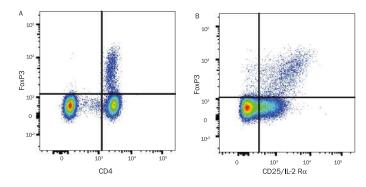
Regulatory T Cells Abs

for Flow Cytometry

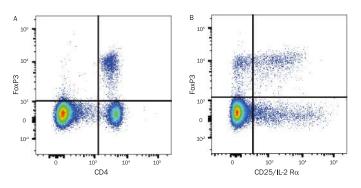


Molecule	Species	Fluorochrome-conjugated Antibodies for Flow Cytometry											
		Unconju -gated	APC	Fluores -cein	PE	PerCP	Alexa Fluor®						
							350	405	488	594	647	700	750
CD3	Human	•	•	•	•	•	•	•	•	•	•	•	•
	Mouse	•	•	•	•	•	•	•	•	•	•	•	•
CD4	Human	•	•	•	•	•	•	•	•	•	•	•	•
	Mouse	•	•	•	•	•	•	•	•	•	•	•	•
FoxP3	Human	•	•		•		•	•	•	•	•	•	•
	Mouse	•	•		•		•	•	•	•	•	•	•
CD25	Human	•	•		•		•	•	•	•	•	•	•
	Mouse	•	•		•		•	•	•	•	•	•	•
CD39	Human	•	•				•	•	•	•	•	•	•
	Mouse	•	•				•	•	•	•	•	•	•
CD73	Human	•	•		•		•	•	•	•	•	•	•
	Mouse	•		•			•	•	•	•	•	•	•
Neuropilin-1	Human	•	•	•	•		•	•	•	•	•	•	•
	Mouse	•	•	•	•		•	•	•	•	•	•	•
CTLA-4	Human	•	•		•		•	•	•	•	•	•	•
	Mouse	•					•	•	•	•	•	•	•



FoxP3+ Regulatory T Cells in Human PBMCs by Flow Cytometry

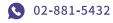
A) Fluorescein-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791F) and B)APC-conjugated Mouse Anti-Human IL-2 Ra/CD25 Monoclonal Antibody (Catalog # FAB1020A) followed by intracellular staining using a PE-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Antigen Affinity-purified Monoclonal Antibody (Catalog # IC8970P)



FoxP3+ Regulatory T Cells in Mouse Splenocytes by Flow Cytometry

A) Alexa Fluor® 488-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (Catalog# FAB554G) and B) APC conjugated Rat Anti-Mouse IL-2 Ra/CD25 Monoclonal Antibody (Catalog#FAB2438A), followed by intracellular staining using a PE-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Monoclonal Antibody (Catalog # IC8970P)









Mechanisms of Regulatory T Cell-mediated Suppression

Secretion of TGF-β1, IL-10, IL-35, and Galectin-1

© CTLA-4-dependent Suppression

N-formylkynurenine

Iryptophan

Dendritic Cell (DC)

Active TGF- $\beta 1$

atent TGF- β

IL-10, IL-35, Galectin-1

_atent TGF-β1

LRRC32

L-2

CD25/IL-2 Ro

IL-2 R_V

- CTLA-4 interacts with B7 (CD80 and CD86) on DCs, triggering indoleamine 2, 3-dioxygenase (IDO) expression (which is also induced by IFN-7 receptor stimulation)
- IDO catabolizes tryptophan, depleting stores needed for Teff cell proliferation, and producing the pro-apoptotic metabolite N-formylkynurenine

IFN-γR2

0

TCR-CD3

MHC

Binding of LAG-3 to MHC II

 Induces an immunoreceptor tyrosine-based activation motif (ITAM)-mediated inhibitory signaling pathway, blocking the maturation and immunostimulatory capacity of DCs

NRP-1

CD4⁺CD25⁺ Treg Cell

Granzyme A/B

@

Granzyme A/B Secretion

 Granzyme A/B induces apoptosis in DCs and Teff cells in both a perforin-dependent and -independent manner

LRRC32

Latent lGF-B

0

(

Induction of Infectious Tolerance

 In mice, membrane-associated LAP-TGF-\(\beta\)1 converts non-Treg cells into functional, FoxP3-expressing Treg cells

Effector T Cells (Teff Cells)

IL-35 and TGF-β1 induce IL-10 production and regulate FoxP3 expression,

promoting the maintenance and expansion of CD4*CD25* Treg cells

Suppresses cytokine production by Teff cells

• Inhibits the differentiation, proliferation, and activation of Teff cells

Membrane-associated LAP-TGF-β1 (Latent TGF-β1)

 Cell surface LAP-TGF-β1(latent TGF-β1) complexed with LRRC32, suppresses the proliferation of activated Teff cells

3 High Levels of CD25/IL-2 Rα Expression

- Depletes local IL-2, inhibiting activation and proliferation of Teff cells
- IL-2 maintains CD4*CD25*Treg cell populations

IL-2 Rß

@

AMP Adenosine Adenosine

♠ Generation of Extracellular Adenosine by Cell Surface Expression of CD39/CD73

 Activation of A_x receptors by Adenosine blocks the expression of costimulatory molecules and growth factor receptors in Teff cells, inhibiting Teff cell activation, proliferation, and expansion

Transfer of Inhibitory cAMP through Gap Junctions

• Inhibits Teff cell proliferation and IL-2 gene expression



• Binds to GM1 ganglioside on Teff cells

FoxP3

 Induces cross-linking of associated integrins, triggering TRPC5 channel activation and calcium influx, inhibiting Teff cell proliferation



