of these tools in bioassays allows life science researchers to explore biologic systems and examine:

- Initiation of signaling cascades via receptor-ligand interactions
- Induction of cell activation, differentiation, migration or survival
- Expression of unique surface and functional phenotypes
- Pattern assessment of protein expression

eBioscience offers an expansive list of high quality Recombinant Proteins and Functional Grade Antibodies designed for optimal results when used *in vitro* or *in vivo* for activation, blocking or neutralization studies, enabling you to explore a myriad of biologic systems with consistent, reproducible results.

### Advantages of our Recombinant Proteins and Functional Grade Antibodies

- Exhibit functional activity for activation, blocking and neutralization studies
- Endotoxin levels significantly lower than the industry standard
- Always preservative-free for *in vivo* or *in vitro* applications



Staining of 3-day Anti-Mouse CD3 and Anti-Mouse CD28 Functional Grade Purified (cat. no. 16-0031 and 16-0281)-stimulated (right) or unstimulated (left) mouse splenocytes with Anti-Mouse/Rat CD278 (ICOS) APC (cat. no. 17-9949) and Anti-Mouse CD279 (PD-1) APC-eFluor® 780 (cat. no. 47-9985). Total viable cells, as determined by Fixable Viability Dye eFluor® 450 (cat. no. 65-0863), were used for analysis.

# Functional Grade Antibodies

The term “functional grade antibody” refers to antibodies that are ready for *in vivo* or *in vitro* use and often either mimic or interrupt the natural biological effects associated with receptor-ligand interactions. Functional Grade Antibodies are used for activation, blocking and/or neutralization studies. These reagents are provided in appropriate buffers containing extremely low endotoxin levels and are preservative (sodium azide)-free.

## Performance: Quality First

Quality, performance and value are paramount when choosing reagents for your studies. At eBioscience, we manufacture our products to meet stringent quality specifications, so you can be confident that the performance of a new lot of antibody is consistent with that of previous lots.

### Functional Grade Purified Antibodies

- SDS-PAGE and HPLC testing ensures purity and integrity of the antibody
- Flow cytometry or bioassay testing ensures specificity/functionality of the antibody
- Endotoxins levels must be < 0.001 ng/μg of antibody as determined by LAL testing
- Sterile-filtered

### Hybridoma Cell Banks

- Cell lines are tested for specificity, absence of mycoplasma and isotype
- Clonality of the antibody produced from hybridoma is ensured

### Cell Culture

- Functional Grade purified antibodies are expressed in tissue culture, never ascites, to reduce the risk of contaminants and the presence of non-specific antibodies.

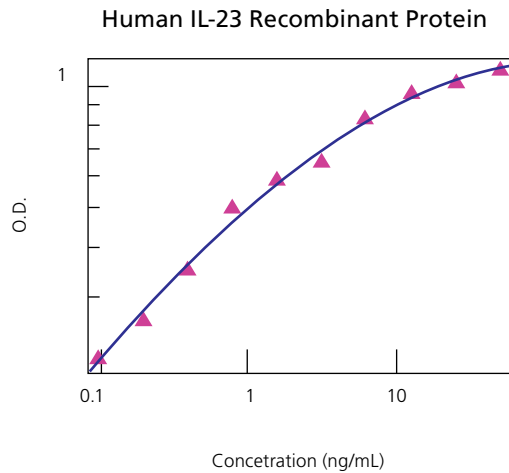
# Recombinant Proteins

Recombinant proteins have best-in-class performance for cell culture maintenance, proliferation, and/or cell differentiation. A wide variety of cytokines and chemokines are found in our catalog for inducing growth and/or differentiation, stimulating migration and promoting survival. Most of our proteins are available in multiple formats, including carrier-free options, which all surpass industry-standard endotoxin specifications for *in vivo* or *in vitro* applications.

## Quality Assurance

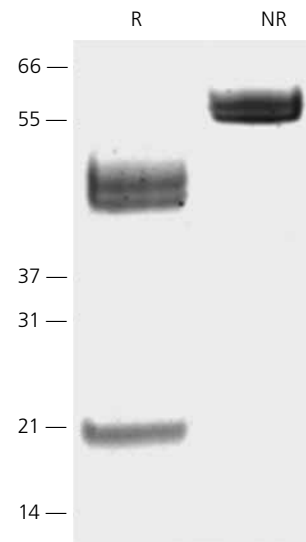
Only eBioscience recombinant proteins meet the following criteria to ensure you receive the best protein possible:

- Endotoxin levels must be < 0.01 ng/μg of protein
- Bioactivity analysis data from a relevant bioassay for each lot



The ED50 of this protein, as measured by induction of IL-17A in mouse splenocytes,  $\leq 3.5$  ng/mL. This corresponds to a specific activity of  $\geq 2.9 \times 10^5$  Units/mg.

## Mouse IL-23 Recombinant Protein



Under non-reducing SDS-PAGE conditions, the heterodimeric cysteine-linked Mouse IL-23 Recombinant Protein migrates as a 60 kDa protein (Lane NR). Under DTT-reducing conditions it migrates as 43 kDa and 21 kDa polypeptides (Lane R).

# T Cell and B Cell Activation

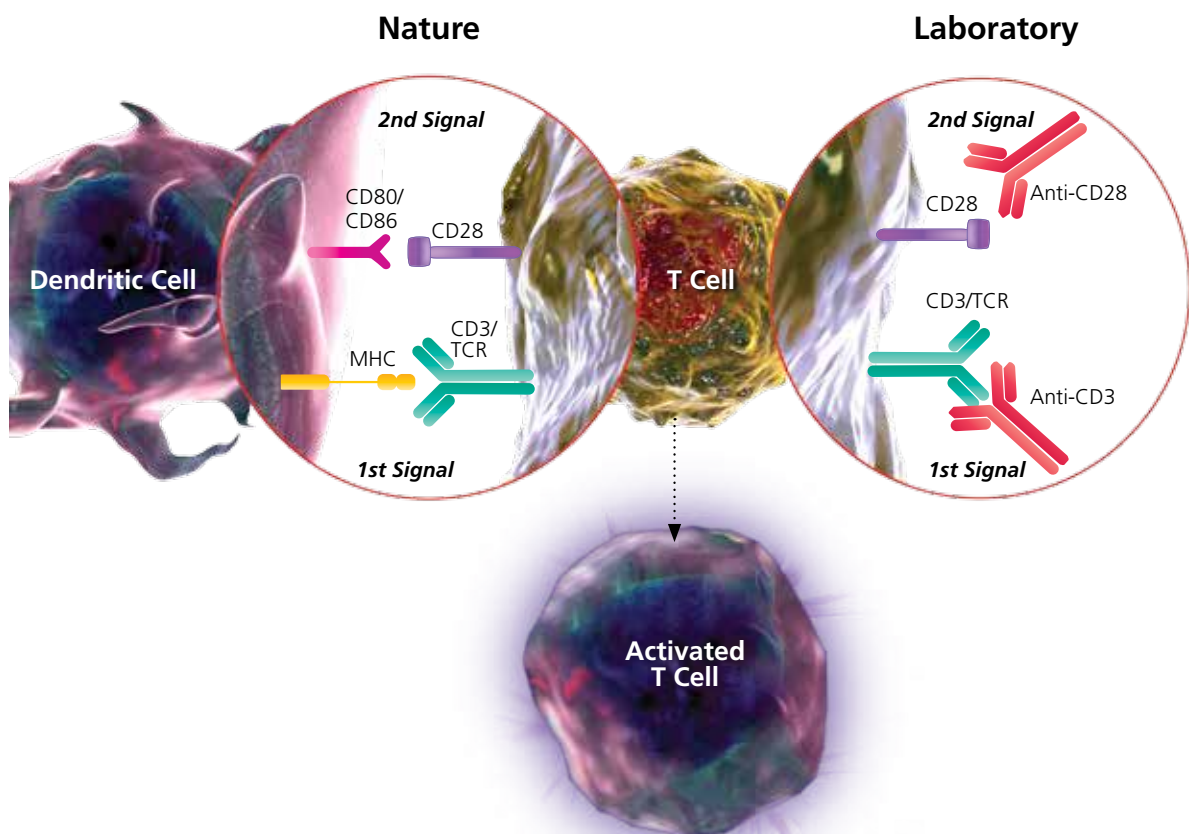
## Regulators and Effectors of the Adaptive Immune System

### T Cell Activation

A fully activated T cell requires at least two signals. The first signal involves engagement of the T Cell Receptor (TCR) with a peptide-Major Histocompatibility Complex (MHC) presented by an Antigen Presenting Cell (APC). The second T cell-activating signal is generated when co-stimulatory molecules expressed on the surface of T cells associate with ligands expressed on the APC. Together, these activating signals are transmitted to the nucleus of the T cell and result in:

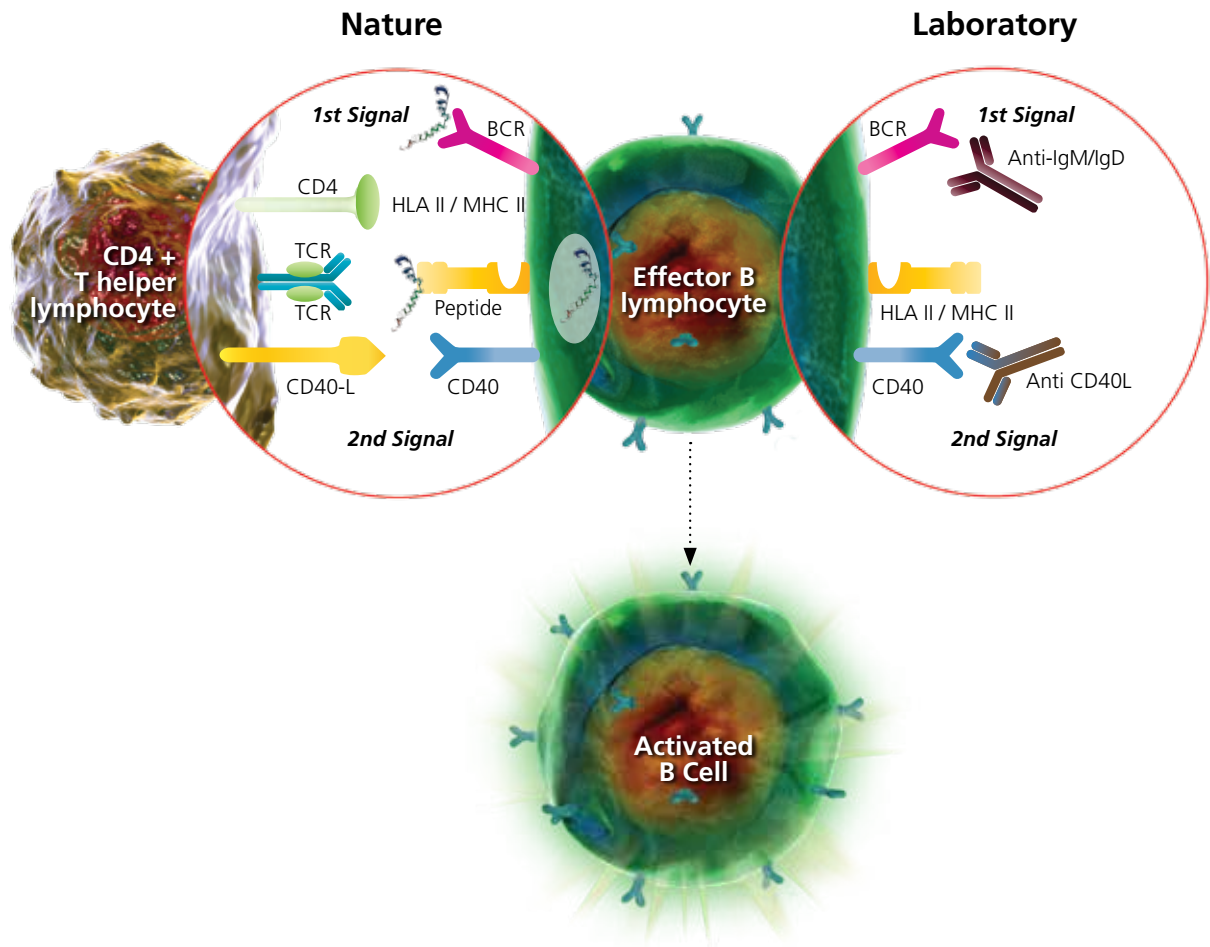
- 1) Clonal expansion of T cells
- 2) Upregulation of activation marker expression on the T cell surface
- 3) Differentiation into effector cells in the presence of additional factors
- 4) Induction of cytotoxicity or cytokine secretion
- 5) Induction of apoptosis

A common way to assess T cell activation is to measure T cell proliferation upon *in vitro* stimulation of T cells using agonistic antibodies to the TCR.



## B Cell Activation

As with T cell activation, B cell activation also requires two signals. The first signal is provided by the B Cell Receptor (BCR), a surface-expressed antibody binding to its cognate antigen. This signal may also be mimicked using anti-IgM or IgD antibodies. The second signal is achieved through engagement of co-stimulatory molecules such as CD40 and cytokine signaling. Alternatively, components of bacterial cell walls, such as Lipopolysaccharide (LPS), and antigens with highly repetitive molecules may signal B cell activation directly.



<b>T Cell Activation</b>		
<b>Antibodies</b>	<b>Clone</b>	<b>Cat. No.</b>
Anti-Human CD3 Functional Grade Purified*	HIT3a	16-0039
Anti-Human CD3 Functional Grade Purified*	OKT3	16-0037
Anti-Human CD3 Functional Grade Purified	SK7	16-0036
Anti-Human CD3 Functional Grade Purified	UCHT1	16-0038
Anti-Human CD28 Functional Grade Purified*	CD28.2	16-0289
Anti-Human CD28 Functional Grade Purified	CD28.6	16-0288
Anti-Mouse CD3e Functional Grade Purified*	145-2C11	16-0031
Anti-Mouse CD3e Functional Grade Biotin	145-2C11	36-0031
Anti-Mouse CD3 Functional Grade Purified	17A2	16-0032
Anti-Mouse CD3e Functional Grade Purified	eBio500A2	16-0033
Anti-Mouse CD28 Functional Grade Purified*	37.51	16-0281
Anti-Rat CD3 Functional Grade Purified	eBioG4.18	16-0030
Anti-Rat CD28 Functional Grade Purified	JJ319	16-0280
Anti-Canine CD28 Functional Grade Purified	5B8	16-0282
<b>Small Molecules</b>		
Cell Stimulation Cocktail (500X)	--	00-4970
Cell Stimulation Cocktail (plus protein transport inhibitors) (500X)	--	00-4975

\* For human T cell activation, we recommend Anti-CD3, clone OKT3 or HIT3a, and Anti-CD28, clone 28.2

\* For mouse T cell activation, we recommend Anti-CD3, clone 145-2C11, and Anti-CD28, clone 37.51

<b>B Cell Activation</b>		
<b>Antibodies</b>	<b>Clone</b>	<b>Cat. No.</b>
Anti-Mouse IgD Functional Grade Purified	10.4.22	16-5924
Anti-Mouse IgD antiserum	Polyclonal	24-5093
Anti-Mouse IgM $\mu$ -chain	Polyclonal	16-5092
Anti-Mouse CD40 Functional Grade Purified	1C10	16-0401
Anti-Mouse/Rat CD40 Functional Grade Purified	HM40-3	16-0402
Anti-Human CD40 Functional Grade Purified	5C3	16-0409
Anti-Mouse CD180 (RP105) Functional Grade Purified	RP/14	16-1801
Anti-Human CD180 (RP105) Functional Grade Purified	MHR73-11	16-1809
<b>Recombinant Proteins</b>		<b>Cat. No.</b>
Human CD257 (BAFF) Recombinant Protein		14-8943
Mouse IFN gamma Recombinant Protein		14-8311
		34-8311
Human IFN gamma Recombinant Protein		14-8319
		34-8319
Mouse IL-4 Recombinant Protein		14-8041
		34-8041
Human IL-4 Recombinant Protein		14-8049
		34-8049
Mouse IL-5 Recombinant Protein		14-8051
		34-8051
Mouse TGF-beta 1 Recombinant Protein		14-8342
		34-8342
Human TGF-beta 1 Recombinant Protein		14-8349
		34-8349

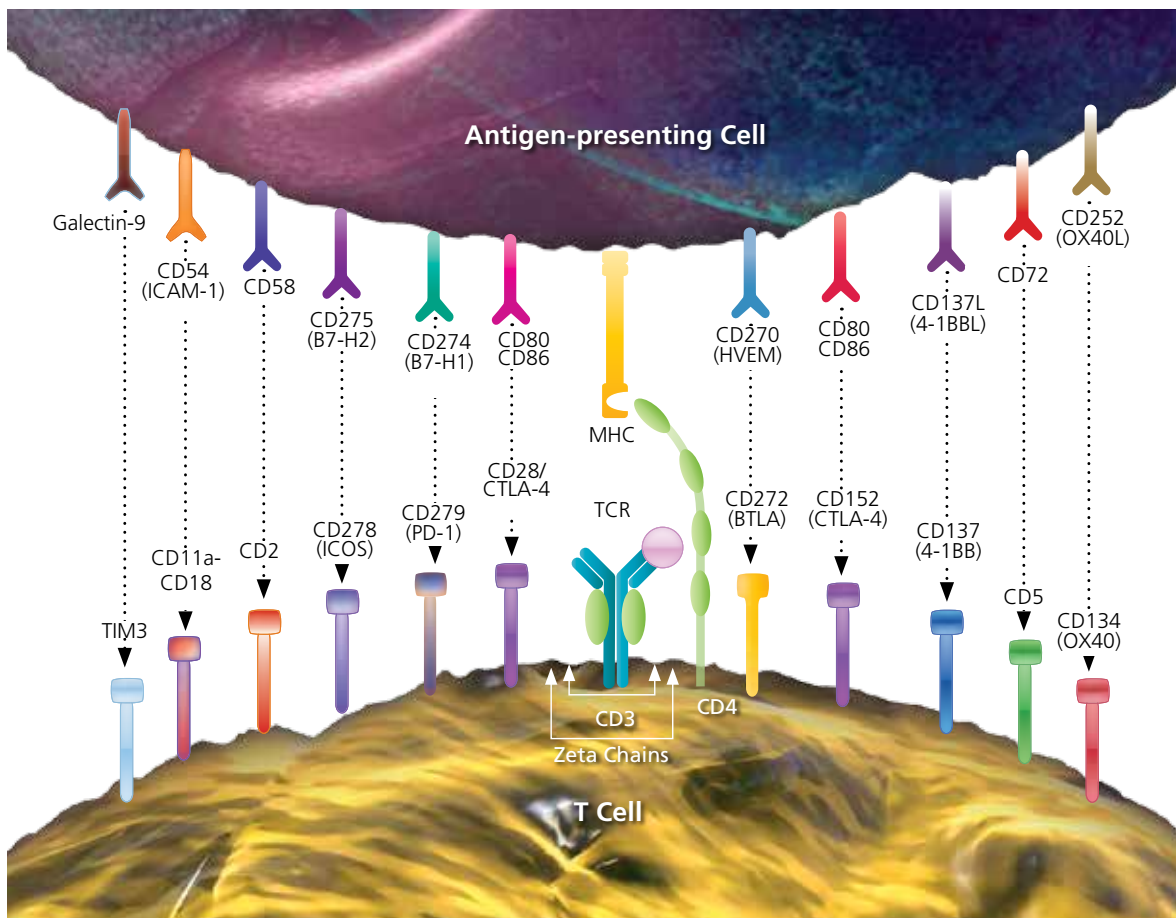


## Co-stimulation

T cell function is dependent upon and controlled by a variety of inducible structures that may have stimulatory (ICOS, OX40, and 4-1BB) or inhibitory (cytotoxic T-lymphocyte antigen [CTLA-4/CD152], B and T lymphocyte attenuator [BTLA/CD272]) potential. Some of these receptors act as either immune inhibitors or activators depending upon the stimulatory environment.

Most co-stimulatory and inhibitory molecules are organized into two families based upon their sequence similarities. The Ig superfamily includes the co-stimulatory receptor, CD28 and inducible costimulator (ICOS/CD278), and the inhibitory receptors, (CD152), programmed cell death-1 (PD-1/CD279), CD272 and CD160. The tumor necrosis factor (TNF) superfamily includes the co-stimulatory receptors CD40, CD134 (OX40), and herpes virus entry mediator (CD270 HVEM).

Co-stimulatory receptor-ligand pairs present on T cells and antigen presenting cells.



Co-stimulation - Receptor - Ligand Quick Guide									
Receptor	Human FG Ab Cat. No.	Mouse FG Ab Cat. No.	Family	Expression	Signal	Ligand	Human FG Ab Cat. No.	Mouse FG Ab Cat. No.	Ligand Expression
CD2	16-0029	16-0021	IgSF	T, B and NK cells	+	CD58 (LFA-3)	16-0578	--	Hematopoietic, non-hematopoietic cells
CD4	16-0049	16-0041	IgSF	T subset and Macro/Mono	+ and -	MHC class II (I-A/I-E)	--	16-5321	B, Marco/Mono, DC and human T cells
CD28	16-0289	16-0281	IgSF	T cells	+	CD80 (B7-1) CD86 (B7-2)	16-0809 16-0869	16-0801 16-0861 16-0862	B, Macro/Mono, DC and activated T cells
CD152 (CTLA-4)	16-1529	16-1521	IgSF	B and activated T cells	-	CD80 (B7-1) CD86 (B7-2)	16-0809 16-0869	16-0801 16-0861 16-0862	B, Marco/Mono, DC and activated T cells
CD160	1609 (clone BY55)	16-1601	IgSF	T, NK and NKT cells	-	CD270 (HVEM) MHC class I (H-2Db)	5969 (clone HVEM-122) 9958 (clone A4)	5962 (clone LH1) 16-5957 16-5999	T, B, DC, Macro/Mono and NK cells
CD223 (Lag-3)		16-2231	IgSF	Activated T, B and NK cells	-	MHC class II (I-A/I-E)	--	16-5321	B, Marco/Mono, DC and human T cells
CD244 (2B4)	16-5838 16-2449	244F4	IgSF	NK, T subset, Basophils and Macro/Mono	+ and -	CD48	16-0489	16-0481	Most leukocytes
CD272 (BTLA)	16-5979	16-5950	IgSF	Activated T, Th1, B and DC cells	-	CD270 (HVEM)	5969 (clone HVEM-122)	5962 (clone LH1)	T, B, DC, Macro/Mono and NK cells
CD274 (B7-H1/PD-L1)	--	--	IgSF	B, Marco/Mono, DC, T and NK cells	-	CD80 (B7-1)	16-0809	16-0801	B, Macro/Mono, DC and activated T cells
CD278 (ICOS)	16-9948	16-9942 16-9949	IgSF	Activated T, Th2 and NKT cells	+	CD275 (B7-H2, ICOS-L)	16-5889	16-5985	B, Marco/Mono, DC and endothelial cells
CD279 (PD-1)	16-9989	16-5982 16-9985	IgSF	Activated T, activated B, DC and Macro/Mono	-	CD274 (B7-H1, PD-L1) CD273 (B7-DC, PD-L2)	16-5983 16-5888	16-9982 16-5986*	B, Marco/Mono, DC, T and NK, Marco/Mono and DC cells
TIM3	3109 (clone F38-2E2)	16-5871	IgSF	T, B, DC, Macro/Mono and NK cells	-	Galectin 9	--	--	T, Treg and others
CD27	16-0271 16-0272	16-0271 16-0272	TNFRSF	T, B subset, NK subset and HSC	+	CD70	--	16-0701	Activated T, B and DC cells
CD30	0309 (clone Ber-H2)	16-0301	TNFRSF	Activated T, B and NK cells	+	CD153 (CD30L)	--	16-1531	B and activated T cells
CD134 (OX40)	1347 (clone ACT-35)	16-1341	TNFRSF	Activated T, B, DC and Marco/Monos	+ (- for Treg)	CD252 (OX40 Ligand)	--	16-5905	Activated T, Treg, NKT, NK and neutrophils
CD137 (4-1BB)	1379 (clone 4B4)	16-1371	TNFRSF	Activated T, Macro/Mono, DC and NK cells	+	CD137 Ligand (4-1BBL)	--	16-5901	B, DC and Macro/Mono
CD154 (CD40L)	16-1548	16-1541	TNFRSF	Activated T, B and platelets cells	+	CD40	16-0409	16-0402	B, DC, Macro/Mono and endothelial cells
CD270 (HVEM)	5969 (clone HVEM-122)	5962 (clone LH1)	TNFR	T, B, DC, Macro/Mono, and NK cells	+	CD258 (LIGHT) CD272 (BTLA) CD160	2589 (clone 7-3) 16-5979 1609 (clone BY55)	-- 16-5950 16-1601	Activated T and B, DC, Marco/Monos and NK cells
CD357 (GITR)	5875 (clone AITR)	16-5874	TNFRSF	Activated T, Treg and NK cells	+	GITR Ligand	--	5854 (clone YGL 386)	B, DC, Macro/Mono and endothelial cells
Lymphotoxin $\beta$ Receptor	--	16-5671	TNFRSF	DC, Macro/Mono and stromal cells	+	CD258 (LIGHT) LT $\alpha\beta$	2589 (clone 7-3) --	--	Activated T and B, DC, Marco/Monos and NK cells

# Cell Differentiation

## T helper (Th) Cells

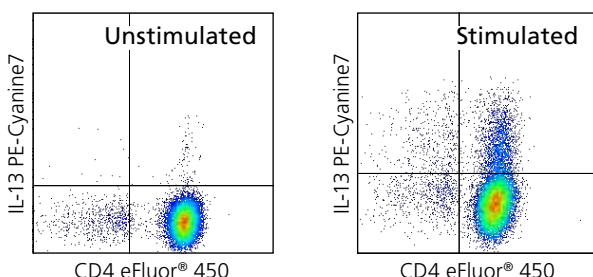
Th1 lymphocytes play an important role in host responses against intracellular microbes and viruses. Th1 cell promoting factors include IFN $\gamma$  and IL-12 (p70), and the activation of the transcription factors STAT1 and STAT4. IL-12 receptor activation increases IFN $\gamma$  expression, through STAT1-mediated signaling to induce the Th1 master regulator T-bet. T-bet-driven transcription further increases IFN $\gamma$  expression, while suppressing production of IL-4.

Th2 cells mediate the activation and maintenance of the humoral, or antibody-mediated, immune response against extracellular parasites, bacteria, allergens and microbial toxins. IL-4 signals through STAT6 to upregulate expression of GATA3, the master regulator of Th2 cell differentiation. Repression of GATA-3 results in the failure of the development of IL-4 producing cells. IL-4 also suppresses Th1 and Th17 cell responses through the upregulation of transcriptional repressor(s) of IFN $\gamma$  and IL-17 production. Of note, the IL-4/STAT6 pathway is not completely required for Th2 cell differentiation as Th2 cell differentiation can also occur through other cytokines such as TSLP, IL-17E (IL-25) and IL-33.

Th17 cells are a subset of activated CD4+ T cells that are responsive to IL-1R1 and IL-23R signaling. They act as a bridge between adaptive and innate immunity by promoting neutrophil activation, immunity to pathogens and inflammation. While Th17 cell differentiation is driven by TGF $\beta$  and IL-6 *in vitro*, it has been shown that IL-1 $\beta$  and IL-23 are also necessary for Th17 differentiation *in vivo*. Th17 differentiation is driven and regulated by the lineage-defining transcription factors AHR, BATF, I $\kappa$ B $\zeta$ , IRF4, c-Maf, ROR $\alpha$ , ROR $\gamma$ t and STAT3. It is important to note, IL-23 is required for Th17 expansion and stabilization.

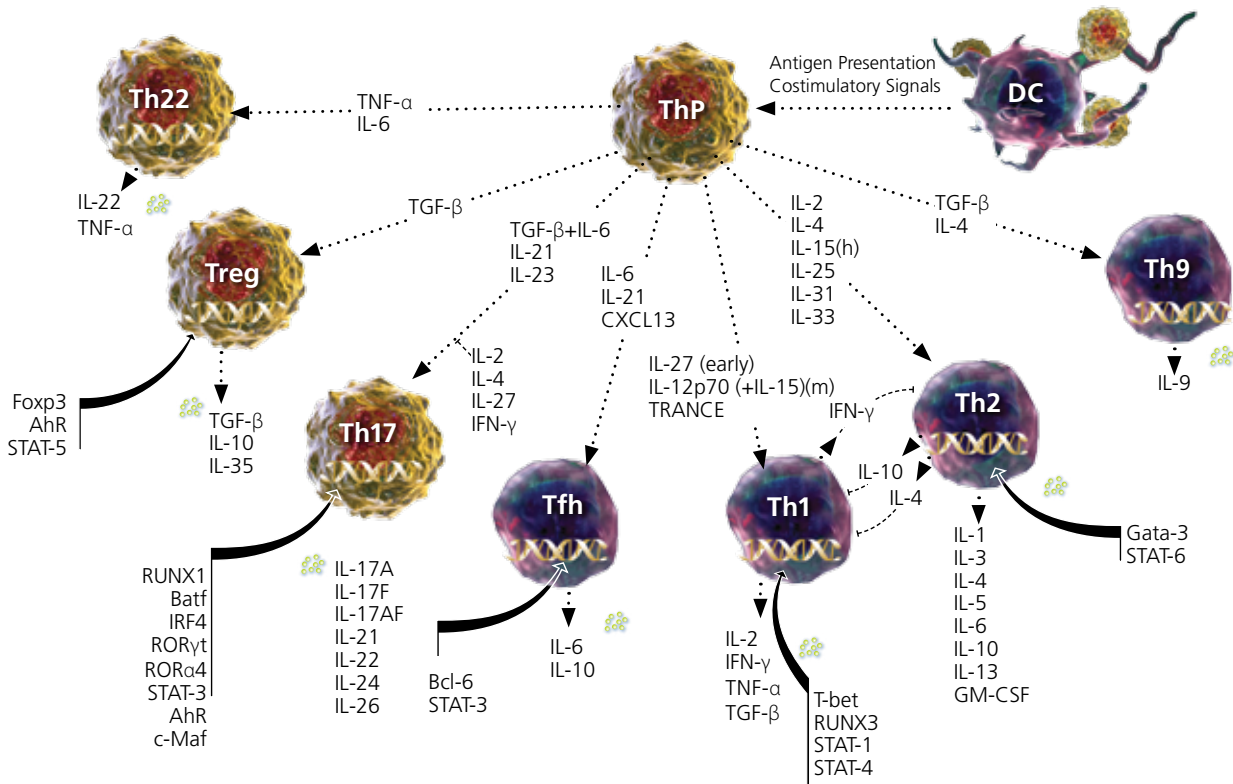
T follicular helper (Tfh) cells require IL-6, IL-21 and B cell interaction for complete development. A key transcription factor involved in the differentiation of Tfh cells is Bcl-6, which regulates the changes in CXCR5 and CCR7 expression required for T cell migration into follicles. Tfh cells are a regulatory class of specialized effector T helper cells that are essential for the development of antigen-specific effector and memory B cell responses.

Regulatory T (Treg) cells are specialized CD4+ T cells that function to maintain self-tolerance and immune homeostasis by suppressing the activation, proliferation and effector functions of various immune cells. Treg cells secrete TGF $\beta$  to induce Foxp3 expression causing conventional CD4+ T cells to differentiate into Treg cells, thereby skewing the ratio of Treg to T helper cells during an immune response.



CD4+ BALB/c splenocytes were stimulated for 10-days under Th2 polarizing conditions. Cells were then unstimulated (left) or restimulated (right) with Cell Stimulation Cocktail (plus protein transport inhibitors) (cat. no. 00-4975) for 5 hours. Cells were subsequently fixed and permeabilized using the Intracellular Fixation & Permeabilization Buffer Set (cat. no. 88-8824) and then stained with Anti-Mouse CD4 eFluor $^{\text{®}}$  450 (cat. no. 48-0042) and Anti-Mouse IL-13 PE-Cyanine7 (cat. no. 25-7133). Viable cells, as determined by Fixable Viability Dye eFluor $^{\text{®}}$  660 (cat. no. 65-0864), were used for analysis.

## Helper T Cell Paradigm



Th1 Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
IL-2	14-8029	14-8021	- Expressed by activated T cells, but not resting T cells
	34-8029	34-8021	- Mediates proliferation of activated T cells
IL-12 p70	14-8129	14-8121	- Produced by activated macrophages
	34-8129	34-8121	- Promotes survival and growth of Th1 cells - Sustains sufficient number of memory/effector Th1 cells - Inhibits the formation of Th2 cells
IL-18	--	--	- Produced by monocytes, macrophages, dendritic cells, keratinocytes and epithelial cells
			- Critical inducer of IFNγ - Functions as a key growth and differentiation factor
IL-27	14-8279	14-8271	- Produced by activated monocytes, macrophages and dendritic cells
	34-8279	34-8271	- Synergizes with IL-12 to cause the production of IFNγ by naïve Th cells - Increases proliferation of cells without affecting memory T cells
IFN gamma	14-8319	14-8311	- Autocrine factor in the establishment of Th1 cells
	34-8319	34-8311	- Enhanced by the action of IL-12
Neutralizing Antibodies	Human Cat. No.	Mouse Cat. No.	Role
Anti-IL-4	16-7048	16-7041	Inhibits Th1 differentiation and promotes Th2 differentiation

Th2 Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
IL-2	14-8029	14-8021	- Expressed by activated T cells
	34-8029	34-8021	- Mediates activated T cell proliferation and clonal expansion
IL-4	14-8049	14-8041	- Required for Th2 priming and maturation
	34-8049	34-8041	- Autocrine of Th2 cells during their maturation - High concentrations can block the generation of Th1 cells from naïve T cells
IL-6	14-8069	14-8061	- Released by antigen presenting cells
	34-8069	34-8061	- Initiates maturation of Th2 cells from Th0 in conjunction with IL-4 - High concentration can block the generation of Th1 cells in a similar fashion to IL-4
IL-17E (IL-25)	- -	39-9175	- Induces cytokine expression - Helps maintain Th2 function - Plays a critical role in the formation of Th2 memory
	14-8310	14-8531	- Expressed by activated CD4+ cells
IL-31	34-8310	34-8531	- Associated with enhanced IL-4 and IL-13 expression by Th2
	14-8338	14-8332	Necessary for Th2 cytokine production
IL-33	34-8338	34-8332	
TSLP	14-8497	14-8498	Drives Th2 differentiation via the induction of IL-4 production
	34-8497	34-8498	
Neutralizing Antibodies	Human Cat. No.	Mouse Cat. No.	Role
Anti-IFN gamma	16-7318	16-7312	Inhibits Th2 differentiation and promotes Th1 differentiation
Anti-IL-12 p70	16-7129	16-7123	

Th9 Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
TGF beta 1	14-8348	14-8342	Essential to the reprogramming of Th0 cells into mature Th9 cells
	34-8348	34-8342	
IL-4	14-8049	14-8041	Blocks the generation of TGFβ-induced Foxp3+ Treg cells and induces Th9 cell formation
	34-8049	34-8041	
IL-1 beta	14-8018	14-8012	Enhances IL-9 expression of cultured Th9 cells
	34-8018	34-8012	
IL-6	14-8069	14-8061	
	34-8069	34-8061	
IL-10	14-8109	14-8101	
	34-8109	34-8101	
IL-21	14-8219	14-8211	
	34-8219	34-8211	
IFN alpha	- -	148312	
		34-8312	
		14-8313	
		34-8313	
Neutralizing Antibodies	Human Cat. No.	Mouse Cat. No.	Role
Anti-IFN gamma	16-7318	16-7312	Inhibits IL-9 production in human Th9 cells (Anti IL-27 is not required with purified naïve T cell culture)
Anti-IL-27 p28	16-7285	16-7285	

Th17 Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
TGF beta 1	14-8348 34-8348	14-8342 34-8342	Essential factor for Th0 to Th17 development in concert with IL-6 and IL-23
IL-1 beta	14-8018 34-8018	14-8012 34-8012	- Involved in early Th17 cell differentiation - Upregulates ROR $\gamma$ t and IRF4 - Helps maintain Th17 cytokine profile post-polarization
IL-6	14-8069 34-8069	14-8061 34-8061	Essential in the activation of IL-17 specific transcription factor ROR $\gamma$ t and IL-21 expression that then activates the expression of IL-17A, IL-17F, and IL-23R on Th17 cells
IL-21	14-8219 34-8219	14-8211 34-8211	Expression and autocrine feedback through STAT3, IRF4 and ROR $\gamma$ t lead to upregulation of the IL-23R, thereby preparing Th17 cells for maturation and maintenance by the inflammatory cytokine IL-23
IL-23	14-8239 34-8239	14-8231 34-8231	- Decreases the ability of de-differentiation and plasticity in Th17 cells - Induces expression of the characteristic Th17 cytokines - Essential for survival and expansion
Neutralizing Antibodies	Human Cat. No.	Mouse Cat. No.	Role
Anti-IFN gamma	16-7318	16-7312	Inhibits Th17 cell differentiation (Anti IL-27 is not required with purified naïve T cell culture)
Anti-IL-2	16-7027	16-7022	
Anti-IL-4	16-7048	16-7041	
Anti-IL-27p28	16-7285	16-7285	

Th22 Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
IL-6	14-8069 34-8069	14-8061 34-8061	Necessary for the maintenance of Th22 cells <i>in vitro</i>
TNF alpha	14-8329 34-8329	14-8321 34-8321	- Necessary for induction and maintenance of Th22 cells <i>in vitro</i> - Associated with both pro and anti-inflammatory activities of T cells, including Th22 cells

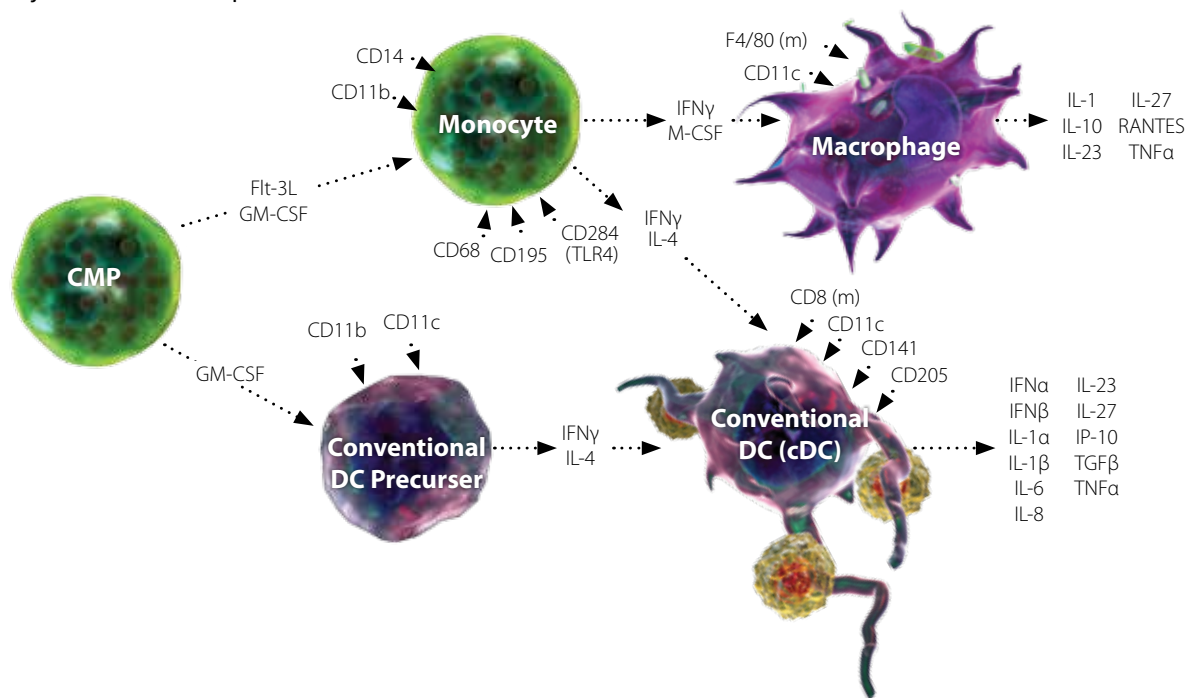
Tfh Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
IL-6	14-8069 34-8069	14-8061 34-8061	Essential for inducing Tfh cell differentiation
IL-21	14-8219 34-8219	14-8211 34-8211	- Essential for inducing Tfh cell differentiation - Acts as an autocrine growth factor to maintain Tfh survival
CXCL13	--	--	- Critical for recruiting activated CD4+ T cells to the follicles of secondary lymphoid tissues B cell - Interaction with B cells within the follicle is required for complete Tfh cell development

Treg Differentiation Profile			
Recombinant Protein	Human Cat. No.	Mouse Cat. No.	Role
IL-2	14-8029 34-8029	14-8021 34-8021	Supports the development of Treg in the thymus and maintains peripheral homeostasis by signaling through CD122 (IL-2R $\beta$ )
TGF beta 1	14-8348 34-8348	14-8342 34-8342	Induces Foxp3 expression. Necessary for conversion of Th0 cells to Treg cells in the presence of antigen stimulation at the appropriate level

# Monocyte, Macrophage and Dendritic Cell Differentiation

Monocytes, macrophages and dendritic cells (DCs) are innate immune cells that arise from myeloid precursors and act as professional phagocytes. Macrophages and DCs are termed Antigen Presenting Cells (APCs) because of their ability to process and present protein-derived antigens to T cells in the context of Major Histocompatibility Complex (MHC) molecules. Mainly formed in the bone marrow, these cells circulate in the blood and can migrate into tissues. The migration of monocytes into tissues causes cell differentiation into tissue-resident macrophages such as brain microglia, bone osteoclasts, epidermal Langerhans cells and liver kupffer cells.

## Myeloid Cell Development

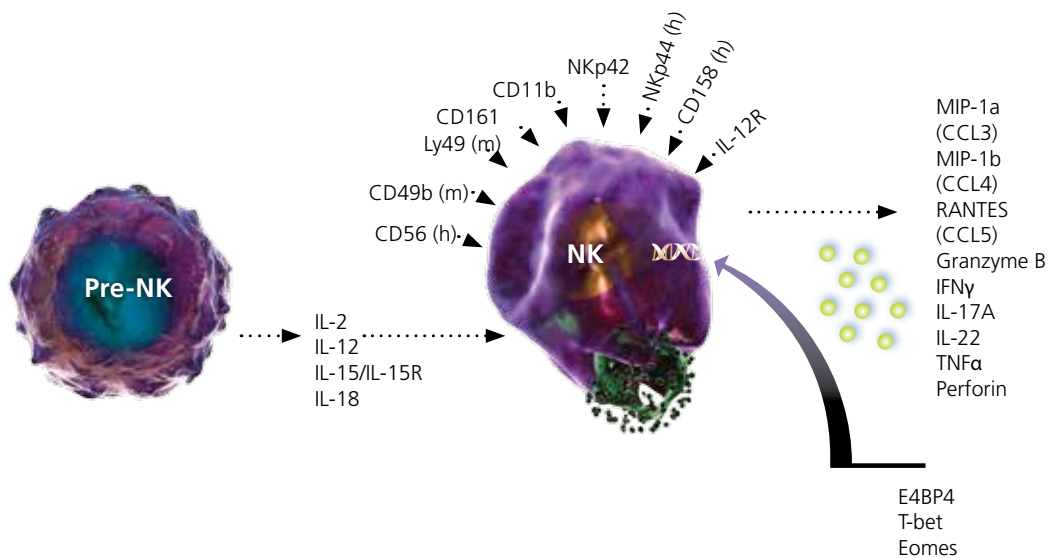


Myeloid Cell Differentiation Profiles			
Cell Type	Recombinant Protein	Human Cat. No.	Mouse Cat. No.
Monocyte	Flt3 Ligand	14-8513 34-8513	14-8001 34-8001
	GM-CSF	14-8339 34-8339	14-8331 34-8331
Macrophage	M-CSF	14-8789 34-8789	14-8983 34-8983
	IFN gamma	14-8319 34-8319	14-8311 34-8311
Dendritic Cell	GM-CSF	14-8339 34-8339	14-8331 34-8331
	IL-4	14-8049 34-8049	14-8041 34-8041
	IFN gamma	14-8319 34-8319	14-8311 34-8311

# Natural Killer Cell Differentiation

Natural Killer (NK) cells are lymphoid cells poised and ready to assist in the destruction of virally infected cells and tumor cells. Unlike most lymphoid cells, NK cells are part of the innate immune system and mediate their effect in an antigen independent manner that most often does not give rise to immunological memory or protective immunity. NK cells become activated upon stimulation by the cytokines IL-2, IL-15, IL-15 Receptor  $\alpha$  in complex with IL-15, IL-18 and IL-12 to produce a large variety of cytokines and chemokines.

## Natural Killer Cell Maturation



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 $\gamma$ 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



# Product Guide

## Functional Grade Antibodies by Cell Type

Induce, Neutralize or Block Cell Signaling or Protein-Protein Interactions

B Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD19	HIB19	16-0199	MB19-1 1D3	16-0191 16-0193
CD20	--	--	AISB12	16-0201
CD21/35	--	--	eBio4E3	16-0212
CD24	--	--	M1/69	16-0242
CD27	LF.7F9	16-0271	LF.7F9	16-0271
	LG.3A10	16-0272	LG.3A10	16-0272
CD32	6C4 (CD32)	16-0329	93	16-0161
CD34	4H11	14-0349	RAM34	16-0341
CD38	--	--	90	16-0381
CD40	5C3	16-0409	HM40-3	16-0402
CD45R (B220)	--	--	RA3-6B2	16-0452
CD48	156-4H9	16-0489	HM48-1	16-0481
CD49b (Integrin alpha 2)	--	--	HMa2	16-0491
CD49d (Integrin alpha 4)	9F10	16-0499	R1-2	16-0492
CD80 (B7-1)	2D10.4	16-0809	16-10A1	16-0801
CD86 (B7-2)	IT2.2	16-0869	PO3.1	16-0861
			GL1	16-0862
CD124	X2/45-12	16-1249	--	--
CD133	--	--	13A4	16-1331
CD137 (41BB)	--	--	17B5	16-1371
CD153	--	--	RM153	16-1531
CD154	24-31	16-1548	MR1	16-1541
CD180 (RP105)	MHR73-11	16-1809	RP/14	16-1801
CD184 (CXCR4)	12G5	16-9999	2B11	16-9991
CD252 (OX40 Ligand)	--	--	RM134L	16-5905
CD253 (TRAIL)	RIK-2	16-9927	N2B2	16-5951
CD267 (TACI)	11H3	16-9217	--	--
CD268 (BAFF-R)	8A7	16-9117	--	--
CD272 (BTLA)	MIH26	16-5979	6F7	16-5950
CD275 (B7-H2)	MIH12	16-5889	HK5.3	16-5985
CD279 (PD-1)	J116	16-9989	RMP1-30	16-9981
			RMP1-14	16-9982
			J43	16-9985
CD317 (BST2)	26F8	16-3179	eBio927	16-3172
IgD	--	--	10.4.22	16-5924
	--	--	Polyclonal	24-5093
IgM	--	--	Polyclonal	24-5092

General T Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD2	RPA-2.10	16-0029	RM2-5	16-0021
CD3	HIT3a	16-0039	145-2C11	16-0031
	UCHT1	16-0038	17A2	16-0032
	OKT3	16-0037	500A2	16-0033
	SK7	16-0038		
CD4	RPA-T4	16-0049	GK1.5	16-0041
	OKT4	16-0048	RM4-5	16-0042
			RM4-4	16-0043
CD27	LG.3A10	16-0272	LG.7F9	16-0271
			LG.3A10	16-0272
CD28	CD28.2	16-0289	37.51	16-0281
CD30	--	--	mCD30.1	16-0301
CD40	5C3	16-0409	HM40-3	16-0402
CD43	--	--	eBioR2/60	16-0431
CD57	TB01	16-0577	--	--
CD62L (L-Selectin)	DREG-56	16-0629	MEL-14	16-0621
CD81	1D6-CD81	16-0819	Eat-2	16-0811
CD90 (Thy-1)	--	--	G7	16-0901
CD90.2 (Thy-1.2)	--	--	30-H12	16-0903
CD100	--	--	BMA12	16-1001
CD103 (Integrin alpha E)	--	--	2E7	16-1031
CD122	--	--	TM-b1	16-1222
CD127	--	--	A7R34	16-1271
CD130	AN-HH1	16-1307	--	--
CD134 (OX-40)	--	--	OX-86	16-1340
CD150	A12 (7D4)	16-1509	9D1	16-1501
CD184 (CXCR4)	12G5	16-9999	2B11	16-9991
CD197 (CCR7)	--	--	4B12	16-1971
CD226	--	--	10ES	16-2261
CD279 (PD-1)	J116	16-9989	RMP1-30	16-9981
			RMP1-14	16-9982
			J43	16-9985
CD305 (LAIR-1)	--	--	113	16-3051
CD317 (BST2, PDCA-1)	26F8	16-3179	eBio927	16-3172
B7-H4	H74	16-5949	188	16-5972
TCR alpha beta	R73	16-5960	--	--
TCR DO11.10	--	--	KJ1-26	14-5808
TCR gamma delta	B1.1	16-9959	UC7-13D5	16-9951
TCR beta	--	--	H57-597	16-5961
TCR V beta 5.1	LC4	16-5832	--	--

Th1 Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD178 (FasL)	NOK-1	16-9919	MFL3 MFL4	16-5911 16-5912
CD254 (RANK Ligand)	--	--	IK22/5	16-5952
CD278 (ICOS)	ISA-3	16-9948	C398.4A 7E.17G9	16-9949 16-9942
Tim-3	--	--	8B.2C12	16-5871
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-2	AB12-3G4	16-7027	JES6-5H4 JES6-1A12	16-7021 16-7022
IL-10	JES3-9D7	16-7108	JES5-16E3 JES5-2A5	16-7101 16-7102
IFN gamma	MD-1 NIB42	16-7317 16-7318	XMG1.2 R4-6A2 AN-18	16-7311 16-7312 16-7313
TNF alpha	MAb1	16-7348	MP6-XT22 MP6-XT3 TN3-19 1F3F3D4	16-7321 16-7322 16-7323 16-7325
TNF beta	359-238-8	14-7329	--	--

Th2 Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD124	X2/45-12	16-1249	--	--
ST2 (IL-33R)	--	--	RMST2-33	16-9333
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-4	MP4-25D2	16-7048	11B11	16-7041
IL-5	JES1-5A10	16-7059	TRFK5	16-7052
IL-6	MQ2-13A5	16-7069	MP5-20F3	16-7061
IL-10	JES3-9D7	16-7108	JES5-16E3 JES5-2A5	16-7101 16-7102
IL-13	PVM13-1	16-7139	eBio13A eBio1316H	16-7133 16-7135

Th9 Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD124	X2/45-12	16-1249	--	--
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-9	--	--	D9302C12	16-7093
IL-10	JES3-9D7	16-7108	JES5-16E3 JES5-2A5	16-7101 16-7102

Th17 Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD161	--	--	PK136	16-5941
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-17A	eBio64CAP17	16-7178	eBioMM17F3	16-7173
IL-17F	SHLR17	16-7169	RN17	16-7473
IL-21			FFA21	16-7211
IL-22	IL22JOP	16-7222	IL22JOP	16-7222
GM-CSF	--	--	MP1-22E9	16-7331
TNF alpha	MAB1	16-7348	MP6-XT22	16-7321
			MP6-XT3	16-7322
			TN3-19	16-7323
			1F3F3D4	16-7325
Th22 Cell-Related Functional Grade Antibodies				
Secreted Cytokines	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
IL-22	IL22JOP	16-7222	IL22JOP	16-7222
TNF alpha	MAB1	16-7348	MP6-XT22	16-7321
			MP6-XT3	16-7322
			TN3-19	16-7323
			1F3F3D4	16-7325
T Follicular Helper Cells (TFh) Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD126	--	--	D7715A7	16-1261
CD185 (CXCR5)	--	--	MU5UBEE	14-9185
CD252 (OX40L)	--	--	RM134L	16-5905
CD272 (BTLA)	MIH26	16-5979	6F7	16-5950
CD278 (ICOS)	ISA-3	16-9948	7E.17G9	16-9942
			C398.4A	16-9949
CD279 (PD-1)	J116	16-9989	RMP1-30	16-9981
			RMP1-14	16-9982
			J43	16-9985
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-4	MP4-25D2	16-7048	11B11	16-7041
IL-10	JES3-9D7	16-7108	JES5-16E3	16-7101
			JES5-2A5	16-7102
IL-12	C8.6	16-7129	C17.8	16-7123
IL-21	--	--	FFA21	16-7211
Treg Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD25	--	--	PC61.5	16-0251
CD39	--	--	24DMS1	16-0391
CD73	--	--	TY/11.8	16-0731
CD127	--	--	A7R34	16-1271
CD152 (CTLA-4)	14D3	16-1529	9H10	16-1521
CD183 (CXCR3)	--	--	CXCR3-173	16-1831
CD357 (GITR)	--	--	DTA-1	16-5874
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-10	JES3-9D7	16-7108	JES5-16E3	16-7101
			JES5-2A5	16-7102
TGF beta	1D11.16.8	16-9243	1D11.16.8	16-9243

CD8+ T Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD8a	HIT8a OKT8	16-0089 16-0086	53-6.7	16-0081
IL-15/IL-15R	--	--	GRW15PLZ	16-8156
IL-15	ct2nu	16-0157	AIO.3	16-7154

Natural Killer (NK) Cell-Related Functional Grade Antibodies				
Cell Markers	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
Asialo GM1	--	--	Polyclonal	16-6507
CD2	RPA-2.10	16-0029	RM2-5	16-0021
CD8a	HIT8a OKT8	16-0089 16-0086	53-6.7	16-0081
CD11b	CBRM1/5	16-0113	M1/70	16-0112
CD16	B73.1 CB16	16-0167 16-0168	93	16-0161
CD27	LG.3A10	16-0272	LG.7F9 LG.3A10	16-0271 16-0272
CD32	6C4 (CD32)	16-0329	93	16-0161
CD34	4H11	14-0349	RAM34	16-0341
CD43	--	--	eBioR2/60	16-0431
CD49b (Integrin alpha 2)	--	--	HMa2	16-0491
CD49d (Integrin alpha 4)	9F10	16-0499	R1-2	16-0492
CD57	TB01	16-0577	--	--
CD62L (L-Selectin)	DREG-56	16-0629	MEL-14	16-0621
CD85j (ILT2)	HP-F1	16-5129	--	--
CD96	--	--	6A6	16-0960
CD122	--	--	TM-b1	16-1222
CD160	--	--	CNX46-3	16-1601
CD161 (NK1.1)	--	--	PK136	16-5941
CD178 (FasL)	NOK-1	16-9919	MFL3 MFL4	16-5911 16-5912
CD223 (Lag-3)	--	--	C9B7W	16-2231
CD226 (DNAM-1)	--	--	eBio10 <sup>5</sup>	16-2261
CD244	PP35 C1.7	16-2449 16-5838	--	--
CD253 (TRIAL)	RIK-2	16-9927	N2B2	16-5951
CD305 (LAIR-1)	--	--	113	16-3051
CD335 (NKp46)	9E2	16-3359	29A1.4	16-3351
CD336 (NKp44)	44.189	16-3369	--	--
GalCer:CD1d Complex	--	--	L363	16-2019
Ly-49C/I/F/H	--	--	14B11	14-5991
Ly-49H	--	--	3D10	14-5886
Ly-49I	--	--	YLI-90	16-5895
Ly-108	--	--	13G3-19D	16-1508
Secreted Cytokines	Clone	Cat. No.	Clone	Cat. No.
IL-15/IL-15R	--	--	GRW15PLZ	16-8156
IL-15	ct2nu	16-0157	AIO.3	16-7154
IL-17A	eBio64CAP17	16-7178	eBioMM17F3	16-7173
IL-22	IL22JOP	16-7222	IL22JOP	16-7222
TNF alpha	MAb1	16-7348	MP6-XT22	16-7321
			MP6-XT3	16-7322
			TN3-19	16-7323
			1F3F3D4	16-7325

Monocyte, Macrophage and Dendritic Cell-Related Functional Grade Antibodies												
Monocytes, Macrophages and Dendritic Cells	Human		Mouse		Conventional DC	Plasmacytoid DC	Macrophage	Monocyte	Neutrophil	Eosinophil	Mast	Basophil
	Clone	Cat. No.	Clone	Cat. No.								
CD1d			1B1	16-0011	■							
CD2	RPA-2.10	16-0029	RM2-5	16-0021	■							
CD4	RPA-T4	16-0049	GK1.5	16-0041								
	OKT4	16-0048	RM4-5 RM4-4	16-0042 16-0043	■	■						
CD11a	HI111	16-0119	M17/4	16-0111			■					■
CD11b	CBRM1/5	16-0113	M1/70	16-0112		■		■		■	■	■
CD11c			N418	16-0114	■	■	■	■				
CD13	WM-15	16-0138			■			■				■
CD14	61D3	16-0149	Sa2-8	16-0141	■		■	■				
CD16	B73.1	16-0167	93	16-0161			■					■
	CB16	16-0168	1D3	16-0193								
CD32	6C4 (CD32)	16-0329	93	16-0161			■	■	■	■	■	■
CD38			90	16-0381				■				■
CD40	5C3	16-0409	HM40-3	16-0402			■	■				
CD49e			HMa5-1	16-0493				■				
CD58	TS2/9	16-0578					■					
CD64	10.1	16-0649			■			■		■	■	
CD66a			CC1	14-0661					■			
CD80 (B7-1)	2D10.4	16-0809	16-10A1	16-0801	■							
CD85d (ILT4)	27D6	16-5148						■				
	42D1	16-5149										
CD85h (ILT1)	135.4	16-5119						■				
CD85j (ILT2)	HP-F1	16-5129			■			■				
CD85k (ILT3)	ZM4.1	16-5139			■	■		■				
CD86 (B7-2)	IT2.2	16-0869	PO3.1	16-0861	■			■				
			GL1	16-0862								
CD95 (Fas, Apo-1)	EOS9.1	16-0958						■				
CD105 (Endoglin)	SN6	16-1057			■							
CD115 (c-fms)			AFS98	16-1152				■				
CD117 (c-kit)			ACK2	16-1172							■	
CD126	B-R6	BMS135	D7715A7	16-1261							■	
CD142			HTF-1	16-1429				■				
CD147			RL73	16-1471				■				
CD154 (CD40L)	24-31	16-1548	MR1	16-1541								■
CD162	PL-1	BMS164						■				
CD172a			P84	16-1721	■			■				
CD180 (RP105)	MHR73-11	16-1809	RP/14	16-1801				■				
CD184 (CXCR4)	12G5	16-9999	2B11	16-9991				■				
CD197 (CCR7)			4B12	16-1971	■							
CD244	PP35 C1.7	16-2449 16-5838								■	■	
CD281 (TLR1)	GD2.F4	16-9911						■	■			■
CD282 (TLR2)	TL2.1	16-9922	6C2	16-9021	■	■	■	■	■			■
	T2.5	16-9024	T2.5	16-9024								
CD283 (TLR3)	TLR3.7	16-9039			■							
CD284 (TLR4)	HTA125	16-9917	MTS510	16-9924	■	■	■	■	■			■
	HT52	16-9879										
CD300e (IREM-2)			UP-H2	16-3007	■			■				
CD305 (LAIR-1)			113	16-3051				■				
CD317 (BST2, PDCA-1)	26F8	16-3179	eBio927	16-3172				■				
CD357 (GITR)			DTA-1	16-5874			■					
FcεR1			eBioMAR-1	16-5898						■	■	■
IFNAR1			MAR1-5A3	16-5945	■							
Ly-6G (Gr-1)			RB6-8C5	16-5931				■	■			

Megakaryocyte and Erythrocyte Cell-Related Functional Grade Antibodies				
Megakaryocyte	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD31 (PECAM-1)	WM-59	16-0319	390	16-0311
CD41	HIP8	16-0419	MWReg30	16-0411
CD42b	HIP1	16-0429	--	--
CD51 (Integrin alpha V)	--	--	RMV-7	16-0512
CD51/CD61 (Integrin alpha v beta 3)	23C6	16-0519	--	--
CD54 (ICAM-1)	HA58	16-0549	YN1/1.7.4 KAT-1	16-0541 16-0542
CD61 (Integrin beta 3)	VI-PL2	14-0619	2C9.G3	16-0611
CD62p (P-Selectin)	AK-4	14-0628	Psel.KO2.12	16-0622
CD102 (ICAM-2)	CBRIC2/2	14-1029	--	--
CD147	--	--	RL73	16-1471
CD151	50-6	16-1519	--	--
CD154 (CD40L)	24-31	16-1548	MR1	16-1541
CD226 (DNAM-1)	eBio10 <sup>5</sup>	16-2261	--	--
Erythrocyte	Human		Mouse	
	Clone	Cat. No.	Clone	Cat. No.
CD35	--	--	eBio4E3	16-0212
CD44	IM7	16-0441	IM7	16-0441
TER-119 (Ly-76)	--	--	TER-119	16-5921

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