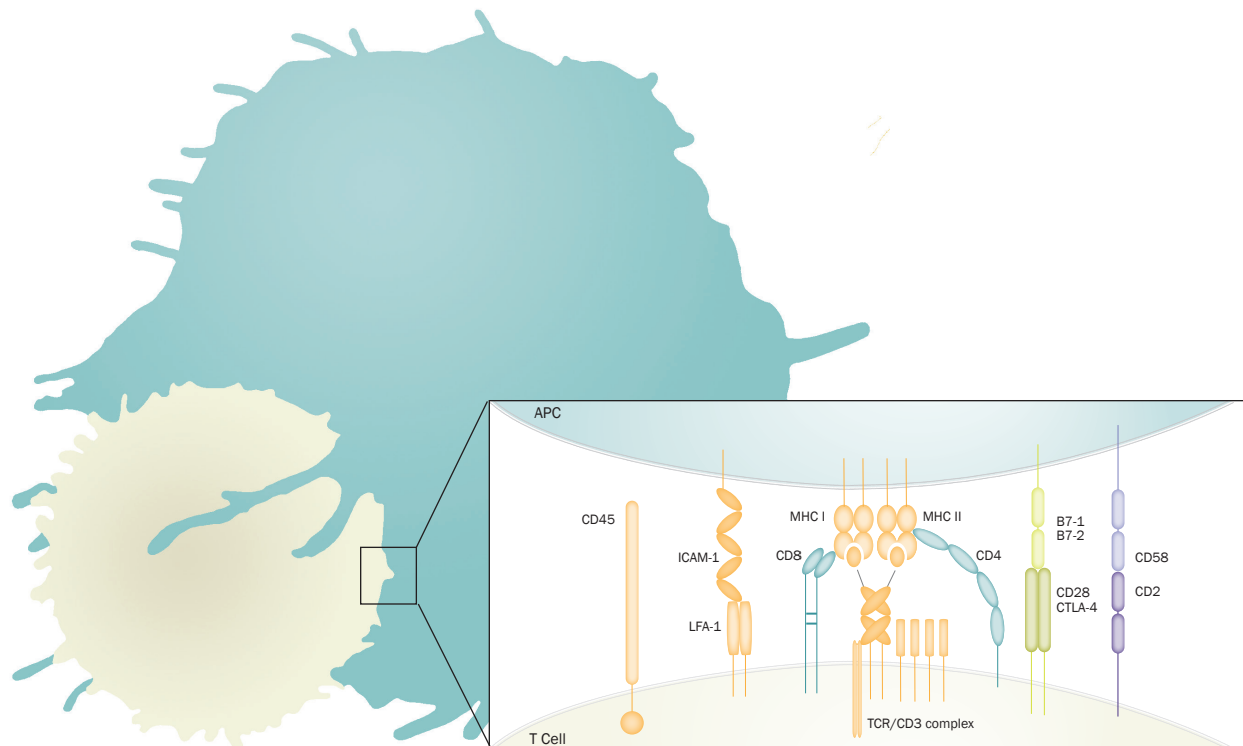


T Cells:

Co-signaling Molecules



Multiple Co-stimulatory and Co-inhibitory Interactions Regulate T Cell Responses

T cell activation occurs through a two-step process initiated by antigen recognition by the T cell receptor (TCR) followed by a second signal mediated by co-signaling molecules expressed on the surface of the antigen-presenting cell.¹ This classical two-signal model has evolved into a complex regulatory system in which integration of the signals derived from the co-stimulatory and co-inhibitory receptors regulates the outcome of the T cell response, including the enhancement or suppression of T cell proliferation, differentiation, and/or cytokine secretion.^{2,3} Most co-signaling molecules (stimulatory or inhibitory) belong to the Immunoglobulin Superfamily (IgSF) or the Tumour Necrosis Factor Superfamily (TNFSF) and can be further classified into specific subfamilies based on their primary amino acid sequence, protein structure, and function. Two inhibitory receptors belonging to the IgSF, Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and Programmed cell death-1 (PD-1) have been actively studied in the context of clinical cancer immunotherapy. Multiple studies have shown that neutralization of CTLA-4 or PD-1 with specific antibodies enhances the potential of an anti-tumor immune response.⁴ These findings suggest new areas of exploration for human cancer immunotherapy.

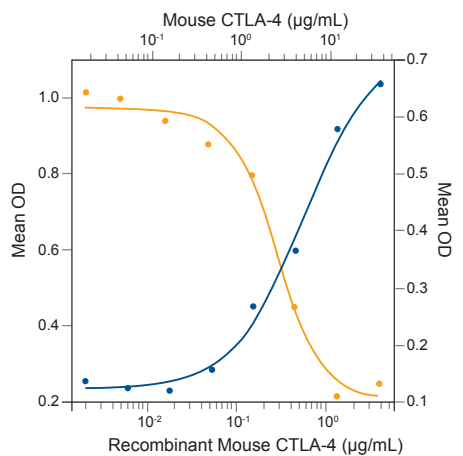
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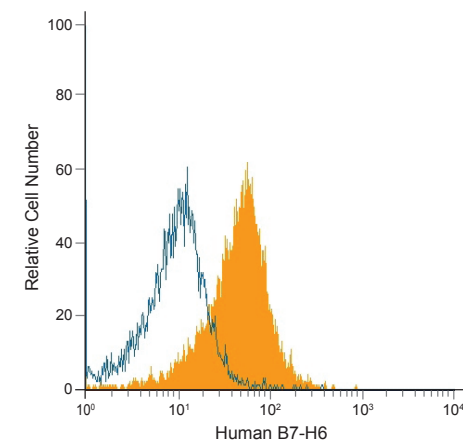
T Cell Co-stimulatory Molecules

Ig Superfamily			
Molecule	Recombinant Proteins	Antibodies	ELISAs
CD28 subfamily			
CD28	H M	H (FA, FC, ICC, WB) M (FC, WB)	
ICOS	H M	H (FC, IHC, WB) M (B/N, FC, WB)	H
B7 subfamily			
B7-1/CD80	H M R	H (B/N, E, FC, IHC, WB) M (B/N, E, FC, ICC, WB) R (ICC, WB)	H M
B7-2/CD86*	H M R	H (B/N, FC, IHC, WB) M (B/N, FC, WB) R (B/N, E, FC, IHC, WB)	R
B7-H2	H M	H (B/N, FC, WB) M (FC, WB, ICC)	
CD226 subfamily			
DNAM-1/CD226	H M	H (B/N, FC, WB) M (FC, WB)	
CD96	M	H (FC, WB) M (FC)	
TIM subfamily			
TIM-1/KIM-1/HAVCR	H M R	H (E, FC, IHC, WB) M (E, FC, WB) R (E, IHC, WB)	H M R
CD2/SLAM subfamily			
CD2		H (FC, ICC, WB) M (FC, WB)	
SLAM/CD150	H M	H (FC, WB) M (E, FC, WB)	H M
CD58/LFA-3	H	H (B/N, FC, IHC, WB)	
CD48/SLAMF2	H M	H (FC, ICC, WB) M (E, FC, ICC, WB)	M
CD229/SLAMF3	H M	H (FC, WB) M (WB)	
2B4/CD244/SLAMF4*	H M	H (E, FA, FC, IHC, WB) M (FC, IHC, WB)	H
CD84/SLAMF5	H M	H (FC, WB)	
Orphans			
NKp30/NCR3	H	H (FA, FC, WB)	
* molecules having co-stimulatory and inhibitory effects			



CTLA-4 Inhibition of B7-1/CD80-induced IL-2 Secretion and Neutralization by Mouse CTLA-4 Antibody. Recombinant Mouse CTLA-4 Fc Chimera (Catalog # 434-CT) inhibits Recombinant Human B7-1/CD80 Fc Chimera (Catalog # 140-B1) induced IL-2 secretion in the Jurkat human acute T cell leukemia cell line in a dose-dependent manner (orange line), as measured by the Human IL-2 Quantikine ELISA Kit (Catalog # D2050). Inhibition of Recombinant Human B7-1/CD80 Fc Chimera (3 µg/mL) activity elicited by Recombinant Mouse CTLA-4 Fc Chimera (1 µg/mL) is neutralized (blue line) by increasing concentrations of Mouse CTLA-4 Monoclonal Antibody (Catalog # MAB434). The ND₅₀ is typically 2.5-10 µg/mL in the presence of PHA (10 µg/mL).

TNF Superfamily			
Molecule	Recombinant Proteins	Antibodies	ELISAs
Type-V Subfamily			
4-1BB/TNFRSF9/CD137	H M R	H (E, FA, FC, ICC, WB) M (B/N, E, FA, FC, WB)	H M
4-1BB Ligand/TNFSF9	H M	H (FC, WB) M (FC, WB)	
OX40/TNFRSF4	H M	H (FC, WB) M (FA, FC, WB)	
OX40 Ligand/TNFSF4	H M	H (B/N, FC, ICC, WB) M (B/N, E, FC, ICC, WB)	M
CD27/TNFRSF7	H M R	H (B/N, FC, IHC, WB) M (E, FC, WB)	M
CD27 Ligand/TNFSF7	M	H (FC, ICC, WB) M (B/N, E, FC, WB)	M
GITR/TNFRSF18	H M	H (B/N, E, FC, IHC, WB) M (E, FC, WB)	H M
GITR Ligand/TNFSF18	H M	H (B/N, E, FC, WB) M (B/N, E, WB)	H M
CD30/TNFRSF8	H M	H (FA, FC, ICC, WB) M (E, FA, ICC, WB)	M
CD30 Ligand/TNFSF8	H M	H (B/N, FC, WB) M (B/N, E, FC, WB)	M
Type-L Subfamily			
HVEM/TNFRSF14	H M	H (E, FC, IHC, WB) M (WB)	H
LIGHT/TNFSF14	H M	H (B/N, E, FC, WB) M (FC, WB)	H
DR3/TNFRSF25	H M	H (FC, WB) M (IHC, WB)	H
CD40/TNFRSF5	H M	H (B/N, FA, FC, IHC, WB) M (E, FA, FC, ICC, IF, IP, WB)	H M
CD40 Ligand/TNFSF5	H M	H (B/N, FC, IHC, WB) M (B/N, E, FC, ICC, WB)	H M
Others			
CD155/PVR*	H M	H (FC, ICC, WB) M (FC, ICC, WB)	
Nectin-2/CD112*	H M	H (FC, WB) M (FC, WB)	
B7-H6	H	H (FC)	
TL1A/TNFSF15	H M	H (WB) M (B/N, WB)	
* molecules having co-stimulatory and inhibitory effects			



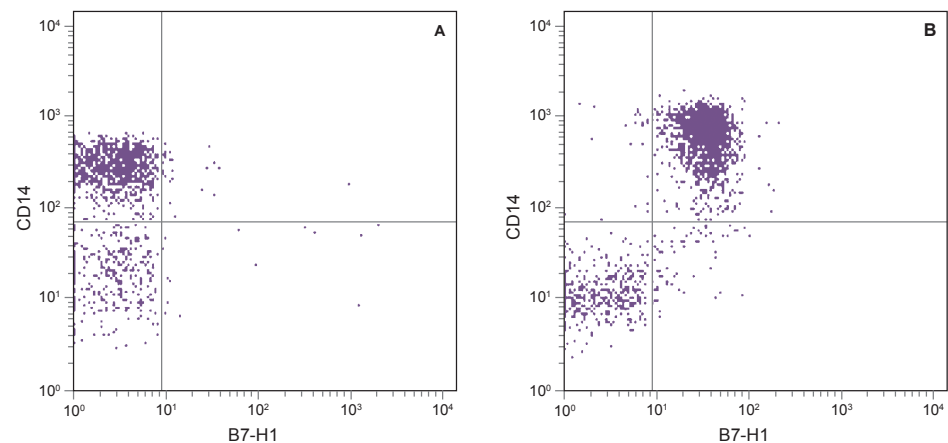
Detection of B7-H6 in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Mouse Anti-Human B7-H6 APC-conjugated Monoclonal Antibody (Catalog # FAB7144A, filled histogram) or isotype control antibody (Catalog # IC002A, open histogram).

Applications Key: B/N Blocking/Neutralization E ELISA
FA Functional Assay FC Flow Cytometry ICC Immunocytochemistry
IHC Immunohistochemistry WB Western blot
Species Key: H Human M Mouse R Rat CM Cynomolgus Macaque

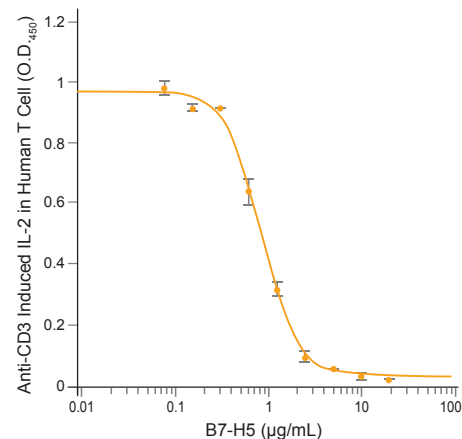
T Cell Co-stimulatory Molecules

Ig Superfamily			
Molecule	Recombinant Proteins	Antibodies	ELISAs
CD28 subfamily			
CTLA-4	H M	H (FC, ICC, WB) M (B/N, E, FC, WB)	M
PD-1	H M	H (B/N, E, FC, IHC, WB) M (FC, IHC, WB)	H M
BTLA	M	H (FC, WB) M (FC, WB)	
B7 subfamily			
B7-H1/PD-L1	H M	H (B/N, FC, IHC, WB) M (FC, IHC, WB)	H
B7-H3	H M	H (FC, IHC, WB) M (B/N, WB)	H
B7-H4	H M	M (FC, WB)	
Gi24/VISTA/B7-H5	H M	H (FC, ICC, WB) M (FC, ICC, WB)	
B7-H7	H		
PD-L2/B7-DC	H M	H (B/N, FC, IHC, WB) M (B/N, FC, IHC, WB)	H
CD226 subfamily			
TIGIT	H M	H (FC) M (FC)	
TIM subfamily			
TIM-2	M	M (WB)	
TIM-3	H M CM	H (FC, WB) M (FC, WB)	H
Orphans			
LAG-3	H M	H (E, FC, WB) M (FC, WB)	H
LAIR1	H M	H (FC, WB)	
CD160	H M	H (FC, WB) M (FC, ICC, WB)	
Others			
Galectin-9	H M	H (ICC, WB) M (FC, ICC, IHC, WB)	H

Applications Key: B/N Blocking/Neutralization E ELISA FA Functional Assay FC Flow Cytometry ICC Immunocytochemistry IHC Immunohistochemistry WB Western blot
Species Key: H Human M Mouse R Rat

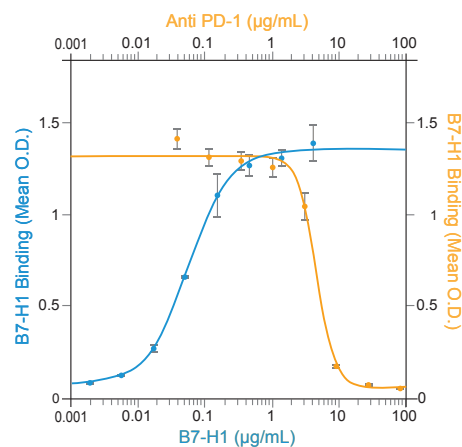


Detection of B7-H1 by Flow Cytometry. Human peripheral blood mononuclear cells A) resting, or B) treated with lipopolysaccharide, were stained with an APC-conjugated Mouse Anti-Human B7-H1 Monoclonal Antibody (Catalog # FAB1561A) and a Fluorescein-conjugated Mouse Anti-Human CD14 Monoclonal Antibody (Catalog # FAB3832F). Quadrant markers were set based upon staining with an APC-conjugated Mouse IgG₁ Isotype Control (Catalog # IC002A).



Gi24/VISTA/B7-H5 Inhibits Anti-CD3-Induced IL-2

Secretion in Human T Lymphocytes. Human T lymphocytes were treated with Mouse Anti-Human CD3 ϵ Monoclonal Antibody (0.5 µg/mL, Catalog # MAB100;) and increasing concentrations of Recombinant Mouse Gi24/VISTA/B7-H5 Fc Chimera (Catalog # 7005-B7). IL-2 secretion was measured using the Human IL-2 Quantikine ELISA Kit (Catalog # D2050).



Detection of PD-1 binding to B7-H1 and antibody-mediated neutralization. Increasing concentrations of Recombinant Human B7-H1 (Catalog # 156-B7) were added to 1 µg/mL of immobilized Recombinant Human PD-1 Fc Chimera (Catalog # 1086-PD). PD-1-bound B7-H1 was detected by the addition of a biotin-conjugated anti-B7-H1 antibody (Catalog # BAF156) and HRP-conjugated streptavidin (Catalog # DY998) (blue line). Increasing concentrations of Goat Anti-Human PD-1 Affinity Purified Polyclonal Antibody (Catalog # AF1086) were added to 1 µg/mL of immobilized Recombinant Human PD-1 Fc Chimera. Antibody-mediated inhibition of PD-1 binding to Recombinant Human B7-H1 (500 ng/mL) was detected by the addition of a biotin-conjugated anti-B7-H1 antibody and HRP-conjugated streptavidin (orange line).



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