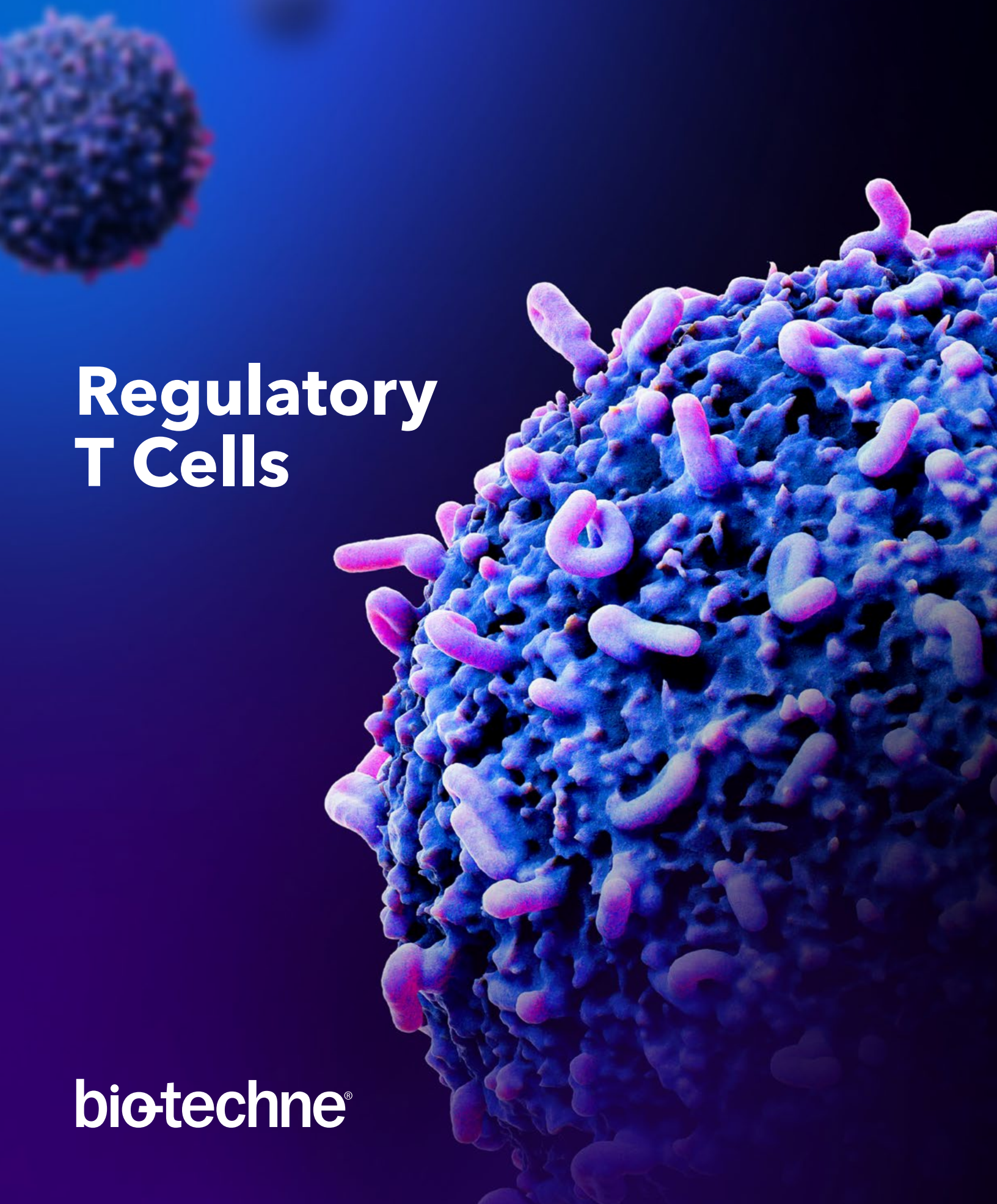
Regulatory T Cells



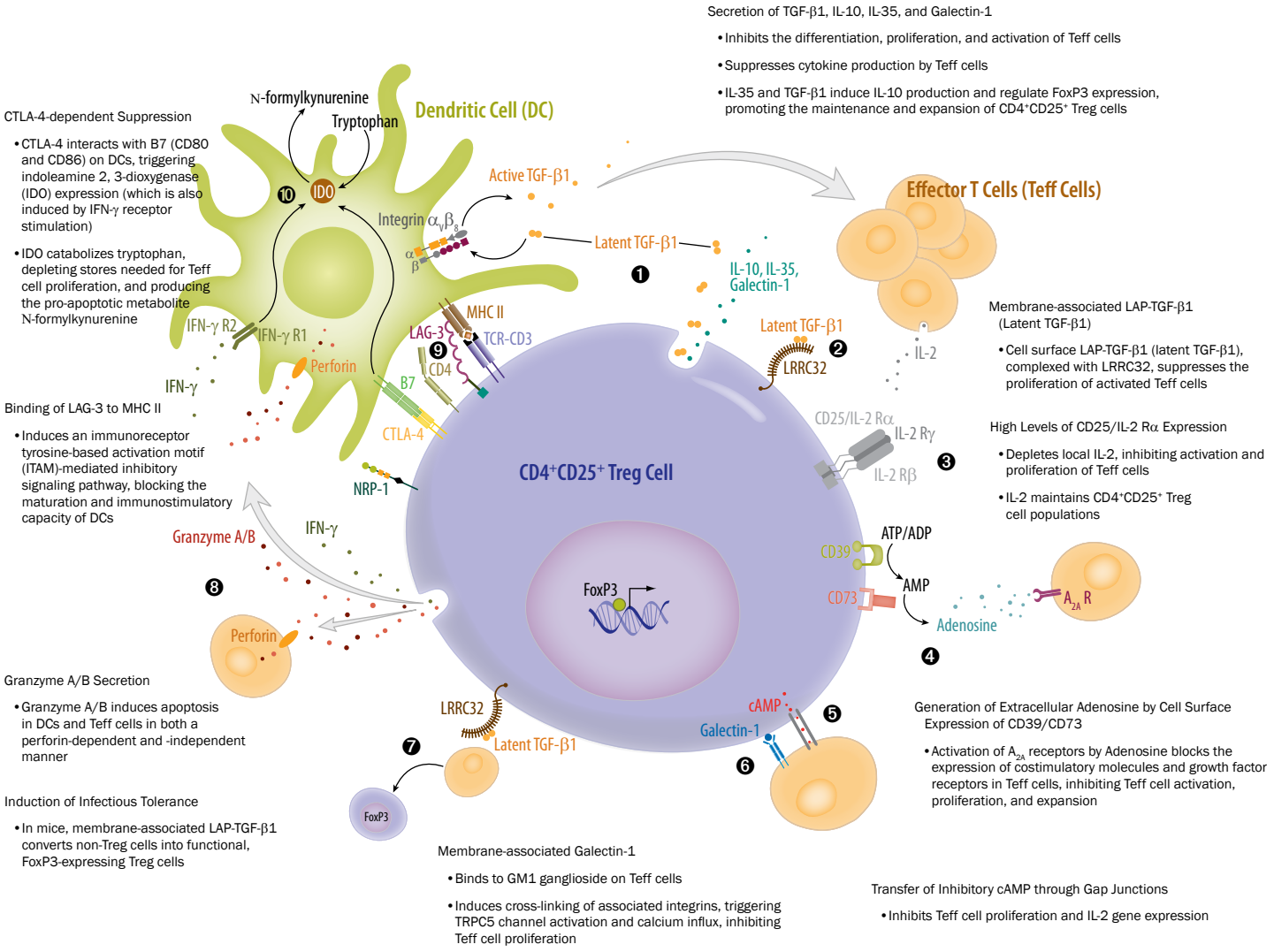
bio-techne®

Regulatory T Cells

Regulatory T cells (Tregs) are a heterogeneous subset of $CD4^+$ T cells with suppressive properties that play a central role in maintaining immune homeostasis and self-tolerance, dampening inflammation, and preventing autoimmunity. They function by inhibiting the activities of $CD4^+$ and $CD8^+$ effector T cells, natural killer cells, NKT cells, and antigen-presenting cells through multiple mechanisms including the secretion of immunosuppressive cytokines (IL-10, IL-35, and TGF-β) and metabolites (Adenosine), production of cytolytic factors (Granzymes A/B and Perforin), disruption of cell metabolism (*i.e.* IL-2 deprivation), and suppression of effector functions through direct cell-cell contact. As a result, they provide a system by which pro-inflammatory immune responses can be counterbalanced. Reduced Treg activity is associated with inflammatory and autoimmune diseases such as rheumatoid arthritis, type I diabetes, multiple sclerosis, and systemic lupus erythematosus. Conversely, Tregs can also be pathogenic under conditions where they suppress beneficial anti-viral or anti-tumor immune responses.

Several subsets of regulatory T cells have been described in the literature. These include naturally occurring $CD4^+CD25^+FoxP3^+$ cells that develop in the thymus (tTregs), peripherally-derived Tregs (pTregs) that are generated from $FoxP3^-$ conventional T cells at sites outside of the thymus, and induced regulatory T cells (iTregs) that are generated *in vitro* by stimulation of mouse conventional T cells with TGF-β. Cells in the pTreg group have been further classified as either central Tregs (cTregs), effector Tregs (eTregs), or tissue-resident Tregs. Additionally, $CD4^+FoxP3^-$ type I regulatory T cells (Tr1), $CD8^+$ Tregs, and follicular Treg cells (T_{FR}) have been described. Characteristics that distinguish these subsets as well as differences in their development and functional activity are active areas of investigation. R&D Systems offers a wide selection of reagents for culturing and characterizing regulatory T cells including cell selection and differentiation kits, recombinant and natural proteins, and ELISA Kits. Together with Novus Biologicals, we also offer the widest selection of unlabeled and fluorochrome-conjugated antibodies for identifying regulatory T cells and investigating their functions.

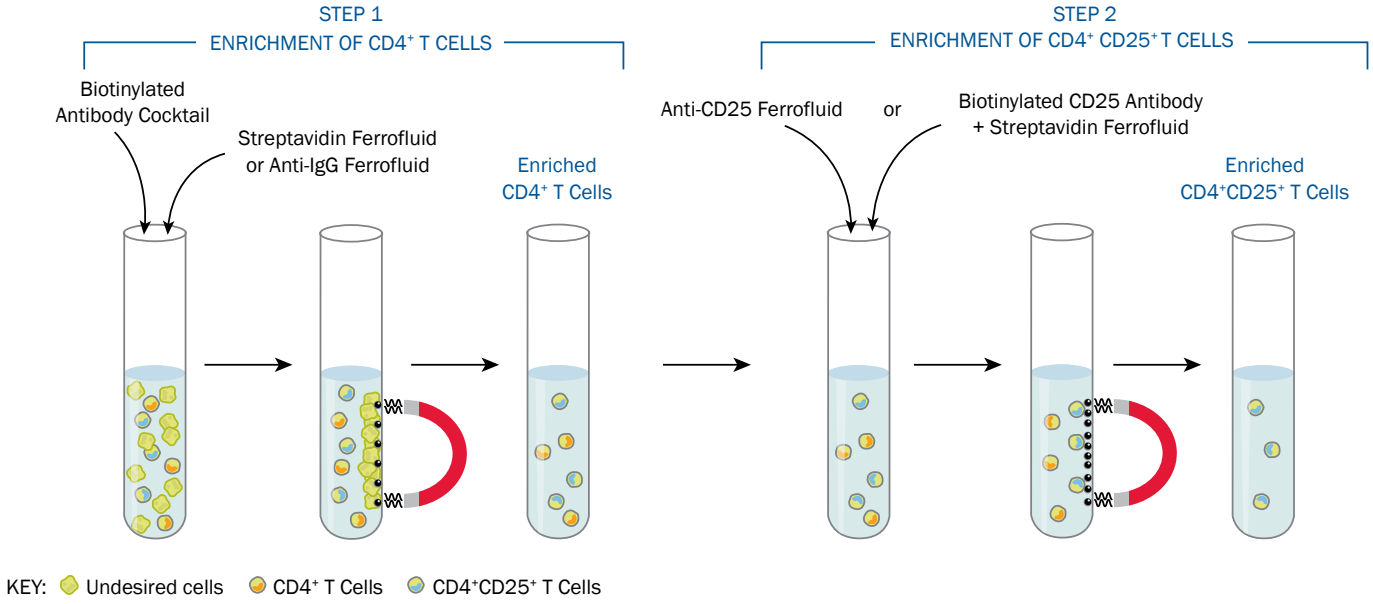
Mechanisms of Regulatory T Cell-mediated Suppression



R&D Systems® MagCellect™ CD4+CD25+ Regulatory T Cell Selection Kits

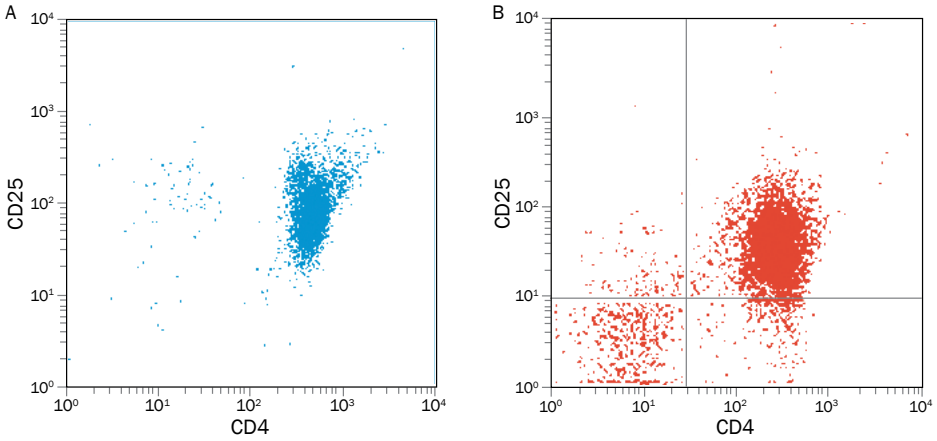
R&D Systems® MagCellect™ Cell Selection Kits are designed to isolate human or mouse CD4+CD25+ regulatory T cells using a two-step procedure. In the first step, CD4+ T cells are isolated by tagging unwanted cells with a biotinylated antibody cocktail followed by the addition of streptavidin ferrofluid. The cell suspension is subsequently placed in a magnetic field and the desired cell population is isolated by aspiration. In the second step, CD25+ cells are isolated from the CD4+ cell fraction by positive selection using a biotinylated CD25 antibody and streptavidin ferrofluid. The typical purity of the recovered CD4+CD25+ regulatory T cells ranges between 85–95% for the human kit and 84–94% for the mouse kit.

Assay Principle



Regulatory T Cell Selection Kits

CD4+CD25+ Regulatory T Cell Isolation Kits	
Kit	**Catalog #**
MagCellect™ Human CD4+CD25+ Regulatory T Cell Isolation Kit	MAGH104
MagCellect™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit	MAGM208



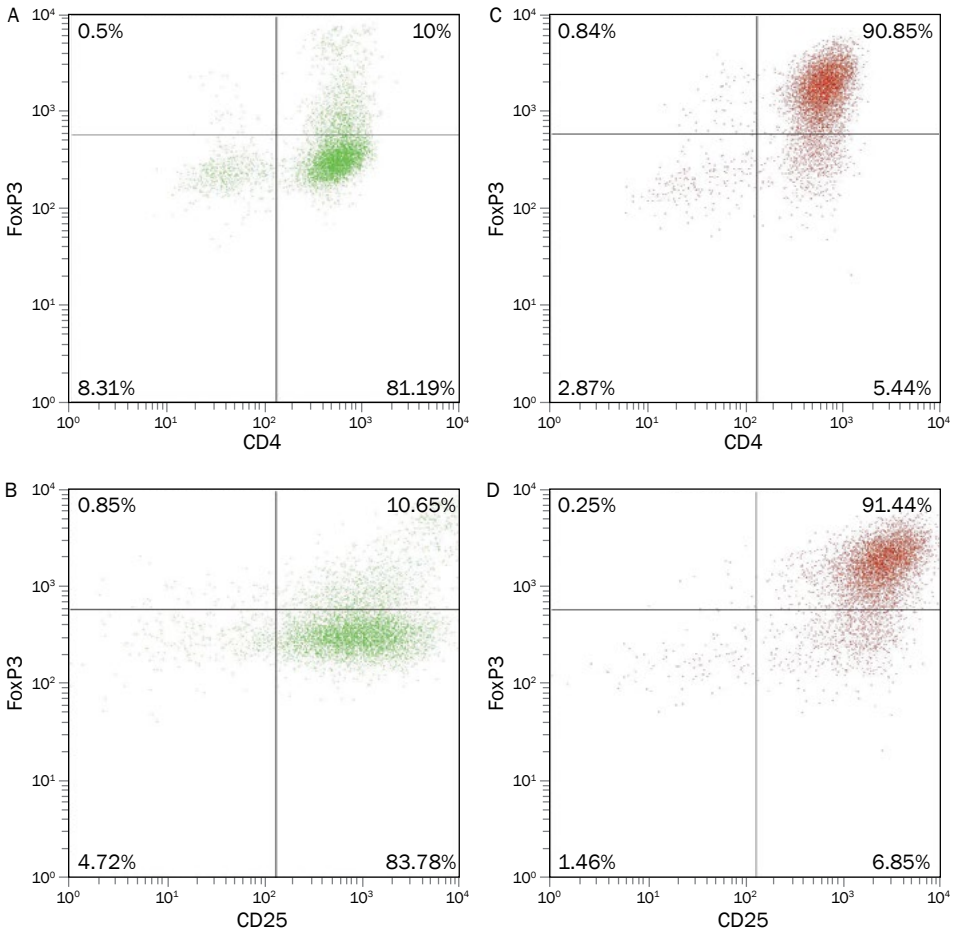
Isolation of CD4+CD25+ Regulatory T Cells using the MagCellect™ Kits. CD4+CD25+ regulatory T cells were isolated from (**A**) human PBMCs using the MagCellect™ Human CD4+CD25+ Regulatory T Cell Isolation Kit (R&D Systems, Catalog # MAGH104) or (**B**) mouse splenocytes using the MagCellect™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit (R&D Systems, Catalog # MAGM208). Total CD4+CD25+ regulatory T cells were detected using fluorescein-conjugated anti-human or anti-mouse CD4 antibodies and PE-conjugated anti-human or anti-mouse CD25 antibodies.

New! R&D Systems® CellXVivo™ Regulatory T Cell Differentiation Kits

R&D Systems CellXVivo™ Human and Mouse Regulatory T Cell Differentiation Kits contain high quality growth factors and other optimized reagents necessary to differentiate human or mouse naïve $CD4^+$ T cells into $FoxP3^+CD25^+$ regulatory T cells. The kits provide sufficient reagents for the differentiation of two 24-well plates and validated, straight-forward procedures.

Regulatory T Cell Differentiation Kits

Kit	Catalog #
CellXVivo™ Human Treg Cell Differentiation Kit	CDK006
CellXVivo™ Mouse Treg Cell Differentiation Kit	CDK007



Flow Cytometric Analysis of $CD4^+$ Regulatory T Cells Following Differentiation with the CellXVivo™ Human Regulatory T Cell Differentiation Kit. Human peripheral blood naïve $CD4^+$ T cells were left untreated (**A, B**) or treated for five days with the differentiation reagents (**C, D**) included in the CellXVivo™ Human Regulatory T Cell Differentiation Kit (R&D Systems, Catalog # CDK006). Five days after the differentiation was initiated, the cells were fixed, permeabilized and stained using antibodies included in the FlowX Human Regulatory T Cell Multi-Color Flow Cytometry Kit (R&D Systems, Catalog # FMC021). Quadrants were set based on samples stained with the appropriate isotype controls.

Reagents for *In vitro* Induction & Expansion of Regulatory T Cells

In addition to our CellXVivo™ Regulatory T Cell Differentiation Kits, R&D Systems also offers individual proteins and antibodies for *in vitro* induction and expansion of regulatory T cells.

Reagents for *In vitro* Induction & Expansion of Regulatory T Cells				
	Antibodies			
	Molecule	**Species**	**Clone**	**Catalog # (Applications)**
■	CD3ε	Human	UCHT1	MAB100 (FA, FC, ICC/IF, IP)
		Mouse	145-2C11	MAB484 (Depl., FA, FC, IP)
■	CD28	Human	37407	MAB342 (FA, FC, WB)
		Human	Polyclonal	AF-342-PB (FA, FC, ICC/IF, WB)
		Mouse	61109	MAB4831 (WB)
		Mouse	Polyclonal	AF483 (WB)

Recombinant Proteins from R&D Systems			
Molecule	**Species**	**Source**	**Catalog #**
IL-2	Human	*E. coli*	202-IL
	Mouse	*E. coli*	402-ML
IL-27	Human	NS0	2526-IL
	Mouse	NS0	2799-ML
IL-33	Human	*E. coli*	3625-IL
	Mouse	*E. coli*	3626-ML
TGF-β1	Human	CHO	240-B
		HEK293	7754-BH
	Mouse	CHO	7666-MB
TGF-β2	Human	NS0	302-B2
	Mouse	CHO	7346-B2
TGF-β3	Human	*Sf*21 (baculovirus)	243-B3
	Human	CHO	8420-B3

■ Indicates an R&D Systems antibody.

Application Key: **Depl** Depletion **FA** Functional Assay **FC** Flow Cytometry **ICC/IF** Immunocytochemistry/Immunofluorescence **IHC** Immunohistochemistry **IP** Immunoprecipitation **WB** Western Blot

Fluorochrome-conjugated and Unlabeled Antibodies for Regulatory T Cell Identification & Characterization

R&D Systems and Novus Biologicals offer a wide selection of unlabeled and fluorochrome-conjugated antibodies for the identification and characterization of human and mouse regulatory T cells. Additionally, R&D Systems offers Human and Mouse Regulatory T Cell Multi-Color Flow Cytometry Kits, which provide three different fluorochrome-conjugated antibodies that can be used together for single-step staining of either human or mouse regulatory T cells.

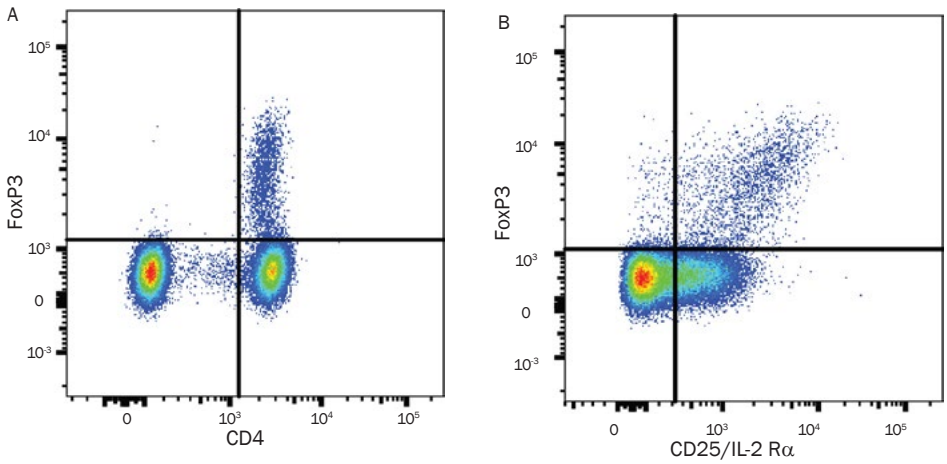
Antibodies Commonly used to Identify Regulatory T Cells by Flow Cytometry

	Molecule	Species	Clone	Fluorochrome-conjugated Antibodies for Flow Cytometry (Catalog #s)							
				APC	Fluorescein	PE	PerCP	Alexa Fluor® 488	Alexa Fluor® 700	Additional Alexa Fluor® conjugates 350/405/594/647/750	Unconjugated Antibodies (Applications)
■	CD3	Human	UCHT1	FAB100A	FAB100F	FAB100P	FAB100C	FB100G	FAB100N	FAB100V/FAB100T/ FAB100R/FAB100S	MAB100 (FA, FC, ICC/IF, IP)
■		Mouse	17A2	FAB4841A	FAB4841F	FAB4841P	FAB4841C	FAB4841G	FAB4841N	FAB4841V/FAB4841T/ FAB4841R/FAB4841S	MAB4841 (FA, FC, ICC/IF, IHC, IP)
■		Mouse	145-2C11	NBP2-30149APC		NBP2-30149PE	NBP2-30149PCP	FAB484G	FAB484N	FAB484U/FAB484V/ FAB484T/FAB484R/ FAB484S	NBP2-30151 (FC); MAB484 (Depl, FA, FC, IP)
■	CD4	Human	11830	FAB3791A	FAB3791F	FAB3791P	FAB3791C	FAB3791G	FAB3791N		
■		Human	RPA-T4	NBP2-27245	NBP2-27247	NBP2-27248	NBP2-27216PCP	NBP2-27216AF488	NBP2-27216AF700	NBP2-27216AF405/ NBP2-27216AF647	NBP2-25199 (B/N, FC, IHC, IV)
■		Mouse	GK1.5	FAB554A	FAB554F	FAB554P	FAB554C	FAB554G	FAB554N	FAB554V/FAB554T/ FAB554R/FAB554S	MAB554 (Depl, FA, FC, IHC, IP)
■	CD25/IL-2 Rα	Human	24212	FAB1020A		FAB1020P		FAB1020G			MAB1020 (FC, WB)
■		Human	BC96	NBP1-43049	NB100-77772	NBP1-43879					NBP1-43430 (FC)
■		Mouse	280406	FAB2438A		FAB2438P	FAB2438C	FAB2438G			MAB2438 (FC)
■		Mouse	PC61	NBP2-30135	NBP2-30134	NBP2-27426	NBP2-27425PCP	NBP2-27425AF488	NBP2-27425AF700	NBP2-27425AF405/ NBP2-27425AF647	NBP2-27425 (FC)
■	FoxP3	Human/ Mouse	1054C	IC8214A		IC8214P		IC8214G	IC8214N	IC8214R	MAB8214 (FC, ICC/ IF, IHC)
■		Human/ Mouse/ Rat	376209			IC8970P				IC8970R	
■		Human/ Mouse	3G3	NBP2-26671	NBP2-26668	NBP2-33297PE	NB100-56582PCP	NB100-56582AF488	NB100-56582AF700	NB100-56582AF405/ NB100-56582AF647	NB100-56582 (FC, WB)

■ Indicates an R&D Systems antibody. ■ Indicates a Novus Biologicals antibody.
Application Key: **Depl** Depletion **FA** Functional Assay **FC** Flow Cytometry **ICC/IF** Immunocytochemistry/Immunofluorescence **IHC** Immunohistochemistry **IP** Immunoprecipitation **WB** Western Blot

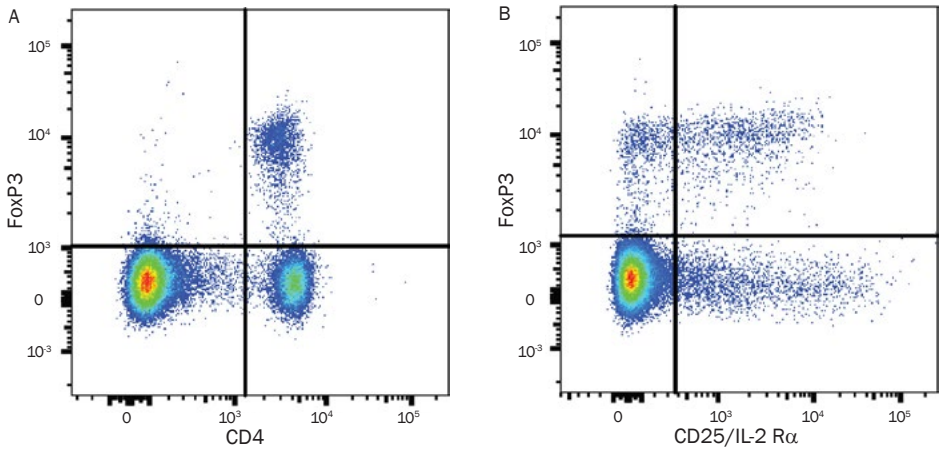
Regulatory T Cell Multi-Color Flow Cytometry Kits from R&D Systems	
FlowX Human Regulatory T Cell Multi-Color Flow Kit	**Catalog # FMC021**
Kit Contents	
APC-conjugated CD25 (clone 24212)	
Fluorescein-conjugated CD4 (clone 11830)	
PE-conjugated FoxP3 (clone 1054C)	
PE-conjugated Rabbit IgG Control	
All necessary staining buffers	
FlowX Mouse Regulatory T Cell Multi-Color Flow Kit	**Catalog # FMC022**
Kit Contents	
APC-conjugated CD25 (clone 280406)	
Fluorescein-conjugated CD4 (clone GK1.5)	
PE-conjugated FoxP3 (clone 1054C)	
PE-conjugated Rabbit IgG Control	
All necessary staining buffers	

Identification of Human FoxP3⁺ Regulatory T Cells in PBMCs by Flow Cytometry



Detection of FoxP3⁺ Regulatory T Cells in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells were surface stained with (**A**) a Fluorescein-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (R&D Systems, Catalog # FAB3791F) and (**B**) an APC-conjugated Mouse Anti-Human IL-2 Rα/CD25 Monoclonal Antibody (R&D Systems, Catalog # FAB1020A), followed by intracellular staining using a PE-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Antigen Affinity-purified Monoclonal Antibody (R&D Systems, Catalog # IC8970P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation and Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Cells were gated on lymphocytes.

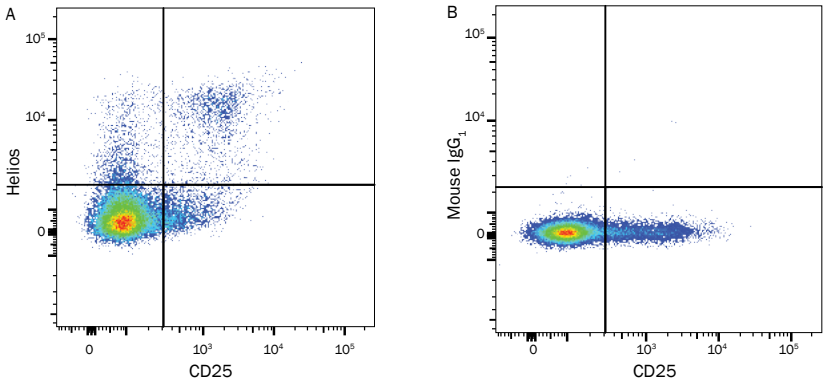
Identification of Mouse FoxP3⁺ Regulatory T Cells by Flow Cytometry



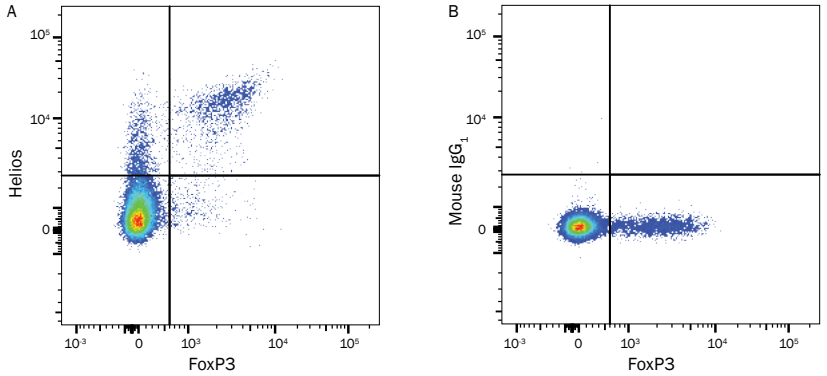
Detection of FoxP3⁺ Regulatory T Cells in Mouse Splenocytes by Flow Cytometry. C57BL/6 mouse splenocytes were surface stained with (**A**) an Alexa Fluor® 488-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (R&D Systems, Catalog # FAB554G) and (**B**) an APC conjugated Rat Anti-Mouse IL-2 Rα/CD25 Monoclonal Antibody (R&D Systems, Catalog# FAB2438A), followed by intracellular staining using a PE-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8970P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation and Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Cells were gated on lymphocytes.

Analysis of Additional Markers on Regulatory T Cells by Flow Cytometry

Detection of Helios on Human CD25$^+$ or FoxP3$^+$ Cells

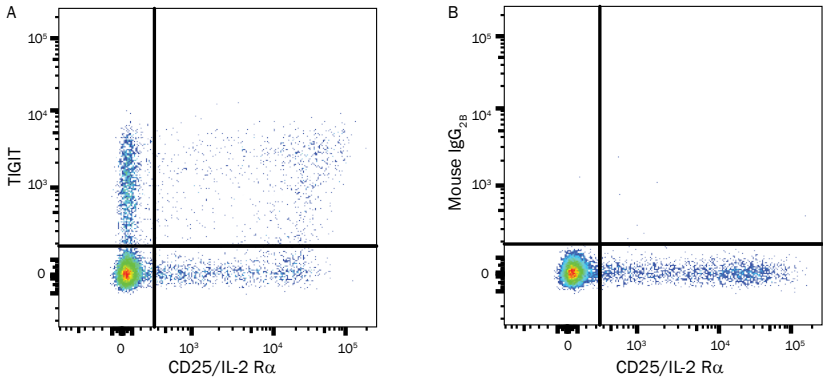


Detection of Helios$^+$CD25$^+$ Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells were stained with an Alexa Fluor® 488-conjugated Mouse Anti-Human CD25/IL-2 Rα Monoclonal Antibody (R&D Systems, Catalog # FAB1020G) and either (**A**) a PE-conjugated Mouse Anti-Human Helios Monoclonal Antibody (R&D Systems, Catalog # IC73092P) or (**B**) a PE-conjugated Mouse IgG_1 Isotype Control (R&D Systems, Catalog # IC002P). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (R&D Systems, Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (R&D Systems, Catalog # FC005).

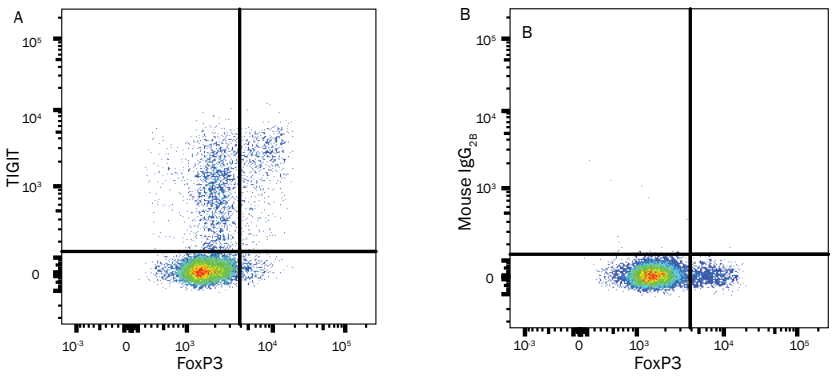


Detection of Helios$^+$FoxP3$^+$ Human Regulatory T Cells by Flow Cytometry. Human peripheral blood mononuclear cells were stained with an Alexa Fluor® 647-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8970R) and either (A) a PE-conjugated Mouse Anti-Human Helios Monoclonal Antibody (R&D Systems, Catalog # IC73092P) or (**B**) a PE-conjugated Mouse IgG_1 Isotype Control (R&D Systems, Catalog # IC002P). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (R&D Systems, Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (R&D Systems, Catalog # FC005).

Detection of TIGIT on Human CD25$^+$ or FoxP3$^+$ Cells

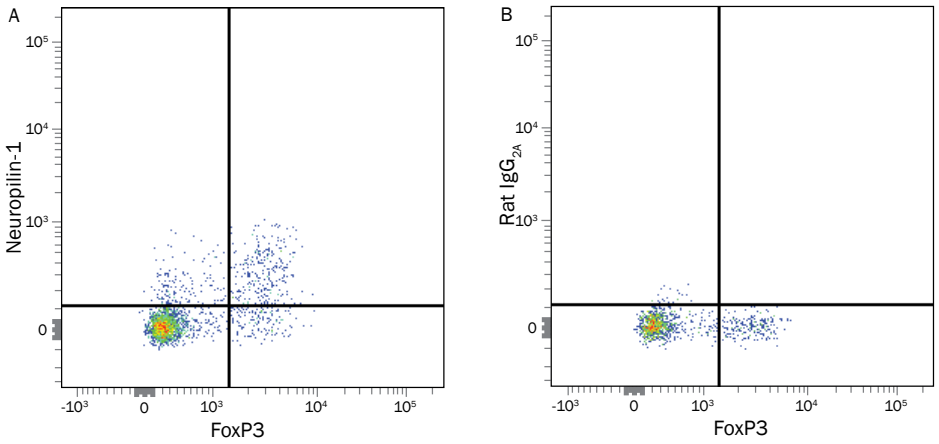


Detection of TIGIT$^+$CD25$^+$ Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells were stained with a PE-conjugated Mouse Anti-Human CD25/IL-2 Rα Monoclonal Antibody (R&D Systems, Catalog # FAB1020P) and either (**A**) an APC-conjugated Mouse Anti-Human TIGIT Monoclonal Antibody (R&D Systems, Catalog # FAB7898A) or (**B**) an APC-conjugated Mouse IgG_{2B} Isotype Control (R&D Systems, Catalog # IC0041A).



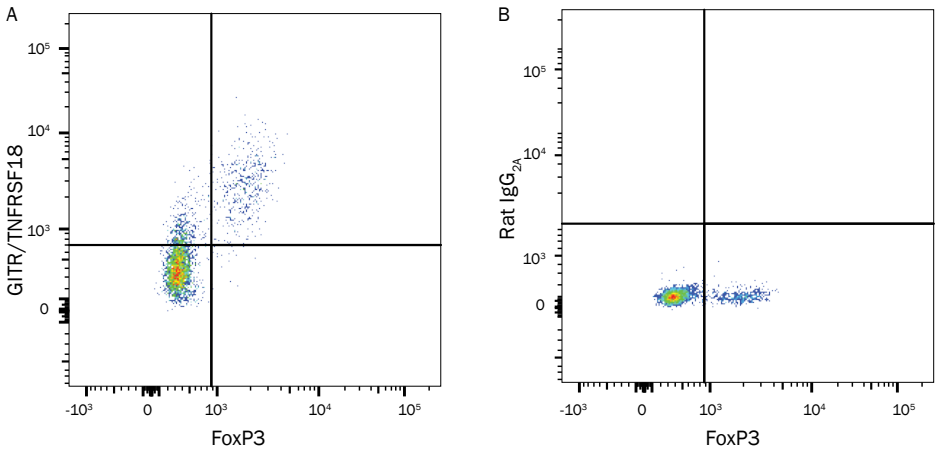
Detection of TIGIT$^+$FoxP3$^+$ Human Regulatory T Cells by Flow Cytometry. Human peripheral blood mononuclear cells were stained with an Alexa Fluor® 488-conjugated Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8214G) and either (**A**) an APC-conjugated Mouse Anti-Human TIGIT Monoclonal Antibody (R&D Systems, Catalog # FAB7898A) or (**B**) an APC-conjugated Mouse IgG_{2B} Isotype Control (R&D Systems, Catalog # IC0041A).

Detection of Neuropilin-1 on CD4$^+$FoxP3$^+$ Mouse Splenocytes



Detection of Neuropilin-1 on CD4$^+$FoxP3$^+$ Mouse Splenocytes by Flow Cytometry. CD4$^+$ mouse splenocytes were stained with an Alexa Fluor® 488-conjugated Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8214G) and either (**A**) an Alexa Fluor® 647-conjugated Rat Anti-Mouse Neuropilin-1 Monoclonal Antibody (R&D Systems, Catalog # FAB5994R) or (**B**) an Alexa Fluor® 647-conjugated Rat IgG$_{2A}$ Isotype Control (R&D Systems, Catalog # IC006R). To facilitate intracellular staining of FoxP3, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Splenocytes were gated on CD4$^+$ cells.

Detection of GITR on CD4$^+$FoxP3$^+$ Mouse Splenocytes



Detection of GITR on CD4$^+$FoxP3$^+$ Mouse Splenocytes by Flow Cytometry. CD4$^+$ mouse splenocytes were stained with an Alexa Fluor® 488-conjugated Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8214G) and either (**A**) a PE-conjugated Rat Anti-Mouse GITR/TNFRSF18 Monoclonal Antibody (R&D Systems, Catalog # FAB5241P) or (**B**) a PE-conjugated Rat IgG$_{2A}$ Isotype Control (R&D Systems, Catalog # IC006P). To facilitate intracellular staining of FoxP3, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Splenocytes were gated on CD4$^+$ cells.

Fluorochrome-conjugated and Unlabeled Antibodies *continued*

Antibodies Commonly used to Characterize Regulatory T Cells by Flow Cytometry

Molecule	Species	Clone	Fluorochrome-conjugated Antibodies for Flow Cytometry (Catalog #s)							Unconjugated Antibodies (Applications)
			APC	Fluorescein	PE	PerCP	Alexa Fluor® 488	Alexa Fluor® 700	Additional Alexa Fluor® conjugates 405/594/647/750	
4-1BB/TNFRSF9	Human	145501			FAB838P					MAB838 (FC, WB)
	Human	4B4-1	NB100-77887APC		NB100-77887PE	NB100-77887PCP	NB100-77887AF488	NB100-77887AF700	NB100-77887AF405/ NB100-77887AF647	NB100-77887 (E, FC, IP)
	Mouse	158332			FAB937P					MAB937 (E, FC, WB)
5' Nucleotidase/ CD73	Human	606112	FAB5795A		FAB5979P					MAB5795 (FC)
	Mouse	496406	FAB4488A	FAB4488F	FAB4488P					MAB4488 (FC)
BLIMP1	Human	646702	IC36081A		IC36081P		IC36081G	IC36081N	IC36081R	MAB36081 (ICC/IF, WB)
CCR2	Human	48607	FAB151A		FAB151P	FAB151C	FAB151G	FAB151N		MAB150(FC, IHC)
	Mouse	475301	FAB5538A	FAB5538F	FAB5538P			FAB5538N	FAB5538T/FAB5538R/ FAB5538S	
CCR7	Human	150503	FAB197A	FAB197F	FAB197P	FAB197C	FAB197G	FAB197N	FAB197T/FAB197R	MAB197 (B/N, FC, ICC/IF)
	Mouse	4B12	FAB3477A		FAB3477P					MAB3477 (B/N, FC, ICC/ IF)
CD8a	Human	37006	FAB1509A	FAB1509F	FAB1509P	FAB1509C	FAB1509G	FAB1509N	FAB1509V/FAB1509T/ FAB1509R/FAB1509S	MAB1059 (FC, ICC/IF)
	Human	C8/144B	NBP2-34588APC		NBP2-34588PE	NBP2-34588PCP	NBP2-34588AF488	NBP2-34588AF700	NBP2-34588AF405/ NBP2-34588AF647	NBP2-32836 (FC, ICC/IF, IHC, IP, WB)
	Human	RPA-T8	NBP2-27246	NBP2-27235	NBP2-27237	NBP2-25195PCP	NBP2-25195AF488	NBP2-25195AF700	NBP2-25195AF405/ NBP2-25195AF647	NBP2-25195 (FC, IHC, IV)
	Mouse	53-6.7	FAB116A	FAB116F	FAB116P	FAB116C	FAB116G		FAB116V/FAB116T/FAB116R/ FAB116S	MAB116 (Depl, FA, FC, ICC/IF, IP)
CD39/ENTPD1	Human	498403	FAB4397A	FAB4397F	FAB4397P					MAB4397 (FC)
	Mouse	495826	FAB4398A	FAB4398F	FAB4398P					MAB4398 (FC, IP, WB)
CD69	Human	298614	FAB23591A	FAB23591F	FAB23591P					MAB23591 (FC, ICC/IF)
	Human	FN50	NBP1-43387APC	NBP1-43992	NBP1-43387PE	NBP1-43387PCP	NBP1-43387AF488	NBP1-43387AF700	NBP1-43387AF405/ NBP1-43387AF647	NBP1-43387 (FC, IHC)
	Mouse	310106	FAB2386A	FAB2386F	FAB2386P					MAB2386 (FC, WB)
	Mouse	H1.2F3	NBP1-28011APC	NBP1-28012	NBP1-28011PE	NBP1-28011PCP	NBP1-28011AF488	NBP1-28011AF700	NBP1-28011AF405/ NBP1-28011AF647	NBP1-28011 (FC, IHC, IP, IV)
CTLA-4	Human	Polyclonal	FAB386A		FAB386P					AF-386-PB (FC, ICC/IF, WB)
	Mouse	63828	FAB434A	FAB434F	FAB434P					MAB434 (B/N, FC, WB)
CXCR5	Human	51505	FAB190A	FAB190F	FAB190P	FAB190C		FAB190N		MAB190 (B/N, FC, ICC/IF, IHC)
	Mouse	614641	FAB6198A	FAB6198F	FAB6198P	FAB6198C				MAB6198 (FC, ICC/IF)
DNAM-1/CD226	Human	102511	FAB666A	FAB666F	FAB666P					MAB666 (B/N, FC, WB)
	Mouse	838216	FAB4436A		FAB4436P					MAB4436 (FC)
FCRL3/FcRH3	Human	546828	FAB3126A		FAB3126P		FAB3126G			MAB3126 (FC)
Galectin-1	Mouse	Polyclonal			IC1245P					AF1245 (ELISA, FC, IHC, WB)
GITR/TNFRSF18	Human	110416	FAB689A	FAB689F	FAB689P		FAB689G	FAB689N		MAB689 (B/N, ELISA, FC, WB)
	Mouse	108619	FAB5241A	FAB5241F	FAB5241P					MAB5241 (FC, WB)
	Mouse	DTA-1	NBP2-26670	NBP2-26664	NBP2-26669	NBP2-26661PCP	NBP2-26661AF488	NBP2-26661AF700	NBP2-26661AF405/ NBP2-26661AF647	NBP2-26661 (FC, IP)
Granzyme A	Human	356412	IC29051A	IC29051F	IC29051P					MAB29051 (FC, ICC/IF, IP)
Granzyme B	Human	351927	IC2906A		IC2906P		IC2906G			MAB2906 (FC, ICC/IF, WB)
Helios	Human	736440			IC73092P					MAB73092 (FC)
	Mouse	22F6								NBP2-37723 (FC)
ICOS	Human	669222	FAB6975A		FAB6975P					MAB6975 (FC)
	Mouse	670306	FAB168A		FAB168P					MAB168 (FC)
IL-7 Rα/CD127	Human	40131	FAB306A		FAB306P	FAB306C	FAB306G	FAB306N		MAB306 (FC, WB)
	Mouse	A7R34	FAB47742A		FAB47742P		FAB47742G	FAB47742N		
IL-10	Human	127107		IC2172F	IC2172P					
	Human	JES3-9D7								NBP2-27574 (E, FC, WB)
	Mouse	AP-MAB0851	NBP1-06673APC		NBP1-06673PE	NBP1-06673PCP	NBP1-06673AF488	NBP1-06673AF700	NBP1-06673AF405/ NBP1-06673AF647	NBP1-06673 (FC, IP)

Application Key: **B/N** Blocking/Neutralization **ChIP** Chromatim Immunoprecipitation **Depl** Depletion **E** ELISA **FA** Functional Assay **FC** Flow Cytometry **ICC/IF** Immunocytochemistry/Immunofluorescence **IHC** Immunohistochemistry **IP** Immunoprecipitation **IV** *In vitro* **WB** Western Blot

Indicates an R&D Systems antibody.
Indicates a Novus Biologicals antibody.

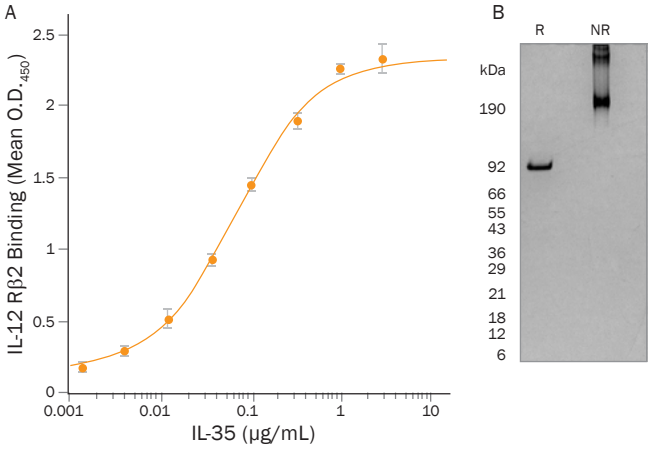
Antibodies Commonly used to Characterize Regulatory T Cells by Flow Cytometry											
Molecule	Species	Clone	Fluorochrome-conjugated Antibodies for Flow Cytometry (Catalog #s)								
			APC	Fluorescein	PE	PerCP	Alexa Fluor®		Additional Alexa Fluor® conjugates 405/594/647/750	Unconjugated Antibodies (Applications)
							488	700		
IL-12/IL-35 p35	Human/ Mouse	27537	IC2191A	IC2191F	IC2191P	IC2191C				MAB1570 (FC, WB)
IL-27 Rα/WSX-1/ TCCR	Human	191106	FAB14791A		FAB14791P		FAB14791G			
	Mouse	263503		FAB21091F	FAB21091P			FAB21091N		MAB21091 (FC, WB)
Integrin α2/CD49b	Human	HAS3			FAB1233P					MAB1233 (FC, ICC/IF, IP)
	Mouse	235033	FAB1740A		FAB1740P					MAB1740 (FC)
	Mouse	DX5	NBP1-28114	NBP1-28110	NBP1-28113					
Integrin αE/CD103	Human	Ber-ACT8	NBP1-97564APC	NBP1-97568	NBP1-97564PE	NBP1-97564PCP	NBP1-97564AF488	NBP1-97564AF700	NBP1-97564AF405/ NBP1-97564AF647	NBP1-97564 (FC, IHC, IP, WB)
	Mouse	Polyclonal	FAB1990A		FAB1990P		FAB1990G			
	Mouse	2 E7	NBP1-43024	NBP1-28124	NBP1-28126					NBP1-28123 (FC, IHC, IP, IV)
KLRG1	Mouse	1151A	FAB6944A		FAB6944P		FAB6944G			MAB6944 (FC)
	Mouse	2F1	NBP1-28115APC	NBP1-28116	NBP1-28115PE	NBP1-28115PCP	NBP1-28115AF488	NBP1-28115AF700	NBP1-28115AF405/ NBP1-28115AF647	NBP1-28115 (FC, IP)
LAG-3	Human	874501	FAB23193A		FAB23193P		FAB23193G	FAB23193N		MAB23193 (FC)
	Human	Polyclonal	FAB2319A	FAB2319F	FAB2319P	FAB2319C				AF2319 (FC, WB)
	Mouse	C9B7W	NB100-63601APC		NB100-63601PE	NB100-63601PCP	NB100-63601AF488	NB100-63601AF700	NB100-63601AF405/ NB100-63601AF647	NB100-63601 (FC)
LAP (TGF-β1)	Human	27232	FAB2463A		FAB2463P	FAB2463C	FAB2463G	FAB2463N		MAB2463 (FC)
LRRC32/GARP	Human	855151	FAB6055A		FAB6055P		FAB6055G			MAB6055 (FC)
	Mouse	725226	FAB62291A			FAB62291C	FAB62291G	FAB62291N		MAB62291 (FC)
Neuropilin-1/BDCA4	Human	446921	FAB3870A	FAB3870F	FAB3870P	FAB3870C		FAB3870N		MAB3870 (FC)
	Mouse	761705	FAB5994A		FAB5994P		FAB5994G	FAB5994N	FAB5994R	MAB5994 (FC)
	Mouse	761704								MAB59941 (B/N, FC)
	Mouse/ Rat	Polyclonal	FAB566A	FAB566F	FAB566P	FAB566C		FAB566N		AF566 (B/N, FC, IHC, WB)
OX40/TNFRSF4	Human	443318	FAB3388A	FAB3388F	FAB3388P					MAB3388 (FC, WB)
	Mouse	Polyclonal			FAB1256P					
	Mouse	OX-86		NB100-63408	NB100-63410					NB100-64847 (FC, IHC)
PD-1	Human	Polyclonal								AF1086 (B/N, E, FC, IHC, WB)
	Human	Polyclonal			FAB7115P		FAB7115G			
	Human	J116	NBP1-43107APC		NBP1-43107PE	NBP1-43107PCP	NBP1-43107AF488	NBP1-43107AF700	NBP1-43107AF405/ NBP1-43107AF647	NBP1-43107 (FC, IHC, IP, WB)
	Mouse	766104	FAB7738A		FAB7738P		FAB7738G			MAB7738 (FC)
	Mouse	Polyclonal		FAB1021F	FAB1021P					AF1021 (FC, IHC, WB)
	Mouse	J43		NBP1-43911						NBP1-43110 (FC, IHC, IP)
L-Selectin/CD62L	Human	4G8		BBA33						BBA24 (ELISA, FC)
	Human	DREG56	NBP1-42795APC	NBP1-42791	NBP1-42795PE	NBP1-42795PCP	NBP1-42795AF488	NBP1-42795AF700	NBP1-42795AF405/ NBP1-42795AF647	NBP1-42795 (FA, FC, IHC, IP, WB)
	Mouse	95218		FAB5761F	FAB5761P					MAB5761 (FC)
	Mouse	MEL-14	NBP1-28010	NBP1-28007	NB100-63971					NBP2-00260 (FC, IHC, IP)
ST2/IL-1 R4	Human	Polyclonal	FAB5231A		FAB5231P					AF523 (B/N, FC, WB)
	Mouse	245707	FAB10041A		FAB10041P			FAB10041N		MAB10041 (B/N, E, FC)
STAT5a	Human	251610		IC21741F	IC21741P					MAB21741 (FC, ICC/IF)
STAT5b	Human	389215	IC1584A							MAB1584 (FC, WB)
TGF-β1	Human	9016	IC240A	IC240F	IC240P					MAB240 (B/N, ELISA, FC, IHC, WB)
	Mouse	860206								MAB7666 (FC)
TGF-β RII	Human	25508	FAB241A	FAB241F	FAB241P	FAB241C		FAB241N		
	Human	Polyclonal	FAB2411A	FAB2411F	FAB2411P			FAB2411N		AF-241-NA (B/N, E, FC, IHC, WB)
	Mouse	Polyclonal	FAB532A	FAB532F	FAB532P	FAB532C		FAB532N		AF532 (FC, WB)
TIGIT	Human	741182	FAB7898A		FAB7898P			FAB7898N		MAB7898 (FC)
	Mouse	Polyclonal	FAB7267A				FAB7267G			AF7267 (FC)
VEGF R1	Human	49560	FAB321A		FAB321P					MAB321 (FC, WB)
	Mouse	141522	FAB4711A		FAB4711P		FAB4711G	FAB4711N		MAB4711 (FC, WB)

Recombinant Proteins for Functional Characterization of Regulatory T Cell-Expressed Molecules

R&D Systems offers a wide selection of recombinant and natural proteins that can be used to characterize the effects of proteins expressed by regulatory T cells both on their own activity and on other immune cells. Stringent production and purification standards ensure that R&D Systems® proteins will provide researchers with industry-leading bioactivity and lot-to-lot consistency. Our current portfolio includes more than 4,800 proteins that we manufacture under standard conditions, along with Animal-Free™ and GMP-grade recombinant proteins as well as custom protein development services.

New! Bioactive Recombinant Human IL-35 Fc Chimera Protein

IL-35 is receiving increasing attention due to its immunosuppressive functions. R&D Systems now offers a Recombinant Human IL-35 Fc Chimera Protein (Catalog # 8608-IL) produced from HEK293 cells with activity in the low ng/mL range.



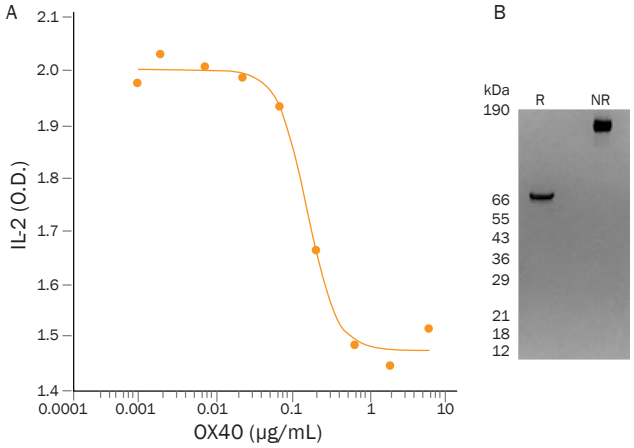
Recombinant Human IL-35 Binds IL-12 Rβ2. (**A**) Recombinant Human IL-12 Rβ2 Fc Chimera (R&D Systems, Catalog # 1959-B2) was coated onto microplate wells at 5 µg/mL and the indicated concentrations of Recombinant Human IL-35 Fc Chimera (R&D Systems, Catalog # 8608-IL) were added. The concentration of Recombinant Human IL-35 Fc Chimera that produces 50% optimal binding response is approximately 20–120 ng/mL. (**B**) The purity of Recombinant Human IL-35 Fc Chimera (R&D Systems, Catalog # 8608-IL) was assessed by SDS-PAGE analysis under reducing (R) and non-reducing (NR) conditions and visualized by silver staining.

Select Recombinant Proteins from R&D Systems for Functional Characterization of Regulatory T Cell-Expressed Molecules

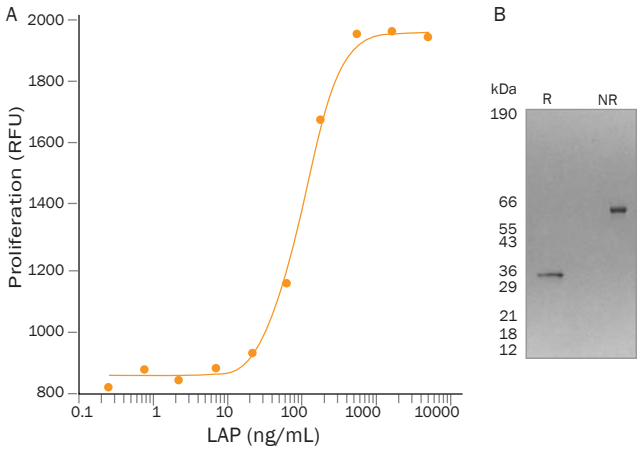
Molecule	Species	Source	Catalog #
4-1BB/TNFRSF9	Human	NS0	838-4B
	Mouse	NS0	937-4B
4-1BB Ligand/TNFSF9	Human	*E. coli*	2295-4L
	Mouse	NS0	1246-4L
B7-1/CD80	Human	NS0	140-B1
	Mouse	NS0	740-B1
B7-2/CD86	Human	NS0	141-B2
	Mouse	*Sf*21	741-B2
CD155/PVR	Human	NS0	2530-CD
	Mouse	NS0	6909-CD
CTLA-4	Human	CHO	7268-CT
	Human	*Sf*21	325-CT
	Mouse	NS0	434-CT
DNAM-1/CD226	Human	NS0	666-DN
	Mouse	NS0	4436-DN

Select Recombinant Proteins from R&D Systems for Functional Characterization of Regulatory T Cell-Expressed Molecules			
Molecule	**Species**	**Source**	**Catalog #**
Galectin-1	Human	*E. coli*	1152-GA
	Mouse	*E. coli*	1245-GA
GITR/TNFRSF18	Human	NS0	689-GR
	Mouse	NS0	524-GR
GITR Ligand/TNFSF18	Human	CHO	6987-GL
	Human	*Sf*21	694-GL
	Mouse	NS0	2177-GL
IL-10	Human	*Sf*21 (baculovirus)	217-IL
	Human	*Sf*21 (stably transfected)	217-ILB
	Human	*E. coli*	1064-IL
	Mouse	*E. coli*	417-ML
IL-35	Human	HEK293	8608-IL
LAG-3	Human	NS0	2319-L3
	Mouse	NS0	3328-L3
LAP (TGF-β1)	Human	*Sf*21	246-LP
LRRC32/GARP	Human	CHO	6055-LR
	Mouse	CHO	6229-LR
Neuropilin-1	Human	NS0	3870-N1
	Mouse	NS0	5994-N1
OX40/TNFRSF4	Human	NS0	3388-OX
	Mouse	NS0	1256-OX
OX40 Ligand/TNFSF4	Human	NS0	1054-OX
	Mouse	NS0	1236-OX
TIGIT	Human	CHO	7898-TG
	Mouse	NS0	7267-TG
TGF-β1	Human	CHO	240-B
		HEK293	7754-BH
	Mouse	CHO	7666-MB
TGF-β2	Human	NS0	302-B2
	Mouse	CHO	7346-B2
TGF-β3	Human	*Sf*21 (baculovirus)	243-B3
	Human	CHO	8420-B3

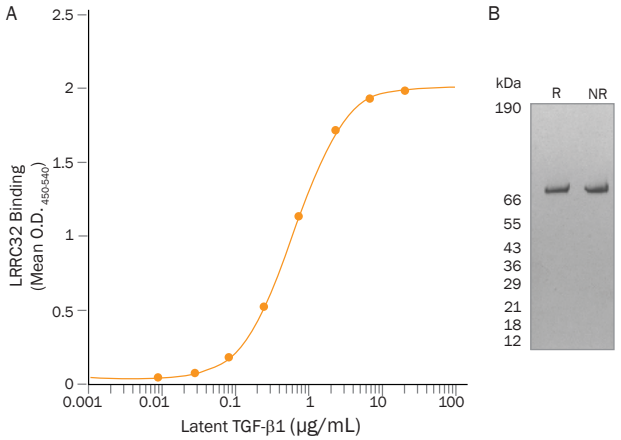
Data for Additional Select Regulatory T Cell-related Recombinant Proteins from R&D Systems



OX40 Suppresses OX40 Ligand-Induced IL-2 Production by Mouse T Cells. (**A**) Mouse T cells were treated with Recombinant Mouse OX40 Ligand (R&D Systems, Catalog # 1236-OX; 30 ng/mL) and the indicated concentrations of Recombinant Mouse OX40 Fc Chimera (R&D Systems, Catalog # 1256-OX). IL-2 secretion was measured in cell culture supernatants using the Mouse IL-2 Quantikine® ELISA Kit (R&D Systems, Catalog # M2000). (**B**) The purity of Recombinant Mouse OX40 Fc Chimera (R&D Systems, Catalog # 1256-OX) was assessed by SDS-PAGE analysis under reducing (R) and non-reducing (NR) conditions and visualized by silver staining.



LAP Suppresses TGF-β1-Mediated Inhibition of Helper T Cell Proliferation. (**A**) The mouse HT2 helper T cell line was treated with Recombinant Human TGF-β1 (R&D Systems, Catalog # 240-B; 1 ng/mL) and the indicated concentrations of Recombinant Human LAP (R&D Systems, Catalog # 246-LP). T cell proliferation was measured using Resazurin (R&D Systems, Catalog # AR002). (**B**) The purity of Recombinant Human LAP (R&D Systems, Catalog # 246-LP) was assessed by SDS-PAGE analysis under reducing (R) and non-reducing (NR) conditions and visualized by silver staining.



Recombinant Human LRRC32 Binds to Human Latent TGF-β1. (**A**) Recombinant Human LRRC32 (R&D Systems, Catalog # 6055-LR) was coated onto microplate wells at 5 µg/mL and the indicated concentrations of Recombinant Human Latent TGF-β1 (R&D Systems, Catalog # 299-LT) were added. Latent TGF-β1 bound to LRRC32 in a dose-dependent manner with an apparent K_D < 30 nM. (**B**) The purity of Recombinant Human LRRC32 (R&D Systems, Catalog # 6055-LR; 1 µg/lane) was assessed by SDS-PAGE analysis under reducing (R) and non-reducing (NR) conditions and visualized by silver staining.

Regulatory T Cell-related ELISA Kits

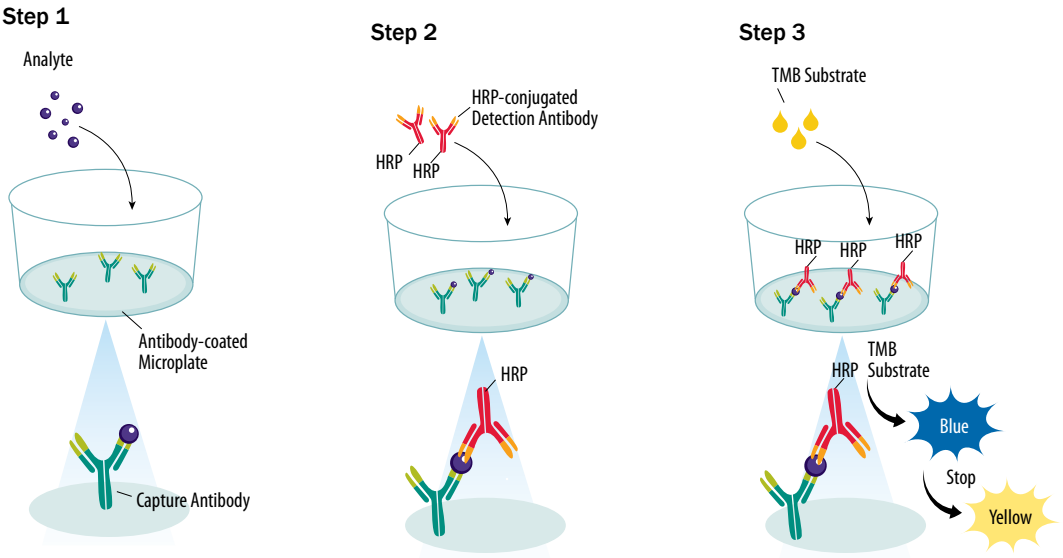
R&D Systems offers complete, ready-to-run Quantikine® ELISA Kits and the more flexible DuoSet® ELISA Development Systems for detecting molecules secreted by regulatory T cells.

Quantikine® ELISA Kits

Features

- Complete, ready-to-use kits
- Exhaustively tested for superior quality and reproducibility
- Detailed protocol booklets
- Colorimetric detection

Assay Principle



A microplate pre-coated with capture antibody is provided. Samples or standards are added and any analyte present is bound by the immobilized antibody. Unbound materials are washed away (Step 1). A second HRP-labeled detection antibody is added and binds to the captured analyte. Unbound detection antibody is washed away (Step 2). Tetramethylbenzidine (TMB) substrate solution is added to the wells and a blue color develops in proportion to the amount of analyte present in the sample. Color development is stopped turning the color in the wells to yellow. The absorbance of the color at 450 nm is measured (Step 3).

DuoSet® ELISA Development Systems

When complete kits are not an option, DuoSet® ELISA Development Systems offer an economical alternative. DuoSet® Kits contain the essential components required to develop an immunoassay, but unlike Quantikine® ELISA Kits, they require the user to set up the assay by coating a microplate with the provided capture antibody. DuoSet® Kits also provide a biotinylated detection antibody and streptavidin-HRP, enabling chemiluminescent or colorimetric detection, a mass-calibrated standard, and detailed protocol.

Features

- Provides sufficient reagents for five or fifteen 96-well plates
- Contains carefully selected and validated antibodies, reducing development time
- Includes mass-calibrated recombinant standard, reducing assay variability
- Can be adapted for use across multiple platforms

Select ELISAs for Detecting Molecules Secreted by Regulatory T Cells

Molecule	Species	Quantikine® ELISA Catalog #	DuoSet® ELISA Catalog #
Galectin-1	Human	DGAL10	DY1152
	Mouse		DY1245
Granzyme B	Human		DY2906
	Mouse		DY1865
IL-10	Human	D1000B	DY217B
	Mouse	M1000B	DY417
TGF-β1	Human	DB100B	DY240
	Mouse	MB100B	DY1679
TGF-β2	Human	DB250	DY302
	Mouse	MB200	DY7346
TGF-β3	Human		DY243

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