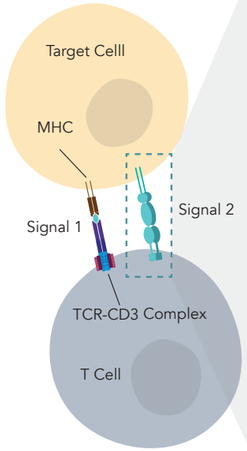
Tools for Investigating Immune Checkpoint Targets

bio-techne®

Multiple Co-Signaling Molecules

Regulate T Cell Activation

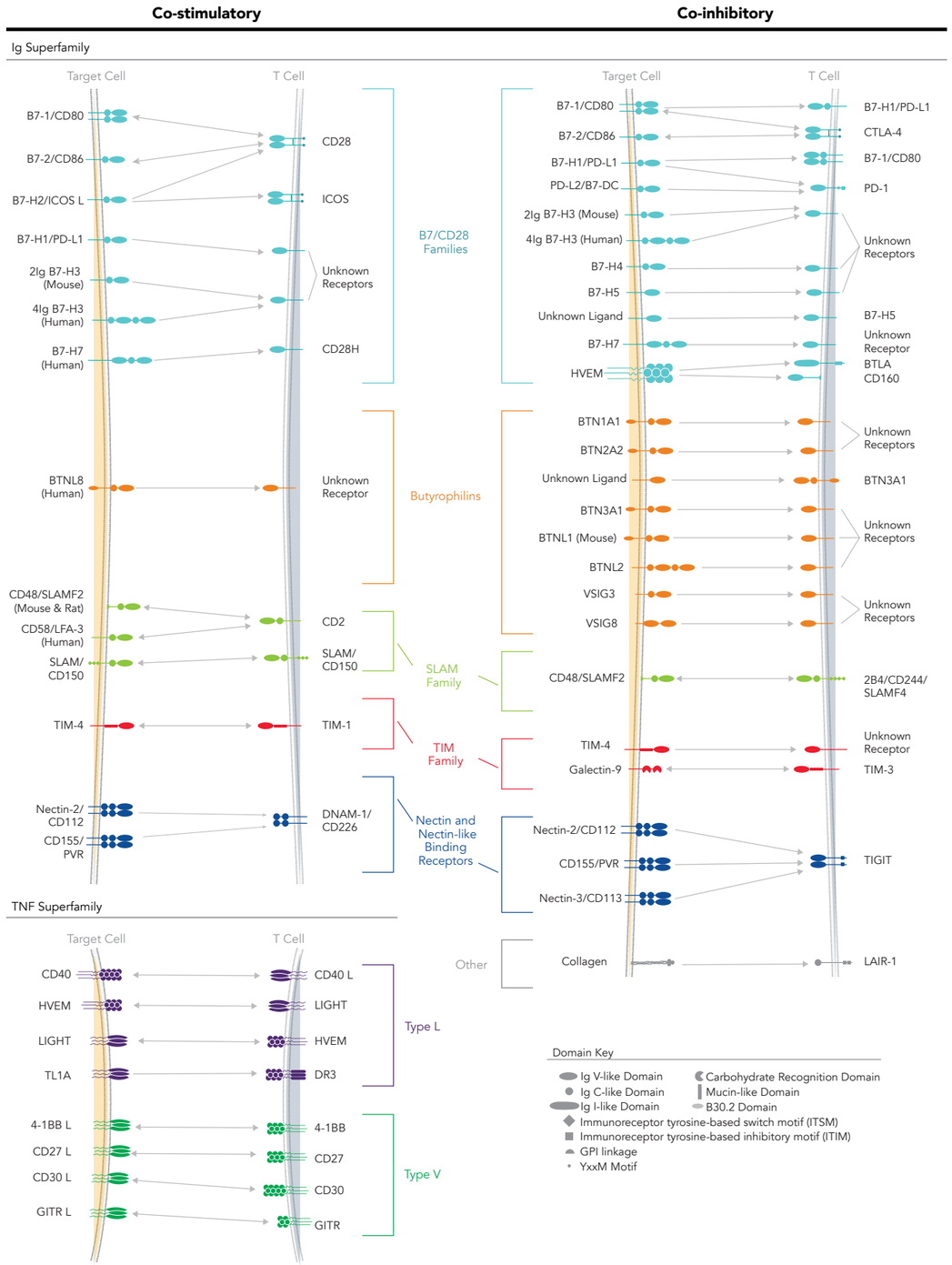


T cell activation requires two signals: 1) recognition of the antigenic peptide/major histocompatibility complex (MHC) by the T cell receptor (TCR) and 2) antigen-independent co-stimulation induced by interactions between co-signaling molecules expressed on target cells, such as antigen-presenting cells (APCs), and their T cell-expressed receptors. Engagement of the TCR in the absence of this second co-stimulatory signal typically results in T cell anergy or apoptosis. In addition, T cell activation can be negatively regulated by co-inhibitory molecules present on APCs. Therefore, integration of the signals transduced by co-stimulatory and co-inhibitory molecules following TCR engagement directs the outcome and magnitude of a T cell response including the enhancement or suppression of T cell proliferation, differentiation, and/or cytokine secretion. Most co-stimulatory and co-inhibitory molecules belong to either the Immunoglobulin (Ig) superfamily or Tumor Necrosis Factor (TNF) receptor superfamily and are further classified as members of the B7/CD28, butyrophilin, CD2/SLAM, TIM, or nectin- and nectin-like binding receptor subfamilies of the Ig superfamily or as members of the type L or type V subfamilies of the TNF receptor superfamily. Many of these proteins are being investigated as potential targets for cancer immunotherapy as multiple studies have shown that the T cell co-stimulatory/co-inhibitory system can be exploited to improve anti-tumor immunity.

B7 proteins are a family of co-signaling molecules that primarily interact with T cell-expressed immune receptors belonging to the CD28 family (CD28, CTLA-4, PD-1, ICOS, and BTLA). The B7 family consists of ten surface glycoproteins including B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-DC/PD-L2, B7-H2/ICOS L, B7-H3, B7-H4, B7-H5/VISTA, B7-H6, and B7-H7/HHLA2. As shown in the graphic on the next page, interactions between B7 and CD28 family members transduce both T cell co-stimulatory and co-inhibitory signals. Additionally, these interactions can have bidirectional effects (indicated by the two-headed arrows).

The butyrophilins are structurally closely related to the B7 family proteins and appear to have similar immunomodulatory functions. To date, thirteen human butyrophilin and butyrophilin-like proteins have been identified including BTN1A1, BTN2A1, BTN2A2, BTN2A3, BTN3A1, BTN3A2, BTN3A3, BTNL2, BTNL3, BTNL8, BTNL9, BTNL10, and SKINT-like (SKINTL). With the exception of BTNL2 and BTN3A2, butyrophilins are type I transmembrane proteins that contain one IgV-like and one IgC-like domain in their extracellular regions and a cytoplasmic B30.2 domain. Most butyrophilin proteins that have been characterized to date, including human BTN1A1, BTN2A2, BTN3A1, BTNL2, and mouse BTNL1, act through unidentified receptors to inhibit T cell proliferation and cytokine production. The exception is BTNL8 which enhances T cell proliferation and cytokine secretion. Further investigation is necessary to identify the butyrophilin receptors and determine the functions of the other butyrophilin family members.

Several members of the CD2/signaling lymphocyte activation molecule (SLAM) subfamily, T cell/transmembrane, immunoglobulin, and mucin (TIM) subfamily, and nectin- or nectin-like binding receptor subfamily of the Ig superfamily have also been shown to regulate T cell activation. Like the B7 family, members of these families can have co-stimulatory and/or co-inhibitory effects. In addition, multiple proteins belonging to the TNF receptor superfamily, including 4-1BB/TNFRSF9, CD27/TNFRSF7, CD30/TNFRSF8, CD40/TNFRSF5, DR3/TNFRSF25, GITR/TNFRSF18, HVEM/TNFRSF14, and OX40/TNFRSF4, have been shown to affect T cell co-signaling.



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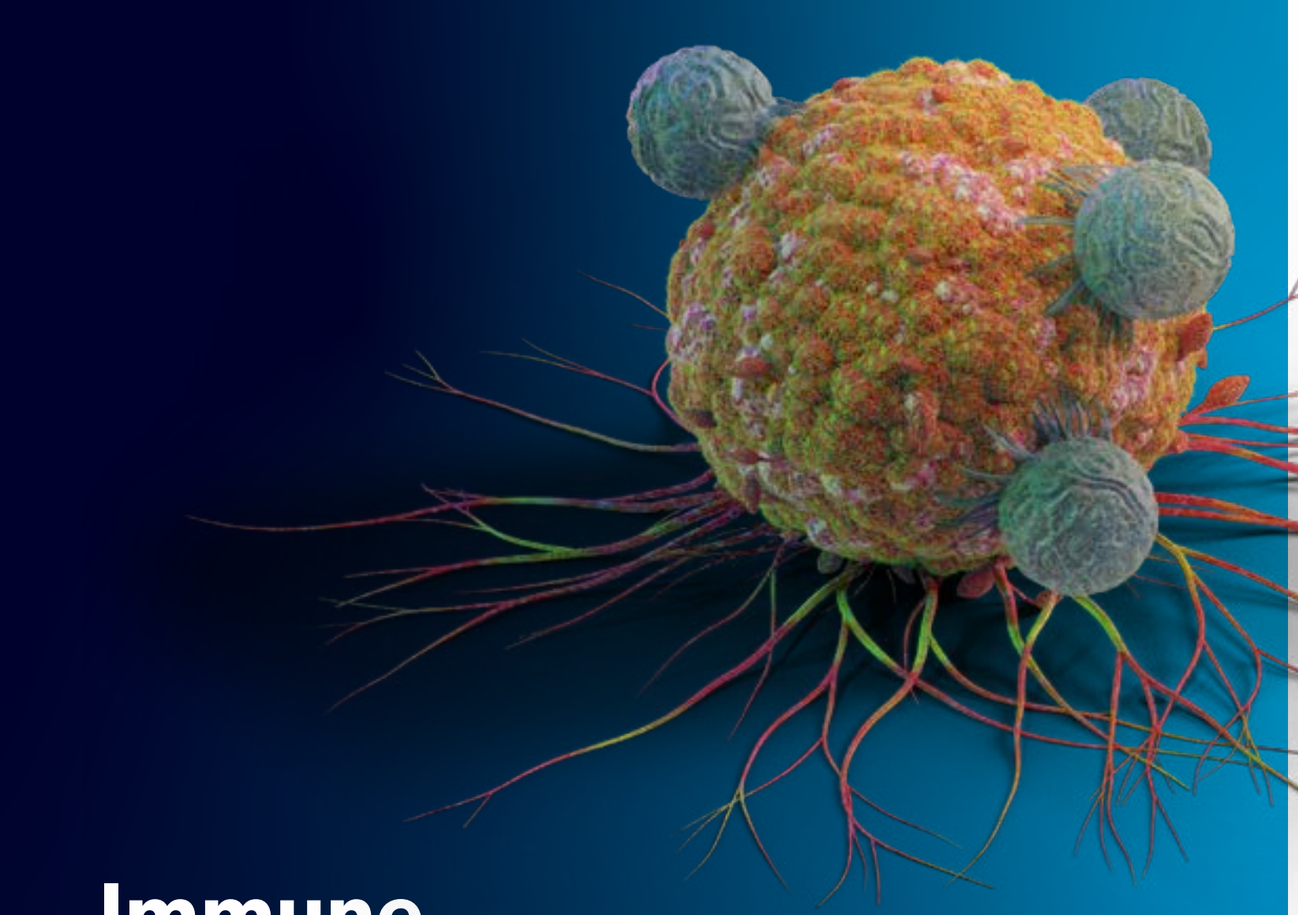


Immune Checkpoint Proteins

Bio-Techne offers a large selection of R&D Systems™ bioactive recombinant proteins for immune checkpoint research.

R&D Systems proteins are validated using a variety of binding and functional assays, and they are rigorously tested to ensure high lot-to-lot consistency. Our expertise in protein purification, scalable production capabilities, and reliable supply chain, enable us to provide the protein quantities needed for both basic research, as well as pharmaceutical development and manufacturing.

Key Benefits of R&D Systems Proteins

- **High Levels of Biological Activity:** Utilizing over 900 different bioassays, the biological activity of every protein we offer is tested in an appropriate bioassay to confirm that it meets our strict QC activity parameters.
- **Lot-to-Lot Consistency:** Minimal lot-to-lot variability is ensured by testing each new lot side-by-side with previous lots and with a master lot, so you don't have to worry whether results will be reproducible over time.
- **High Purity and Low Endotoxin Levels:** Our proteins are typically over 95% pure and have a guaranteed industry-leading endotoxin level of <0.1 EU/ug by the LAL method.
- **Supply Chain Reliability:** Our team has the experience and the capacity to ensure that we can provide you with a stable supply of the proteins you need for your research.
- **Bulk Proteins at Discounted Prices:** We have the capacity and the expertise to scale up the production of any protein and we offer economical pricing on bulk orders.
- **Proteins Beyond the Catalog:** We have tens of thousands of non-catalog proteins that may include different tags or come from different sources than the proteins listed on our website. Contact R&D Systems to see if we may already have the protein that you need.
- **Custom Protein Capabilities:** For specialized protein requests, you can always contact our Custom Protein Services team. We have the expertise and the systems necessary to develop the proteins required to advance your research.
- **Comprehensive Portfolio of Reagents for Your Entire Workflow:** Along with our proteins, Bio-Techne also offers a wide range of other products for immune checkpoint research, including Cultrex™ Basement Membrane Extracts, media, small molecules, antibodies, immunoassays, RNAscope™ ISH assays, and analytical instruments to automate different steps of your workflow.

Proteins for Investigating Immune Checkpoint Targets

Ig Superfamily

B7/CD28 Families

Molecule	Species	Source	Tag	Catalog #
B7-1/CD80	Human	NS0	Fc	10107-B1
	Human	CHO	Fc	10133-B1
	Human	HEK293	His	9050-B1
	Cynomolgus Monkey	HEK293	Fc	9695-B1
	Cynomolgus Monkey	HEK293	His	9244-B1
B7-2/CD86	Human	NS0	Fc, His	141-B2
	Human	CHO	Fc	7625-B2
	Human	HEK293	His	9090-B2
	Cynomolgus Monkey	HEK293	Fc	9798-B2
	Cynomolgus Monkey	HEK293	His	10300-B2
B7-H2/ ICOS L	Human	NS0	Fc	165-B7
	Human	HEK293	His	8206-B7
	Cynomolgus Monkey	HEK293	Fc	9900-B7
	Cynomolgus Monkey	HEK293	His	9327-B7
B7-H3	Human	NS0	Fc	1027-B3
	Human	NS0	His	1949-B3
	Cynomolgus Monkey	HEK293	His	1397-B3
B7-H3 (4Ig)	Human	NS0	Fc	2318-B3
	Human	HEK293	Fc	10472-B3
B7-H4	Human	NS0	His	6576-B7
	Human	HEK293	Fc	8870-B7
B7-H5/VISTA/PD-1H	Human	NS0	Fc	7126-B7
	Human	NS0	His	9057-B7
	Cynomolgus Monkey	HEK293	Fc	9408-B7
	Cynomolgus Monkey	HEK293	His	10473-B7
B7-H6	Human	NS0	Fc	7144-B7
	Human	HEK293	His	9309-B7
	Cynomolgus Monkey	HEK293	Fc	8984-B7
B7-H7/ HHLA2	Human	HEK293	Fc	8084-B7
	Human	HEK293	His	10475-B7
	Cynomolgus Monkey	HEK293	Fc	10108-B7
	Cynomolgus Monkey	HEK293	His	10109-B7

Ig Superfamily

BTLA	Human	NS0	Fc	8385-BT
	Human	HEK293	His	9235-BT
	Human	HEK293	Fc	9509-BT
	Cynomolgus Monkey	HEK293	Fc	10030-BT
CD28	Human/*Cynomolgus Monkey*	NS0	Fc	342-CD
CD28H/ TMIGD2	Human	HEK293	Fc	8316-TR
	Human	HEK293	His	9236-TR
CTLA-4	Human	Sf21 (baculovirus)	His	325-CT
	Human	CHO	Fc	7268-CT
	Cynomolgus Monkey	HEK293	Fc	9336-CT
ICOS	Human	NS0	Fc	169-CS
	Human	CHO	His	9865-CS
	Human	CHO	His	9945-CS
	Cynomolgus Monkey	CHO	Fc	9736-CS
PD-1	Human	NS0	Fc	1086-PD
	Human	HEK293	His	8986-PD
	Cynomolgus Monkey	HEK293	His	8509-PD
	Cynomolgus Monkey	HEK293	Fc	8578-PD
PD-L1/B7-H1	Human	NS0	Fc	156-B7
	Human	HEK293	His	9049-B7
	Cynomolgus Monkey	HEK293	His	10145-B7
	Cynomolgus Monkey	HEK293	Fc	9326-B7
PD-L2/B7-DC	Human	NS0	Fc	1224-PL
	Human	HEK293	His	9075-PL
	Cynomolgus Monkey	HEK293	Fc	9178-PL

Ig Superfamily

Butyrophilins

Molecule	Species	Source	Tag	Catalog #
BTN1A1/Butyrophilin	Human	NS0	His	8467-BT
BTN2A1	Human	HEK293	His	9058-BT
BTN2A2/ Butyrophilin 2A2	Human	HEK293	Fc	8918-BT
BTN3A1/ CD277	Human	HEK293	Fc	8539-BT
BTN3A2	Human	CHO	His	9514-BT
BTN3A3	Human	CHO	His	1350-BT
BTNL3	Human	CHO	Fc	9658-BT
BTNL8	Human	HEK293	Fc	9359-BT
BTNL9	Human	HEK293	Fc	9659-BT
BTNL10/ Butyrophilin-like 10	Human	HEK293	Fc	10014-BT

Ig Superfamily

Nectin and Nectin-like Binding Receptors

Molecule	Species	Source	Tag	Catalog #
CD96	Human	HEK293	Fc	9360-CD
	Cynomolgus Monkey	HEK293	His	10478-CD
CD155/PVR	Human	NS0	His	2530-CD
	Human	HEK293	Fc	9174-CD
	Cynomolgus Monkey	HEK293	Fc	10058-CD
CRTAM	Human	NS0	Fc	1695-CR
DNAM-1/ CD226	Human	NS0	Fc	666-DN
	Human	HEK293	His	9298-DN
	Cynomolgus Monkey	HEK293	Fc	9276-DN
Nectin-2/ CD112	Human	NS0	His	2229-N2
	Human	HEK293	Fc	9317-N2
	Cynomolgus Monkey	CHO	His	10617-N2
	Cynomolgus Monkey	CHO	Fc	10485-N2
Nectin-3/ CD113	Human	NS0	His	3064-N3
TIGIT	Human	CHO	His	9525-TG
	Human	HEK293	Fc	9464-TG
	Human	CHO	Fc	7898-TGB
	Cynomolgus Monkey	HEK293	Fc	9380-TG

Ig Superfamily

SLAM Family

Molecule	Species	Source	Tag	Catalog #
2B4/ CD244/ SLAMF4	Human	NS0	Fc	1039-2B
CD48/ SLAMF2	Human	NS0	His	3644-CD
	Human	HEK293	Fc	9310-CD
	Cynomolgus Monkey	CHO	Fc	10362-CD
	Cynomolgus Monkey	CHO	His	10399-CD
CD58/ LFA-3	Human	NS0	His	1689-CD
	Human	HEK293	Fc	10068-CD
SLAM/ CD150	Human	NS0	His	164-SL
	Cynomolgus Monkey	CHO	Fc	11170-SL
	Cynomolgus Monkey	CHO	His	10971-SL

Ig Superfamily

TIM Family Receptors & Ligands

Molecule	Species	Source	Tag	Catalog #
CEACAM-1/ CD66a	Human	NS0	His	2244-CM
Galectin-3	Human	E. coli	None	1154-GA
	Human	HEK293	None	8259-GA
Galectin-9	Human	HEK293	None	9064-GA
	Human	HEK293	None	2045-GA
TIM-1/KIM-1/HAVCR	Human	NS0	His	1750-TM
	Human	HEK293	Fc	9319-TM
	Human	HEK293	Fc	11273-TM
	Human	HEK293	His	11157-TM
	Cynomolgus Monkey	CHO	Fc	9676-TM
TIM-3	Human	NS0	Fc	2365-TM
	Human	CHO	His	10241-TI
	Cynomolgus Monkey	HEK293	Fc	7914-TM
TIM-4	Human	HEK293	His	9407-TM
	Human	HEK293	Fc	9300-TM
	Cynomolgus Monkey	HEK293	Fc	9384-TM

Proteins for Investigating Immune Checkpoint Targets Cont.

TNF Superfamily

Type V Subfamily Receptors & Ligands

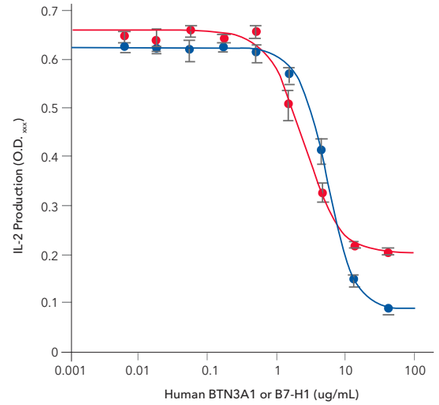
Molecule	Species	Source	Tag	Catalog #
4-1BB/TNFRSF9/CD137	Human	NS0	Fc, His	838-4B
	Human	CHO	His	9220-4B
	Cynomolgus Monkey	HEK293	Fc	9324-4B
4-1BB Ligand/TNFSF9	Human	E. coli	His	2295-4L
	Cynomolgus Monkey	E. coli	His	10439-4L
CD27/TNFRSF7	Human	NS0	Fc, His	382-CD
	Cynomolgus Monkey	CHO	Fc	9904-CD
CD27 Ligand/TNFSF7	Human	HEK293	HA	9328-CL
CD30/TNFRSF8	Human	NS0	Fc, His	813-CD
	Human	NS0	None	6126-CD
	Human	HEK293	Fc	11155-CD
CD30 Ligand/TNFSF8	Human	NS0	His	1028-CL
GITR/TNFRSF18	Human	NS0	Fc	689-GR
	Cynomolgus Monkey	CHO	Fc	9428-GR
GITR Ligand/TNFSF18	Human	CHO	HA	6987-GL
	Human	Sf21 (baculovirus)	His	694-GL
OX40/TNFRSF4	Human	NS0	Fc	3388-OX
	Human	NS0	His	9969-OX
	Cynomolgus Monkey	NS0	His	10137-OX
	Cynomolgus Monkey	HEK293	Fc	10311-OX
OX40 Ligand/TNFSF4	Human	NS0	His	1054-OX

TNF Superfamily

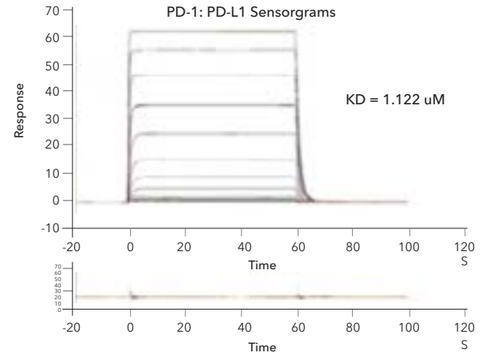
Type L Subfamily Receptors & Ligands

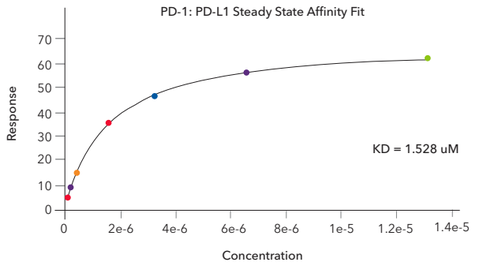
Molecule	Species	Source	Tag	Catalog #
CD40/TNFRSF5	Human	NS0	Fc, His	1493-CDB
	Cynomolgus Monkey	HEK293	Fc	9660-CD
CD40 Ligand/TNFSF5	Human	E. coli	None	6245-CL
	Human	HEK293	HA	6420-CL
	Human	E. coli	His	2706-CL
HVEM/TNFRSF14	Human	NS0	Fc, His	356-HV
	Human	HEK293	Fc	11177-HV
	Cynomolgus Monkey	HEK293	Fc	9197-HV
LIGHT/TNFSF14	Human	NS0	His	664-LI

Bioactivity Data



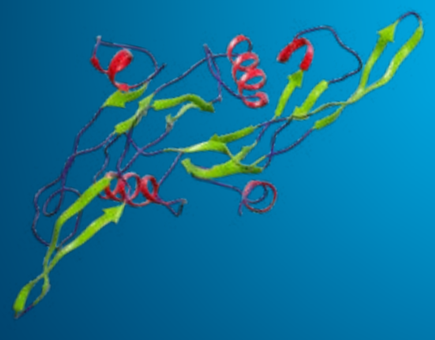
SPR Binding Data

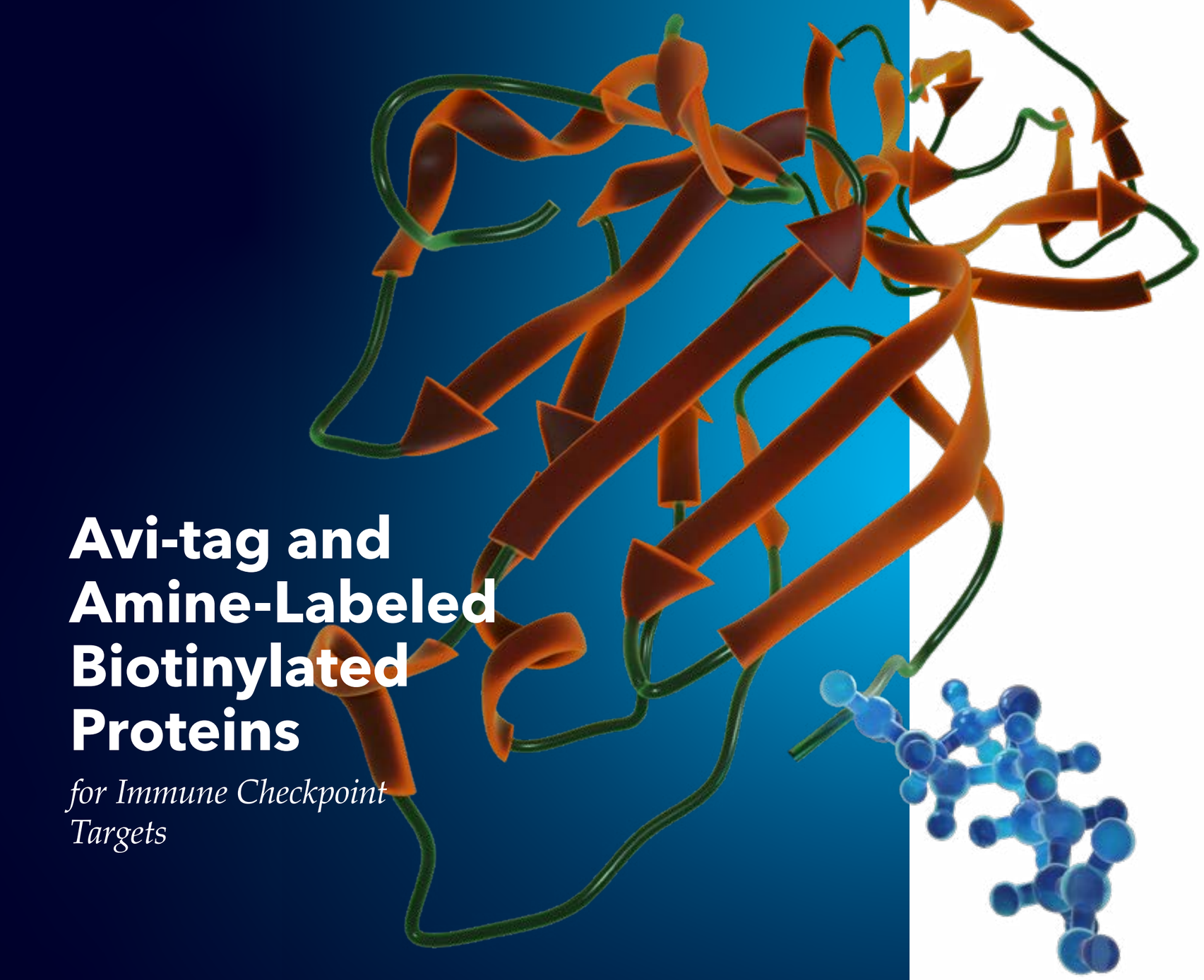




Butyrophilins Can Inhibit IL-2 Production by Human T Cells in a Manner Similar to B7 Family Members. Human T cells were incubated with immobilized Mouse Anti-Human CD3ε Monoclonal Antibody (R&D Systems, Catalog # MAB100; 1 ug/mL) and the indicated concentrations of Recombinant Human BTN3A1 (red line; R&D Systems, Catalog # 8539-BT) or Recombinant Human PD-L1/B7-H1 (blue line; R&D Systems, Catalog # 156-B7). Secretion of IL-2 was measured in cell culture supernatants using the Human IL-2 Quantikine™ ELISA Kit (R&D Systems, Catalog # D2050). The ED_{50} for this effect is approximately 2 ug/mL for BTN3A1 and 5 ug/mL for PD-L1/B7-H1.

Affinity Measurements and Binding Kinetics of the PD-1:PD-L1 Protein Interaction by Surface Plasmon Resonance. Sensorgram data of captured Avi-tag Biotinylated Recombinant Human PD-L1 His-tag (R&D Systems, Catalog # AVI9049) binding to Recombinant Human PD-1 His-tag (R&D Systems, Catalog # 8986-PD). The corresponding overlaid kinetic fits with the residual plot shown below. The concentration of Recombinant Human PD-1 His-tag ranged from 3.2 nM to 13.2 µM. The corresponding steady state affinity fit is shown at the bottom. The experiment was performed on a Biacore T200, GE Healthcare.





Avi-tag and Amine-Labeled Biotinylated Proteins

for Immune Checkpoint Targets

Biotinylated proteins can be powerful tools for assessing protein-protein interactions or screening antibody or small molecule libraries for potential therapeutics. Whether you are looking for an Avi-tag or an amine-labeled biotinylated protein, Bio-Techne's wide selection of R&D Systems™ biotinylated proteins will provide you with the performance that you need and the consistency that you expect to optimize your assay. All our biotinylated proteins are rigorously tested to ensure that they exhibit the same high levels of bioactivity as the corresponding unlabeled protein, and minimal lot-to-lot variability, so you don't have to worry whether your results will be reproducible over time.

Advantages of Avi-tag Biotinylated Proteins

- **Consistent, highly specific labeling:** A single biotin is enzymatically added to a lysine residue in the Avi-tag by BirA biotin ligase resulting in the generation of a homogeneous product.
- **Uniform orientation of the protein:** When bound to a streptavidin-coated surface, the orientation of the Avi-tag biotinylated protein will be uniform due to the precise control over biotinylation.

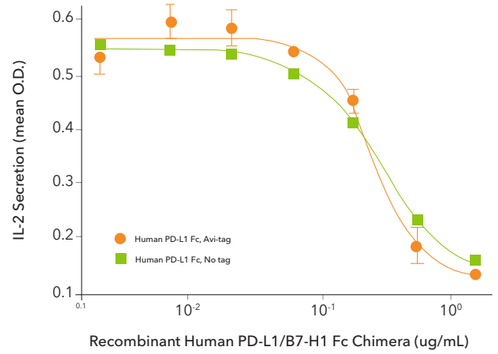
Advantages of Amine-Labeled Biotinylated Proteins

- **High signal strength and binding efficiency to avidin or streptavidin:** Chemical biotinylation labels the protein on amine groups found in lysine residues throughout the protein and at the N-terminus, resulting in multiple biotins being incorporated per protein.

Select Avi-Tag and Amine-Labeled Biotinylated Immune Checkpoint Proteins

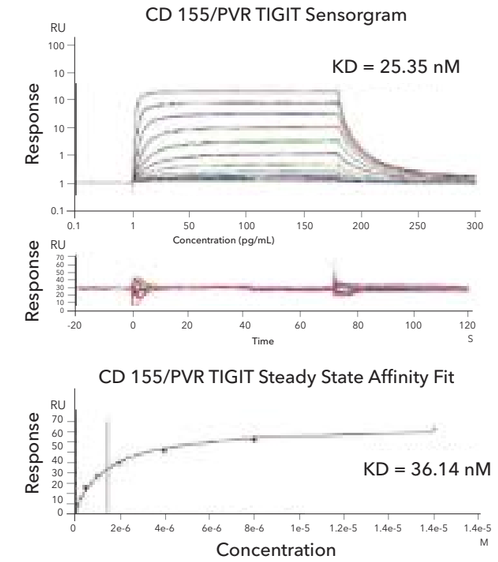
Protein (Human)	Source	Tag	Catalog #
B7-1/CD80	CHO	His, Avi-tag Biotin	AVI9050
		Fc, Avi-tag Biotin	AVI10107
B7-2/CD86	CHO	Fc, Avi-tag Biotin	AVI7625
B7-H2	CHO	Fc, Avi-tag Biotin	AVI165
B7-H3	HEK293	His, Avi-tag Biotin	AVI2318
	CHO	Fc, Avi-tag Biotin	AVI1027
	NS0	Fc, Biotin	BT1027
B7-H4	HEK293	Fc, Avi-tag Biotin	AVI8870
B7-H6	CHO	His, Avi-tag Biotin	AVI9309
	CHO	Fc, Avi-tag Biotin	AVI7144
	NS0	Fc, Biotin	BT7144
CD30/TNFRSF8	HEK293	His, Avi-tag Biotin	AVI10239
	HEK293	Fc, Avi-tag Biotin	AVI10240
CD40/TNFRSF5	CHO	Fc, Avi-tag Biotin	AVI10380
CD47	CHO	Fc, Avi-tag Biotin	AVI4670
CD155/PVR	HEK293	His, Avi-tag Biotin	AVI2530
	HEK293	Fc, Avi-tag Biotin	AVI9174
CTLA-4	HEK293	Fc, Avi-tag Biotin	AVI7268
GITR	CHO	Fc, Avi-tag Biotin	AVI689
LAG-3	CHO	Fc, Avi-tag Biotin	AVI2319
	NS0	Fc, Biotin	BT2319
OX40/TNFRSF4	CHO	His, Avi-tag Biotin	AVI9969
	HEK293	Fc, Avi-tag Biotin	AVI10842
PD-1	HEK293	His, Avi-tag Biotin	AVI8986
	CHO	Fc, Avi-tag Biotin	AVI1086
	NS0	Fc, Biotin	BT1086
PD-L1/B7-H1	HEK293	His, Avi-tag Biotin	AVI9049
	CHO	Fc, Avi-tag Biotin	AVI156
	NS0	Fc, Biotin	BT156
PD-L2/B7-DC	HEK293	His, Avi-tag Biotin	AVI9075
	HEK293	Fc, Avi-tag Biotin	AVI1224
	NS0	Fc, Biotin	BT1224
SIRP-alpha/CD172a	CHO	His, Avi-tag Biotin	AVI9378
	CHO	Fc, Avi-tag Biotin	AVI4546
	CHO	Fc, Biotin	BT4546B
TIGIT (T103)	CHO	His, Avi-tag Biotin	AVI11124
TIM-1/KIM-1/HAVCR	HEK293	Fc, Avi-tag Biotin	AVI9319
TIM-3	CHO	His, Avi-tag Biotin	AVI10241
VISTA/B7-H5/PD-1H	HEK293	His, Avi-tag Biotin	AVI9057
	HEK293	Fc, Avi-tag Biotin	AVI7126
	NS0	Fc, Biotin	BT7126

Bioactivity Data



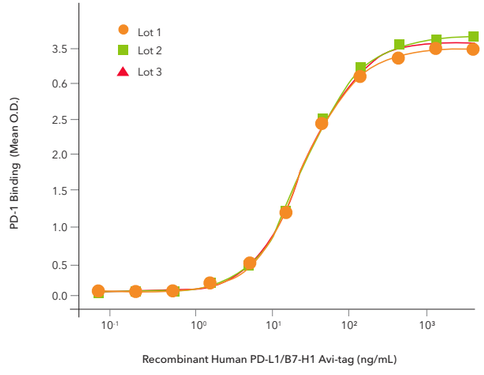
Unlabeled and Avi-tag Biotinylated Recombinant Human PD-L1/B7-H1 Display Comparable Bioactivity. Human T lymphocytes were treated with the indicated concentrations of either unlabeled Recombinant Human PD-L1/B7-H1 Fc Chimera (R&D Systems, Catalog # 156-B7; green line) or Avi-tag Biotinylated Recombinant Human PD-L1/B7-H1 Fc Chimera (R&D Systems, Catalog # AVI156; orange line). IL-2 secretion was measured in cell culture supernatants using the Human IL-2 Quantikine® ELISA Kit (R&D Systems, Catalog # D2050). The similarity in the activities of the two proteins highlights that the Avi-tag biotinylated protein is fully functional.

SPR Binding Data



Affinity Measurements and Binding Kinetics of the CD155/PVR:TIGIT Protein Interaction by Surface Plasmon Resonance. Sensorgram data of captured Avi-tag Biotinylated Recombinant Human CD155/PVR Fc Chimera (R&D Systems, Catalog # AVI9174) binding to Recombinant Human TIGIT His-tag (R&D Systems, Catalog # 9525-TG). The corresponding overlaid kinetic fits with the residual plot shown below. The concentration of Recombinant Human TIGIT His-tag ranged from 0.2 nM to 400 nM. The corresponding steady state affinity fit is shown at the bottom. The experiment was performed on a Biacore T200, GE Healthcare.

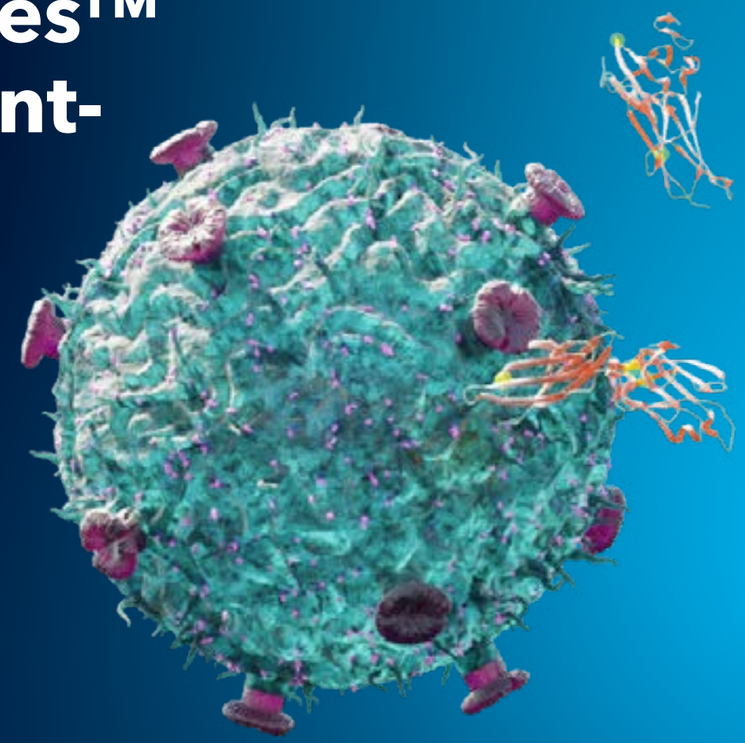
Lot-to-Lot-Consistency



R&D Systems Avi-tag Biotinylated Recombinant Human PD-L1/B7-H1 Displays High Lot-to-Lot Consistency. Three independent lots of Avi-tag Biotinylated Recombinant Human PD-L1/B7-H1 (R&D Systems, Catalog # AVI156) were tested for their ability to bind to Recombinant Human PD-1 (R&D Systems, Catalog # 1086-PD), which was coated at 1 ug/mL. Each trace shown on the graph represents data obtained from Avi-tag Biotinylated Recombinant Human PD-L1/B7-H1 from a different manufacturing run, demonstrating the lot-to-lot consistency of the proteins.

Fluorokines™ Fluorescent-Labeled Proteins

for Immune Checkpoint Research

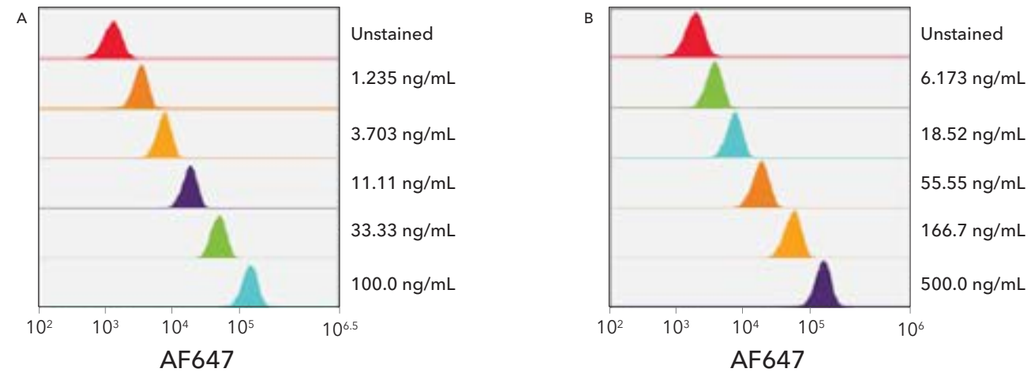


Take advantage of our fluorescent-labeled immune checkpoint ligands to easily identify or sort cells expressing the corresponding immune checkpoint receptors. Fluorescent-labeled ligands bind to cells expressing their cognate receptors in a highly specific manner, allowing these cells to be stained in a single step and detected by flow cytometry.

Advantages of Fluorescent-Labeled Proteins for Detecting Target Molecules

- **Direct detection:** No secondary antibody is needed for Fluorokine detection, reducing processing time and eliminating the possibility of background staining that may occur by indirect detection using a secondary antibody
- **Conjugated to Alexa Fluor® or Atto dyes:** Fluorescent-labeled proteins are conjugated to Alexa Fluor® or Atto dyes, which offer intense fluorescence and excellent photostability
- **High levels of bioactivity and lot-to-lot consistency:** Fluorokines are rigorously tested to ensure that they retain the same high level of bioactivity as the unlabeled protein and each new protein lot is tested side-by-side with previous lots and with a master lot to ensure high lot-to-lot consistency
- **Compatible with fluorochrome-conjugated antibodies:** Fluorokines can be used in combination with fluorochrome-conjugated antibodies for multi-color flow cytometry

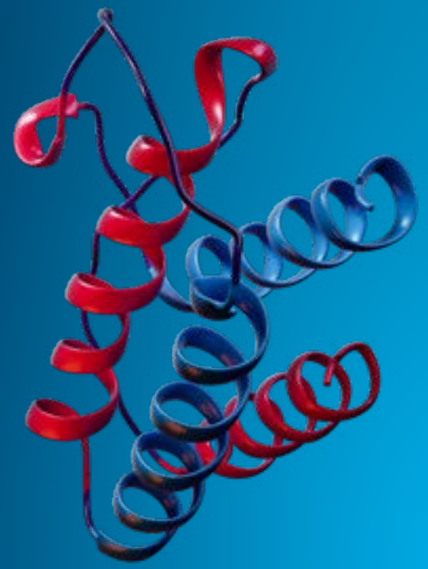
Protein (Human)	Source	Tag	Fluorescent Label	Catalog #
B7-1/CD80	HEK293	His	Alexa Fluor® 488	AFG9050
			Alexa Fluor® 647	AFR9050
	CHO	Fc	Alexa Fluor® 488	AFG10133
			Alexa Fluor® 647	AFR10133
B7-2/CD86	HEK293	His	Alexa Fluor® 647	AFR9090
	CHO	Fc	Alexa Fluor® 488	AFG7625
			Alexa Fluor® 647	AFR7625
B7-H2	NS0	Fc	Alexa Fluor® 488	AFG165
			Alexa Fluor® 647	AFR165
CD155/PVR	NS0	His	Alexa Fluor® 488	AFG2530
			Alexa Fluor® 647	AFR2530
GITR Ligand/TNFSF18	CHO	GCN4-IZ, HA	Alexa Fluor® 488	AFG6987
			Alexa Fluor® 647	AFR6987
HVEM/TNFRSF14	HEK293	Fc	Alexa Fluor® 488	AFG11177
			Alexa Fluor® 647	AFR11177
IgG1	NS0	Fc	Alexa Fluor® 488	AFG110
	NS0	Fc	Alexa Fluor® 647	AFR110
PD-L1/B7-H1	HEK293	His	Alexa Fluor® 488	AFG9049
			Alexa Fluor® 647	AFR9049
PD-L2/B7-DC	NS0	Fc	Alexa Fluor® 488	AFG1224
			Alexa Fluor® 647	AFR1224
	HEK293	His	Alexa Fluor® 488	AFG9075
			Alexa Fluor® 647	AFR9075



Analysis of the Specificity of the Recombinant Human PD-L1/B7-H1 and B7-1/CD80 His-tag Alex Fluor® 647 Proteins. (A) Streptavidin-coated beads conjugated to Biotinylated Anti-Human PD-L1/B7-H1 Monoclonal Antibody were stained with the indicated concentrations of Recombinant Human PD-L1/B7-H1 His-tag Alexa Fluor® 647 Protein (R&D Systems, Catalog AFR9049). (B) Streptavidin-coated beads conjugated to Biotinylated Anti-Human B7-1/CD80 Monoclonal Antibody (R&D Systems, Catalog # BAM1402) were stained with the indicated concentrations of Recombinant Human B7-1/CD80 His-tag Alexa Fluor® 647 Protein (R&D Systems, Catalog # AFR9050).

Animal-Free RUO Proteins

Bio-Techne offers both preclinical Animal-free RUO proteins and GMP-grade Proteins.



Our Animal-free RUO proteins originate from the same clone, sequence, and expression system as our GMP-grade proteins and they are manufactured using the same methods, ensuring a seamless transition from preclinical research into clinical manufacturing. All of our Animal-free proteins are purified and manufactured in dedicated, controlled access, animal-free laboratories using equipment and media that are certified as animal-free, so that at no point in the production process are these proteins exposed to potential contamination by animal components or byproducts. The catalog numbers for our Animal-free RUO proteins are listed alongside the corresponding GMP-grade proteins in the product table on the following page.

GMP-Grade Proteins

GMP-grade proteins are manufactured under guidelines that allow for their use as ancillary materials in cell therapy manufacturing processes. They undergo extensive quality control testing and come with comprehensive documentation and full transparency and traceability of source and manufacturing system. This allows cell therapy manufacturers to be confident that they are using a consistent, safe, and traceable supply of reagents.

Documentation
GMP products manufactured, tested, and released under an ISO 9001:2015 and ISO 13485:2016 certified quality management system, lot-to-lot consistency, materials traceability, employee training and documentation, equipment maintenance and monitoring records, Drug Master Files, and more.

Regulatory Guidelines Followed
GMP proteins are manufactured in compliance of the applicable sections of the World Health Organization:

1. USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
2. Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-base and Gene Therapy Medicinal Products

Quality Control Testing
Mass spectrometry, HPLC, SDS-PAGE, endotoxin, presence of host cell content, adventitious agents, and more.

Satisfied Clients
Clients include more than 300 pharmaceutical and biotech companies and we regularly welcome audits of our facilities.

Preclinical Animal-Free RUO and GMP-Grade Proteins

Protein (Human; Source: *E. coli*)	Animal-Free GMP-Grade Protein (Catalog #)	Animal-Free RUO Protein (Catalog #)
Betacellulin	BT-BTC-GMP*	BT-BTC-AFL
BMP-4	314E-GMP	AFL314E
EGF	236-GMP*	
FGF basic (145 aa)	3718-GMP	AFL3718
Flt-3 Ligand/FLT3L	308E-GMP*	AFL308E
GM-CSF	215-GMP	AFL215
IFN-γ	285-GMP*	AFL285
IGF-I	291-GMP	AFL291
LR3 IGF-I	8335D-GMP	
IL-1β/IL-1F2	201-GMP	AFL201
IL-2	BT-002-GMP*	BT-002-AFL
IL-3	203-GMP	AFL203
IL-4	204-GMP*	AFL204
IL-6	206-GMP	AFL206

Protein (Human; Source: *E. coli*)	Animal-Free GMP-Grade Protein (Catalog #)	Animal-Free RUO Protein (Catalog #)
IL-7	BT-007-GMP*	BT-007-AFL
IL-10	1064-GMP	AFL1064
IL-15	BT-015-GMP*	BT-015-AFL
IL-21	8879-GMP*	AFL8879
M-CSF	216-GMP	AFL216
PDGF-AA	221-GMP	AFL221
PDGF-BB	220-GMP	AFL220
SCF/c-kit Ligand	BT-SCF-GMP	BT-SCF-AFL
Shh N-terminus	1314-GMP	
Shh (C2411) N-terminus	1845-GMP	AFL1845
Thrombopoietin	288E-GMP	AFL288 Coming Soon
TNF-α	210-GMP	AFL210
VEGF	BT-VEGF-GMP	BT-VEGF-AFL

** DMF have been filed for these GMP Proteins. GMP-grade IL-2, IL-7, and IL-15 are available through our joint venture partnership with ScaleReady | scaleready.com*

Additional GMP-grade Proteins Available from Bio-Techne

There are some instances when a protein needs to be produced in a eukaryotic system to maintain activity. This may be due to protein folding or post-translational modifications that can only be accomplished by making the protein in a eukaryotic cell line. These GMP-grade proteins, which are not considered to be animal-free, are listed in the table to the right. Whenever a GMP-grade protein cannot be produced in an animal-free process, it is always clearly indicated on our website.

R&D Systems GMP-grade proteins are intended for use as ancillary materials in GMP manufacturing of investigational or marketed clinical products, such as cell therapy, gene therapy, tissue-engineered products, combination products, or other Advanced Therapy Medicinal Products. They are not therapeutic products or excipient and are not suitable for direct administration to humans.

Protein (Human)	Source	Catalog #
Activin A	CHO	338-GMP*
BMP-2	CHO	355-GMP
GDF-8/Myostatin	NS0	788-GMP
GDNF	NS0	212-GMP
HGF	NS0	294-GMP
KGF/FGF-7	E.coli	251-GMP
Noggin	NS0	3344-GMP
TGF-β1	CHO	240-GMP
Wnt-3a	CHO	5036-GMP

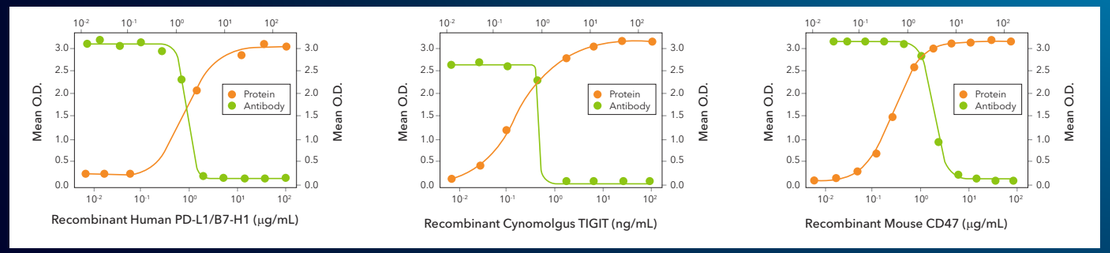
** DMF have been filed for these GMP Proteins.*

Immune Checkpoint Antibodies

Bio-Techne is your trusted full-service partner, dedicated to collaborating with you throughout the entire discovery process. Our antibodies enable progress from benchtop research to clinical applications. We stand behind our products. All Bio-Techne antibodies are backed by a 100% Guarantee.

Antibodies for Blocking/Neutralization

At R&D Systems, we leverage our selection of in-house manufactured proteins to validate our blocking/neutralization antibodies. We utilize our bioassay expertise to perform validation assays, including proliferation, chemotaxis, protein secretion, adhesion, and *in vitro* enzyme neutralization assays to ensure that our antibodies block/neutralize the appropriate cellular function.



Functional ELISA Data Showing Successful Blocking of Receptor-Ligand Interaction with Receptor Blocking Antibodies. (A) Orange line shows recombinant ligand binds to receptor in dose-dependent manner, in the absence of the antibody. At 0.09-0.72 µg/mL, Mouse Anti-Human PD-1 (1015846) (R&D Systems, Catalog # MAB10864) will block 50% of the binding of 5 µg/mL of Recombinant Human PD-L1/B7-H1 Fc Chimera (orange line; R&D Systems, Catalog # 156-B7) to immobilized Recombinant Human PD 1 His-tagged Protein (R&D Systems, Catalog # 8986-PD) coated at 1 µg/mL (100 µL/well). At 5 µg/mL, this antibody will block >90% of the binding. (B) At 70-350 ng/mL, Rabbit Anti-Cynomolgus TIGIT (2629A) (R&D Systems, Catalog # MAB10532) will block 50% of the binding of Recombinant Cynomolgus Monkey TIGIT (R&D Systems, Catalog # 9380-TG) bound to immobilized Recombinant Human CD155/PVR (R&D Systems, Catalog # 2530-CD) coated at 2.5 µg/mL (100 µL/well). (C) At 0.08-0.8 µg/mL, Rat Anti-Mouse CD47 (974222) (R&D Systems, Catalog # MAB18661) will block 50% of the binding of 0.25 µg/mL of Recombinant Mouse CD47 Fc Chimera (orange line; R&D Systems, Catalog # 1866-CD) to immobilized Recombinant Mouse SIRP alpha / CD172a Fc Chimera (R&D Systems, Catalog # 7154-SA) coated at 1 µg/mL (100 µL/well). At 5 µg/mL, this antibody will block >90% of the binding.

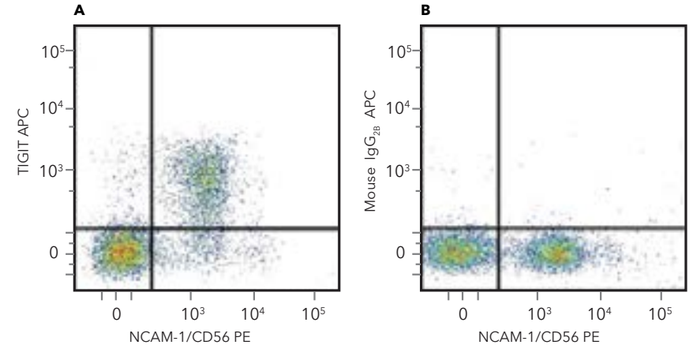
Explore All Blocking/Neutralizing Antibodies

Reliably Detect Immune Checkpoint Markers

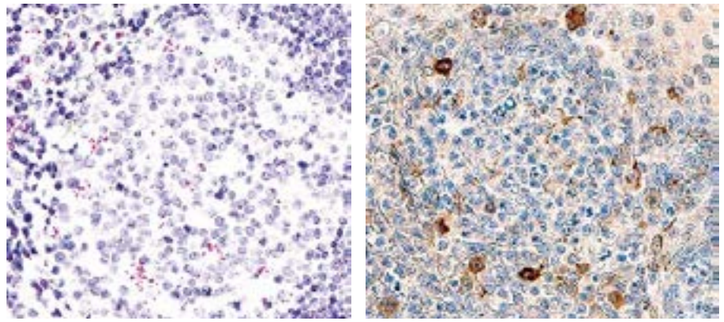
To help you generate reliable results, we've compiled an extensive collection of citations, customer reviews, and application specific data images to showcase antibody performance. Common applications include:

- ELISA
- Flow Cytometry (FC)
- Immunocytochemistry (ICC)
- Immunohistochemistry (IHC)
- Dual RNAScope® in situ hybridization (ISH)/IHC
- Simple Western™ (SW)
- Western Blot (WB)

Complete your experimental setup with secondary antibodies and isotype control antibodies.



Detection of TIGIT in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) gated on CD3- cells were stained with a Mouse Anti-Human NCAM-1/CD56 PE-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB2408P) and either a (A) Mouse Anti-Human TIGIT APC-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB7898A) or (B) Mouse IgG_{2B} Allophycocyanin Isotype Control (R&D Systems, Catalog # IC0041A).



TIM-3 in Human Tonsil Using Dual RNAscope® ISH and IHC. TIM-3 mRNA was detected in formalin-fixed paraffin-embedded tissue sections of human tonsil probed with ACD RNAScope Probe (ACD, Catalog # 560681) and stained using ACD RNAscope 2.5 HD Detection Reagents-Red (top image; ACD, Catalog # 32260). Adjacent tissue section was processed for immunohistochemistry using a Goat Anti-Human TIM-3 Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF2365) at 3 ug/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte HRP Polymer Antibody (R&D Systems, Catalog # VC004) and DAB chromogen (lower image, yellow-brown). Tissues were counterstained with hematoxylin (blue).

Recombinant Antibodies for Immune Checkpoint Markers

Minimize one source of experimental variability by using Recombinant Antibodies. We utilize our expertise in molecular biology and manufacturing and sourcing high-quality antibodies to offer recombinant antibodies and antibody conversion services.

R&D Systems recombinant monoclonal antibody sequences are isolated from a pre-existing hybridoma, rabbit or llama antibody. Recombinant antibodies allow us to provide an immortal supply with consistent results every time.

Search All Recombinant Antibodies

Biosimilar Antibodies
Our research use only (RUO) Biosimilar Antibodies provide a more accessible solution for drug researchers and assay development. Using recombinant antibody engineering technology, our biosimilar antibodies have the same sequence as the therapeutic antibody. Biosimilar antibodies are tested to ensure specific binding to the same targeted molecule as the therapeutic grade antibody.

Learn More About Biosimilar Antibodies | bio-techne.com/reagents/antibodies/biosimilar-antibodies

Biosimilar Antibodies

Biosimilar Antibody	Target
Cetuximab	EGFR
Rituximab	CD20
Trastuzumab	ErB2/Her2
Adalimumab	TNF-α
Vedolizumab	Integrin α4β7/LPAM-1
Bevacizumab	VEGF
Basiliximab	CD25/IL-2 Rα
Alemtuzumab	CD52
Atezolizumab	PD-L1/B7-H1
Gemtuzumab	Siglec-3/CD33
Aducanumab	APP/Protease Nexin II

Find Your Biosimilar Antibody

Antibodies for Top Immune Checkpoint Targets

B7 Family	SLAM Family	Butyrophilins	Nectin and Nectin-like binding Receptors	TNF Superfamily	TIM Family	Others
PD-1	2B4	BTN1A1/Butyrophilin	TIGIT	4-1BB/TNFRSF9/CD137	TIM-1	LAG-3
CTLA-4	CD48/SLAMF2	BTN2A2/Butyrophilin 2A2	CD96	CD30	TIM-3	Galectin-3
VISTA/B7-H5	CD58/LFA-3	BTN3A1/CD277	CD155/PVR	GITR	TIM-4	Galectin-9
PD-L1	CD150	BTN3A1/2	DNAM-1/CD226	OX40		Siglec-10
CD80		BTNL2/Butyrophilin-like 2				CD24
CD86						SIRP alpha
B7-H3/CD276						CD47
B7-H4						
VSIG-3						
B7-H7/HHLA2						

Explore Primary Antibodies I bio-techne.com/reagents/antibodies

Custom Antibody Services

Our custom Antibodies team can provide highly specific, expertly designed products just for you

Our services include:

- Custom engineering services
- Creation of monoclonal antibody panels
- Production of recombinant antibodies
- Antibody conjugation
- GMP conversion
- Anti-idiotype antibody development

Learn More About Custom Antibody Services I bio-techne.com/services/custom-antibody-services

Immunoassays

for Immune Checkpoint Research

From discovery to validation, R&D Systems, A Bio-Techne brand has a broad range of immunoassay solutions for quantifying soluble immune checkpoint proteins. Our immunoassays are vertically integrated. That means that our antibodies, proteins, and diluents are manufactured in-house, thus ensuring a long-term stable supply of immunoassays.

ELISA Kits- Quantikine™ and DuoSet™

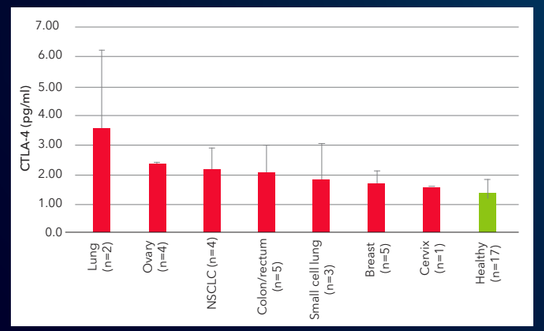
ELISA kits are rigorously validated for reliable performance.

- The most referenced single analyte assay in the literature
- Highly specific and optimized for superior performance
- More than 1,000 targets with new and novel targets across multiple species

Available Assays Include:

- B7/CD28 Families
- TIM Family Receptors and Ligands
- Nectin and Nectin-like Binding Receptors
- Slam Family
- Type V Subfamily Receptors and Ligands
- Type L Subfamily Receptors and Ligands

Serum CTLA-4 in Cancer Patients



The Quantikine CTLA-4 High Sensitivity ELISA (R&D Systems, Catalog # HSCT40) reliably quantifies CTLA-4 in serum from human cancer patients and controls.

Learn More About ELISA Kits | bio-techne.com/reagents/elisa-kits

R&D Systems Luminex® Assays

Luminex® is a registered trademark of Luminex Corporation.

Luminex Assay high plexing capabilities enable you to conserve time, money, and sample volume. Select from more than 450 targets to quantify up to 50 analytes in each sample.

- Robust, reliable, & reproducible data
- Every panel QC tested
- Fast delivery times

- Three flexible formats:
  1. Discovery Assays – Completely customizable
  2. Fixed High Performance Panels – Fully stocked & ready to ship
  3. Configurable High Performance Panels – Select any or all analytes from designed panels

Available Panels Include:

- Human Immunotherapy 25-Plex Fixed Panel
- Human Immuno-Oncology Panel 1 Performance Premix Kit

Build Your Own Luminex Panel | bio-techne.com/luminex-assay-customization-tool

Simple Plex™ Assays on the Ella™ Platform

The bench-top Ella platform automates the ELISA workflow. Simply load your sample, press start, and generate reliable data in less than 90 minutes.

- Powered by R&D Systems, the most trusted brand for quality reagents
- Fully validated, highly sensitive with up to 4 logs of dynamic range
- Simple Plex assays have sub-picogram sensitivity

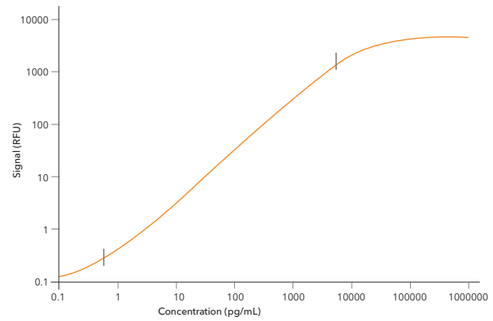
- Choose from more than 200 target analytes in flexible cartridge formats.
- Quantify up to 8 targets simultaneously with customizable cartridges.

Meet Ella and Request Pricing

Available Assays Include:

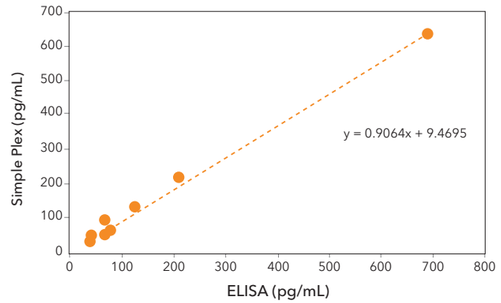
- CTLA-4
- PD-L1
- LAG-3
- TIM-3
- CCL-2
- IL-1
- IL-8
- Angiopoietin-2
- Axl

Simple Plex PD-L1 assay



The Simple Plex PD-L1 assay has a broad dynamic range.

Detection of Endogenous PD-L1 with Simple Plex



Measured levels of PD-L1 in glioma cell supernatant, PBMC, and HDLM-2 samples correlate very well between Simple Plex assays and Quantikine ELISA kits. R2=0.997.

Explore Simple Plex Assays | bio-techne.com/reagents/simple-plex-immunoassays/assay-menu

Where Science Intersects Innovation™

Bio-Techne® | R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ ACD™ ExosomeDx™ Asuragen®

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STRY0290950_MWR_BR_Tools-for-Investigating-Immune-Checkpoint-Targets_PC

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