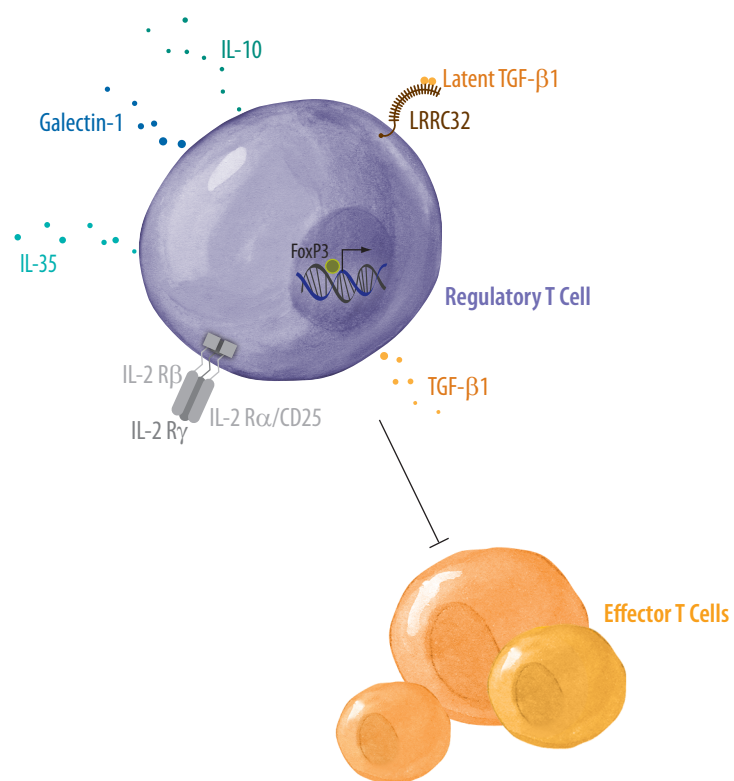


Regulatory T Cells



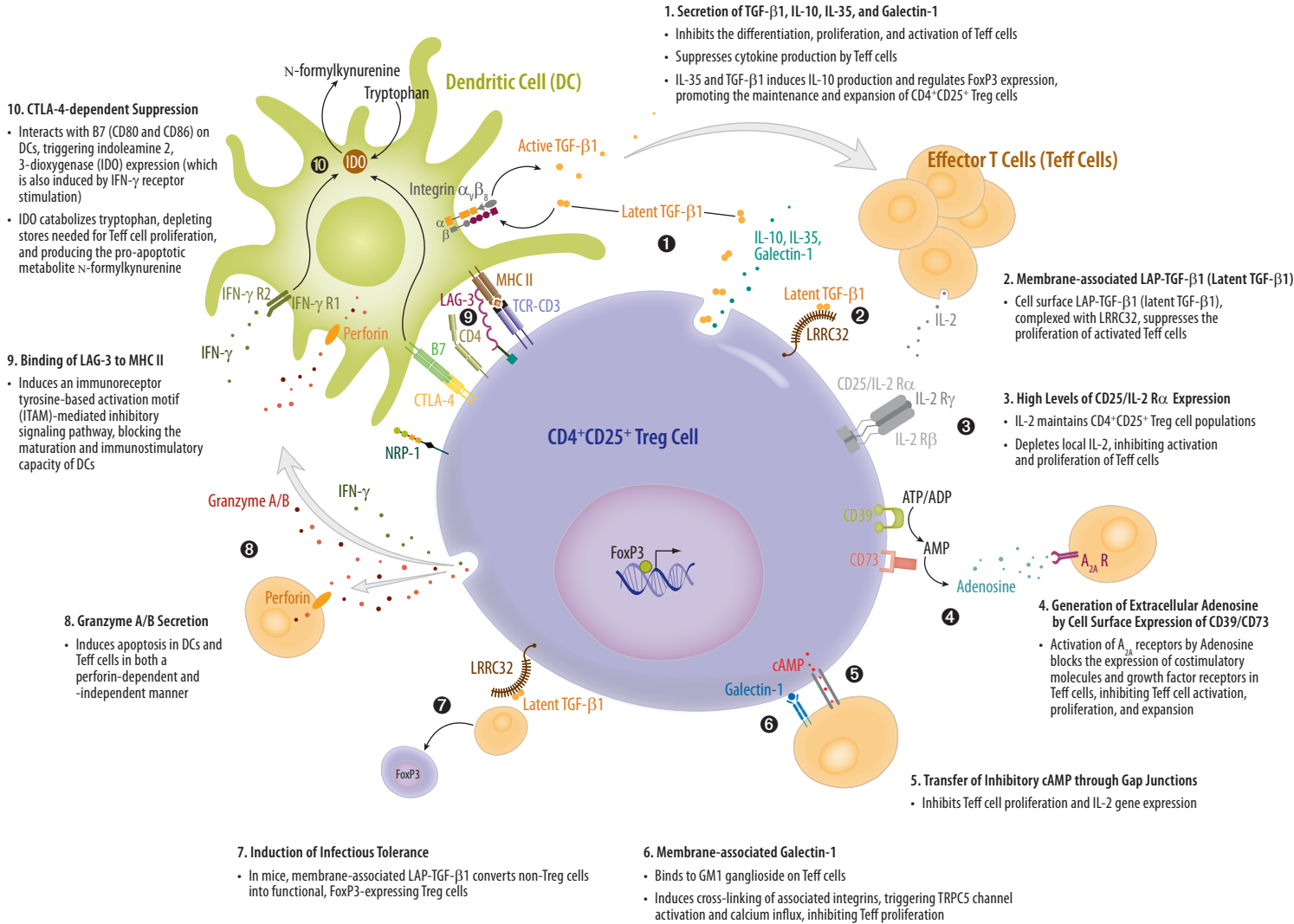
Regulatory T Cells

The immune system has regulatory mechanisms that prevent sustained inflammatory responses and attacks on healthy tissue. Regulatory T cells (Treg cells) play a role in maintaining immune homeostasis, preventing autoimmunity, moderating inflammation, and minimizing collateral damage to tissue. A primary function of Treg cells is to inhibit the function of antigen-presenting cells and effector T cells (Teff cells). Consequently, reduced Treg cell activity may be associated with human autoimmune diseases, including rheumatoid arthritis, type I diabetes, multiple sclerosis, systemic lupus erythematosus, and myasthenia gravis. In addition, Treg cells may play a causative role in aplastic anemia, graft-versus-host disease, and transplant rejection. CD4⁺ Treg cells are traditionally divided into 3 subsets. These include, naturally occurring CD4⁺CD25⁺ Treg cells that develop in the thymus and induced CD4⁺ Tregs, known as Tr1 and Th3 cells, that develop in the periphery. Although the characteristics of these subtypes continue to be defined, they typically have different surface markers, secreted products, and mechanisms of action (Table 1). Studies have suggested that the naturally occurring CD4⁺CD25⁺ Treg cells, which comprise 5-10% of the total peripheral CD4⁺ T cells, have a central role in immune control.

TABLE 1. Characteristics of CD4⁺ Treg Cells

Cell Type	Naturally Occurring Treg Cells CD4 ⁺ CD25 ⁺	Tr1 Cells CD4 ⁺ CD25 ⁻ /variable	Th3 Cells CD4 ⁺ CD25 ^{low} /variable
Differentiation Factors	CD28:B7 signaling IL-2	IL-10	TGF-β1
Associated Markers	FoxP3 ⁺ , CD127 ^{low} , LRRC32/GARP ⁺ , CD39 ⁺ , GITR ⁺ , NRP-1 ⁺ (mouse), CTLA-4 ⁺ , LAP ⁺	CTLA-4 ⁺ , LAP ⁺ , CD45RB ^{low} , FoxP3 ⁻	CTLA-4 ⁺ , LAP ⁺ , CD45RB ^{low} , FoxP3 ⁺
Proposed Regulatory Mechanisms	<ul style="list-style-type: none">Induction of cytotoxicityDisruption of metabolic activitiesInhibition of dendritic cell maturationSecretion of IL-10, IL-35, TGF-β1, Galectin-1	<ul style="list-style-type: none">Cell-cell contactSecretion of IL-10, TGF-β1, IFN-γ	<ul style="list-style-type: none">Secretion of TGF-β1

Suppressive Activities of Treg Cells



This illustration represents general pathways suggested in the scientific literature and is not to be considered comprehensive nor definitive.

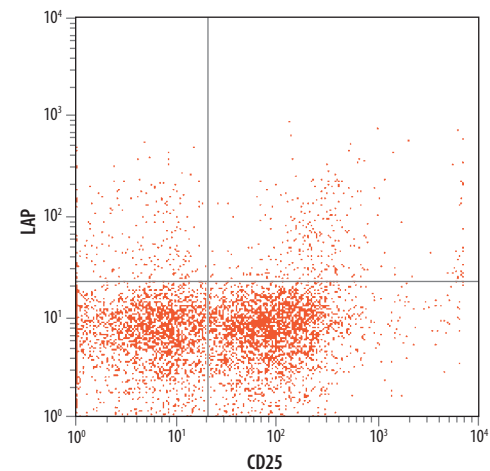
Flow Cytometry Markers for Treg Cells

SPECIES	APC	FLUORESC EIN	PE	PerCP
5'-Nucleotidase/CD73				
H	•		•	
M	•	•	•	
CD4				
H	•	•	•	•
M	•	•	•	•
CD25/IL-2 R α				
H	•		•	
M	•		•	
CD127/IL-7 R α				
H	•		•	
M			•	
CD39/ENTPD1				
H	•	•	•	
M	•	•	•	
CD101/IGSF2				
M	•	•	•	

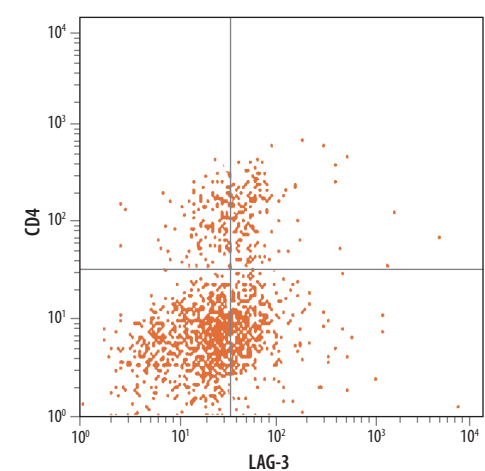
SPECIES	APC	FLUORESC EIN	PE	PerCP
CTLA-4				
H			•	
M		•		
FoxP3				
H/M/R	•			
GITR/TNFRSF18				
H	•	•	•	
M		•	•	
IL-10				
H		•	•	
IL-35/p35				
H/M	•	•	•	•
L-Selectin/CD62L				
H		•		
M		•	•	

SPECIES	APC	FLUORESC EIN	PE	PerCP
LAG-3/CD223				
H		•	•	•
M			•	
LAP (TGF- β 1)				
H/M	•		•	•
NRP-1				
H	•	•	•	•
M/R	•	•		
OX40/TNFRSF4				
H	•	•	•	
TGF- β 1				
Ms	•	•	•	
TGF- β 1, 2, 3				
Ms	•		•	

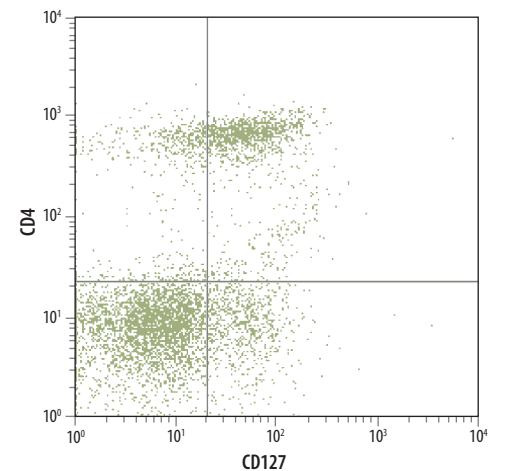
KEY: H: Human M: Mouse R: Rat Ms: Multispecies



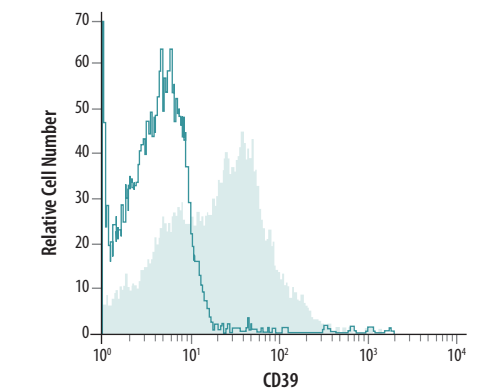
Detection of LAP on CD25⁺ Mouse Splenocytes. Splenocytes from BALB/c mice were labeled using APC-conjugated Mouse CD25/ IL-2 R α Monoclonal Antibody (Catalog # FAB2438A) and PE-conjugated Human/Mouse LAP Monoclonal Antibody (Catalog # FAB2463P). Quadrants were set based on isotype controls.



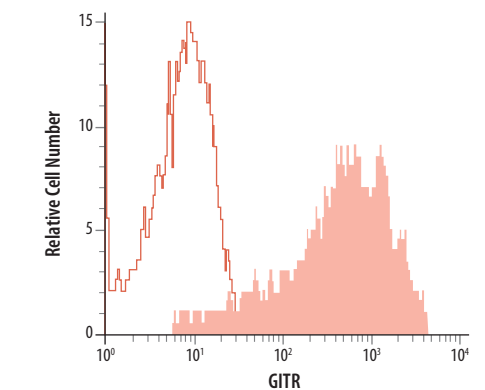
Detection of LAG-3 on Mouse Splenocytes. Mouse splenocytes were treated with PMA/ calcium ionomycin and then labeled using APC-conjugated Mouse CD4 Monoclonal Antibody (Catalog # FAB554A) and PE-conjugated Mouse LAG-3 Polyclonal Antibody (Catalog # FAB3328P). Quadrants were set based on isotype controls.



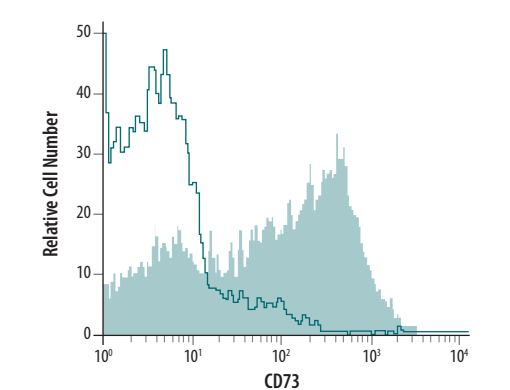
Detection of CD127 on CD4⁺ Mouse Splenocytes. Splenocytes from BALB/c mice were labeled using APC-conjugated Mouse CD4 Monoclonal Antibody (Catalog # FAB554A) and PE-conjugated Mouse CD127/IL-7 R α Antigen Affinity-purified Polyclonal Antibody (Catalog # FAB747P). CD127, which is present on most mature T cells, is absent on CD4⁺CD25⁺ Treg cells. Quadrants were set based on isotype controls.



Detection of CD39 on Mouse Splenocytes. Mouse splenocytes were labeled using APC-conjugated Mouse CD39/ENTPD1 Monoclonal Antibody (Catalog # FAB4398A; filled histogram) or an APC-conjugated Isotype Control Antibody (Catalog # IC005A; open histogram).



Detection of GITR on CD4⁺ Lymphocytes. Human peripheral blood CD4⁺ lymphocytes were stimulated with PHA and then labeled using APC-conjugated Human GITR Monoclonal Antibody (Catalog # FAB689A; filled histogram) or an APC-conjugated Mouse IgG, Isotype Control Antibody (Catalog # IC002A; open histogram).



Detection of CD73 on Mouse CD4⁺ Splenocytes. Mouse CD4⁺ splenocytes were stained using PE-conjugated Mouse 5'-Nucleotidase/CD73 Monoclonal Antibody (Catalog # FAB4488P; filled histogram) or a PE-conjugated Isotype Control Antibody (Catalog # IC003P; open histogram).

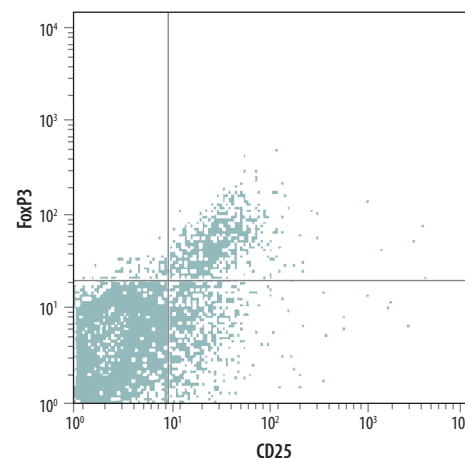
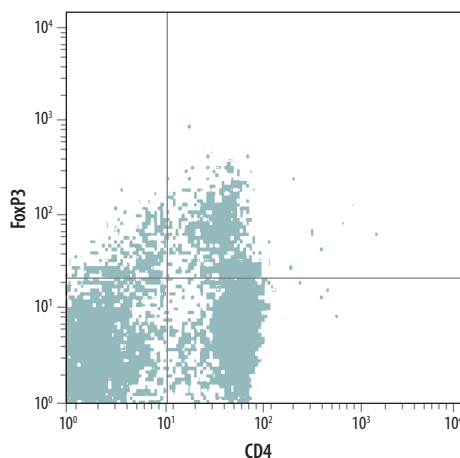
Treg Cell Multi-Color Flow Cytometry Kits

Kit Contents: (Contents also sold separately)

- APC-conjugated anti-FoxP3
- PE-conjugated anti-CD25
- PerCP-conjugated anti-CD4 or FITC-conjugated anti-CD4
- Specifically formulated staining buffers
- Goat IgG-APC isotype control

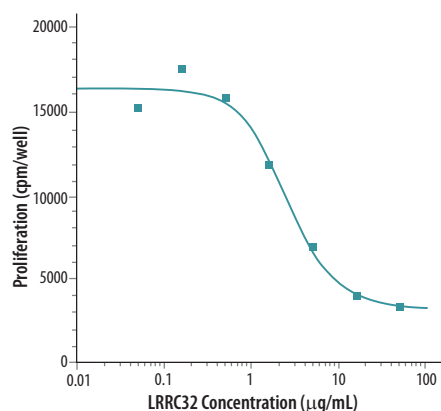
Species (Catalog #): Human (FMC013)
Mouse (FMC014)
Rat (FMC015)

Size: 50 Tests

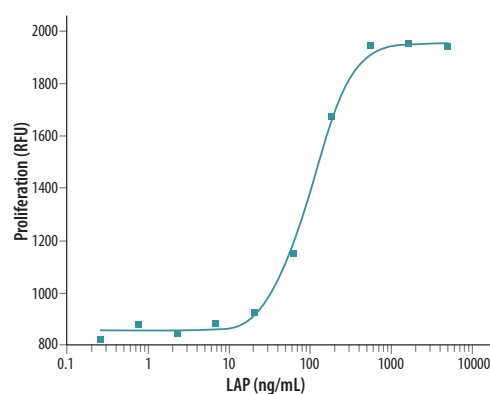


Detection of Human Treg Cells using Multi-Color Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were assessed for FoxP3, CD25, and CD4 expression using antibodies and buffers included in the Human Regulatory T Cell Multi-Color Flow Cytometry Kit (Catalog # FMC013). Quadrants were set based on isotype controls.

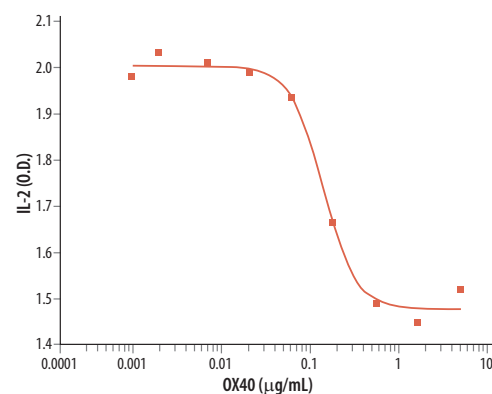
Proteins



Inhibition of T Cell Proliferation by LRRC32. The induction of Human T cell proliferation induced by 2 $\mu\text{g/mL}$ Human CD3 ϵ Monoclonal Antibody (Catalog # MAB100) was inhibited in a dose-dependent manner by Recombinant Human LRRC32 (Catalog # 6055-LR). T cell proliferation was measured by ^3H -thymidine incorporation.

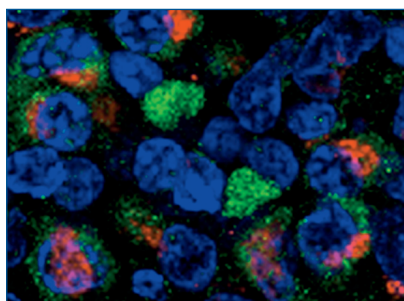


LAP Suppression of TGF- β 1 Activity. The ability of Recombinant Human TGF- β 1 (Catalog # 240-B; 1 ng/mL) to inhibit the proliferation of HT2 mouse helper T cells is suppressed by increasing concentrations of Recombinant Human LAP (Catalog # 246-LP) as measured using Resazurin (Catalog # AR002).



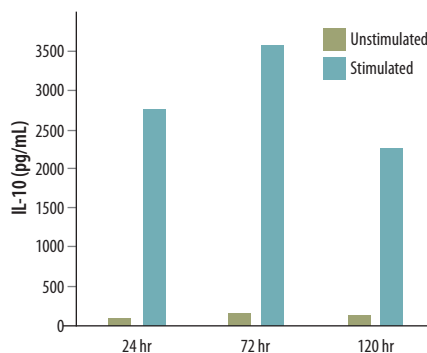
OX40 Suppresses OX40 Ligand-induced IL-2 Production. Increasing concentrations of Recombinant Mouse OX40 Fc Chimera (Catalog # 1256-OX) inhibit IL-2 production induced by Recombinant Mouse OX40 Ligand (Catalog # 1236-OX) in mouse T cell culture supernatants as measured using the Mouse IL-2 Quantikine[®] ELISA Kit (Catalog # M2000).

Antibodies for IHC & ICC



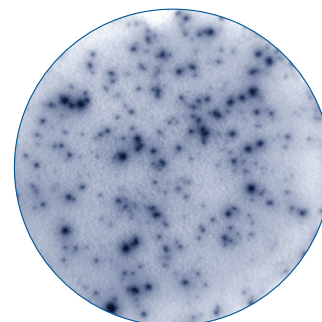
FoxP3 and CD4 in Human Tonsil. FoxP3 was detected in human tonsil tissue using Human FoxP3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3240) followed by staining with NorthernLights[™] 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001; red). CD4 was detected using Human CD4 Monoclonal Antibody (Catalog # MAB379) followed by staining with NorthernLights 493-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # NL009; green). The nuclei were counterstained with DAPI (blue).

ELISAs



Measurement of IL-10 Levels Using the Quantikine ELISA Kit. Human PBMCs were stimulated with 10 $\mu\text{g/mL}$ PHA for 24, 72, or 120 hours. Aliquots of the cell culture supernatants were assayed using the Human IL-10 Quantikine ELISA Kit (Catalog # D1000B).

ELISpot



Detection of Granzyme B-secreting CD4⁺ Cells using ELISpot. Mouse splenocytes enriched for CD4⁺ cells were assessed for Granzyme B secretion using the Mouse CD4⁺/Granzyme B ELISpot Kit (Catalog # EL6024). CD4⁺ cells were first enriched by a short incubation in ELISpot wells coated with anti-CD4. Following a wash, CD4-enriched cells were then stimulated overnight in culture media with PMA/ Ca^{2+} ionomycin. During the incubation, anti-mouse Granzyme B antibodies capture the secreted enzyme which is then visualized as blue spots.

R&D Systems Products for Treg Cell Research

MOLECULE	ANTIBODIES	CELL SELECTION KITS* ELISpot KITS*	RECOMBINANT & NATURAL PROTEINS	ELISAs/ ASSAYS
4-1BB/TNFRSF9/CD137	H M		H M	H M
5'-Nucleotidase/CD73	H M		H M	
B7-1/CD80	H M R		H M R	H M
B7-2/CD86	H M R		H M R	R
B7-H2	H M		H M	
E-Cadherin	H M	H M	H M	H M
cAMP	Ms			Ms
CCL1/I-309/TCA-3	H M		H M	H M
CCL4/MIP-1β	H M Ca CR		H M Ca CR	H M
CCL17/TARC	H M		H M	H M
CCL19/MIP-3β	H M		H M	H M
CCL20/MIP-3α	H M R		H M R	H M R
CCL22/MDC	H M		H M	H M
CCR2	H M			
CCR4	H			
CCR5	H	H		
CCR6	H M			
CCR7	H M			
CCR8	H M R			
CD3	H M	H M R		
CD3ε	H M			
CD4	H M Ca F	H M R	H	
CD5	H M			
CD25/IL-2 Rα	H M R	H M R	H M R	H M
CD27/TNFRSF7	H M		H M	M
CD27 Ligand/TNFSF7	H M		M	M
CD127/IL-7 Rα	H M R		H M R	M
CD28	H M		H M	
CD30/TNFRSF8	H M		H M	M
CD30 Ligand/TNFSF8	H M		H M	M
CD34	R P Ca			
CD38	H M		H M	
CD39/ENTPD1	H M		H M	
CD40/TNFRSF5	H M		H M	M
CD40 Ligand/TNFSF5	H M		H M	H M
CD44	H Ca	H	H	
CD45	H M	H	H M	H
CD45R/B220	M			
CD69	H M			
CD72	M			
CD83	H M		H M	
CD109	H		H	
Common γ Chain/IL-2 Rγ	H M		H M	
CREB	H M R			H M R
CTLA-4	H M		H M	M
CXCL9/MIG	H M	H	H M	H M
CXCL10/IP-10/CRG-2	H M CR	H	H M CR	H M
CXCL12/SDF-1	H M		H M F RM	H M
CXCR3	H M			
CXCR4	H M F	H		
Fas/TNFRSF6/CD95	H M R F		H M R F	H M
FoxP3	H M R			H
Galectin-1	H M		H M	M
GITR/TNFRSF18	H M		H M	H M
GITR Ligand/TNFSF18	H M		H M	H M
Granzyme A	H		H	
Granzyme B	H M	H M	H M	M
HLA-DR	H			
HO-1/HMOX1/HSP32	H M R			H
ICAM-1/CD54	H M R		H M R	H M R
ICOS	H M		H M	
IDO			H	
IFN-α	H M P CR		H M R CR F P RM	H M

MOLECULE	ANTIBODIES	ELISpot KITS* CELL SELECTION KITS*	RECOMBINANT & NATURAL PROTEINS	ELISAs/ ASSAYS
IFN-γ	H M R B Ca CR E F P RM	H M R P Ca E F Pr	H M R B Ca CR E F P RM	H M R B Ca CR E F P Pr
IFN-γ R1/CD119	H M		H M	H M
IFN-γ R2	H M			
IGSF2/CD101	M			
IL-1α/IL-1F1	H M R CR P		H M R CR P	H M R
IL-1β/IL-1F2	H M R Ca CR E F P	H P	H M R Ca CR E F P RM	H M R F P
IL-1 RI/CD121a	H M		H M R	H
IL-1 RII/CD121b	H M		H M	H
IL-2	H M R B Ca CR E F P	H M R Ca E F	H M R B Ca CR E F P	H M R B Ca E F
IL-2 Rβ	H M		H	
IL-4	H M R B Ca CR E F P	H M Ca E	H M R B Ca CR E F P RM	H M R CR E F P
IL-4 Rα	H M		H M	
IL-10	H M R Ca CR E F P V	H M Ca F	H M R Ca CR E F P V	H M R Ca E F P
IL-10 Rα	H M		H M	
IL-10 Rβ	H M		H	
IL-35 p35	H M P			
Integrin αE/CD103	M			
Integrin αEβ7			H	
Integrin αL/CD11a	H			
Integrin αVβ8			H	
Integrin β2/CD18	H M		H	
Jak1	H M R			
Jak3	H			
LAG-3	H M		H	H
LAP (TGF-β1)	H M		H	
LRRC32/GARP	M		H M	
MAdCAM-1	M		M	M
Neuropilin-1/BDCA4	H M R		H M R	
OX40/TNFRSF4	H M		H M	
OX40 Ligand/TNFSF4	H M		H M	M
PD-1	H M		H M	H
PDCD6	H M R			
PD-L1/B7-H1	H M		H M	
PD-L2	H M		H M	
PRAT4A	M			
PRAT4B	M R			
RANK/TNFRSF11A	H M		H M	H
RARα/NR1B1	H			
c-Rel	H M			H
RXRα/NR2B1	H			
E-Selectin/CD62E	H M R		H M	H M
L-Selectin/CD62L	H M R		H M R	H M R
P-Selectin/CD62P	H M		H M	H M
SLAM/CD150	H M		M	
Smad3	H M			H
STAT5a, STAT5b	H M			
TGF-β1	H M Ms	H	H P	H M R Ca P
TGF-β RI/ALK-5	H M		M	
TGF-β RII	H M		H M	H
TGF-β RIIB	H		H	
TLR4	H M		H	
TLR4/MD-2 Complex			H	
TLR7	H			
TNF RII/TNFRSF1B	H M		H M	H M
TOR	H M R			H
TRAIL/TNFSF10	H M		H M	H
TRANCE/TNFSF11/RANK L	H M		H M	M
TSLP	H M		H M	H M
TSLP R	H M		H M	

KEY: H: Human M: Mouse R: Rat B: Bovine Ca: Canine CR: Cotton Rat E: Equine F: Feline Ms: Multispecies P: Porcine Pr: Primate
 RM: Rhesus Macaque V: Viral

* ELISpot Kits & Development Modules * Cell Selection & Detection Kits & Reagents



USA & Canada
R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
Tel: (800) 343-7475 (612) 379-2956
Fax: (612) 656-4400
info@RnDSystems.com

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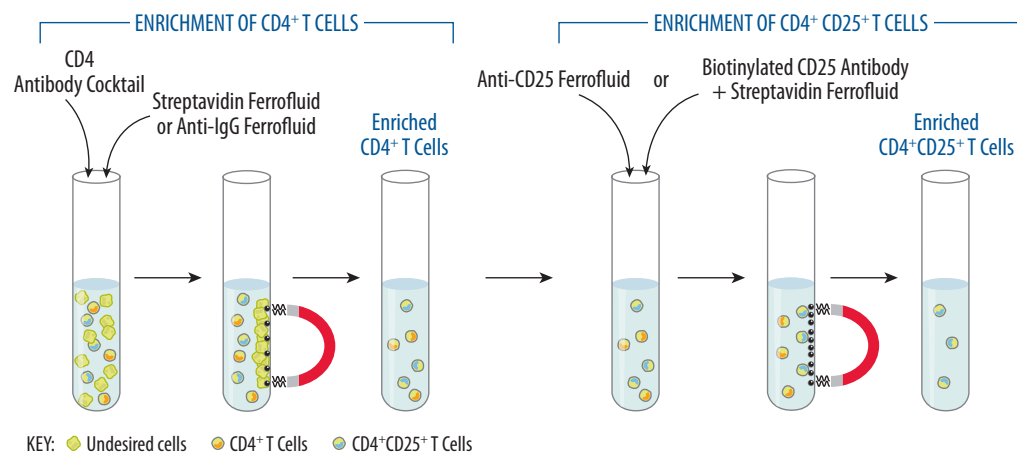
R&D Systems is a registered trademark of TECHNE Corporation.

CD4⁺CD25⁺ Regulatory T Cell Enrichment using MagCollect™ Cell Selection Kits

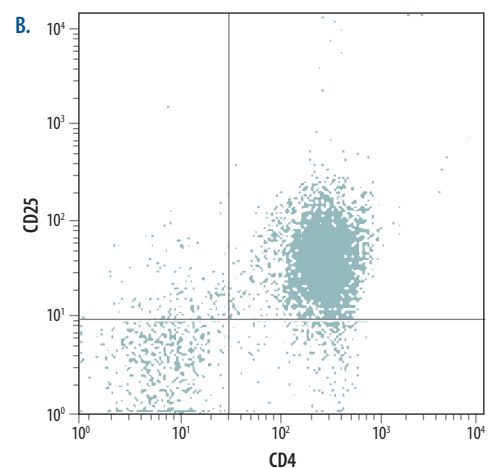
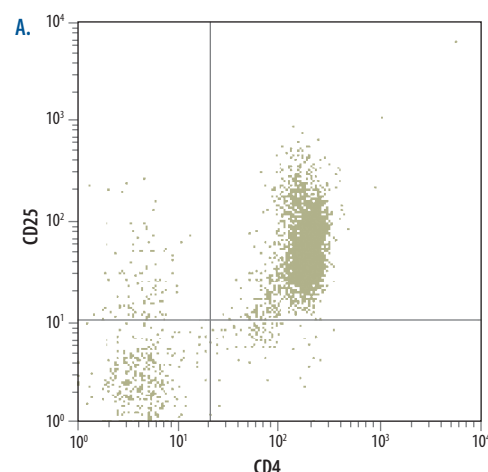
R&D Systems offers MagCollect Cell Selection Kits for human, mouse, or rat CD4⁺CD25⁺ Treg cell isolation. The MagCollect Kits are designed to isolate CD4⁺CD25⁺ Treg cells from a mononuclear cell suspension using a two-step procedure. CD4⁺ T cells are initially enriched by negative selection. CD25⁺ T cells are then isolated by positive selection from the CD4⁺ T cell fraction. The typical purity of the recovered CD4⁺CD25⁺ Treg cells ranges between 85-95% for the human kit, 84-94% for the mouse kit, and 75-85% for the rat kit.

MagCollect KIT	CATALOG #	KIT CONTENTS
Human CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit	MAGH104	Human CD4 ⁺ T Cell Biotinylated Antibody Cocktail, Streptavidin Ferrofluid, Anti-Human CD25 Ferrofluid (Clone 24238), 10X Buffer, Staining Reagent for Human CD4 ⁺ CD25 ⁺ Regulatory T Cells
Mouse CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit	MAGM208	Mouse CD4 ⁺ T Cell Biotinylated Antibody Cocktail, Streptavidin Ferrofluid, Mouse CD25 Biotinylated Antibody (Clone PC61.5), 10X Buffer
Rat CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit	MAGR304	Rat CD4 T Cell Antibody Cocktail, Anti-Mouse IgG Ferrofluid, Streptavidin Ferrofluid, Rat CD25 Biotinylated Antibody, 10X Buffer

Assay Principle



Enrichment of CD4⁺CD25⁺ T cells using the MagCollect CD4⁺CD25⁺ Regulatory T Cell Isolation Kits. The CD4 Antibody Cocktail is added to a mononuclear cell suspension. Undesired cells are bound by the antibodies and then captured by MagCollect Ferrofluid magnetic particles, or equivalent. The undesired cells are isolated from the sample by negative selection using a MagCollect Magnet (Catalog # MAG997), or equivalent, and an enriched CD4⁺ T cell population is aspirated from the sample solution. MagCollect anti-Human CD25 Ferrofluid or Biotinylated CD25 Antibody and Streptavidin Ferrofluid is then added to the CD4⁺ T cell solution. CD4⁺CD25⁺ T cells are captured by applying the MagCollect Magnet, or any compatible magnet system.



Enrichment of CD4⁺CD25⁺ Treg Cells using the MagCollect Kits. CD4⁺CD25⁺ Treg cells were isolated from (A) human PBMCs using the MagCollect Human CD4⁺CD25⁺ Regulatory T Cell Isolation Kit (Catalog # MAGH104) or (B) mouse splenocytes using the MagCollect Mouse CD4⁺CD25⁺ Regulatory T Cell Isolation Kit (Catalog # MAGM208). Total CD4⁺CD25⁺ Treg cells were detected using Fluorescein-conjugated CD4 and PE-conjugated CD25 antibodies.