

# Regulatory T Cells

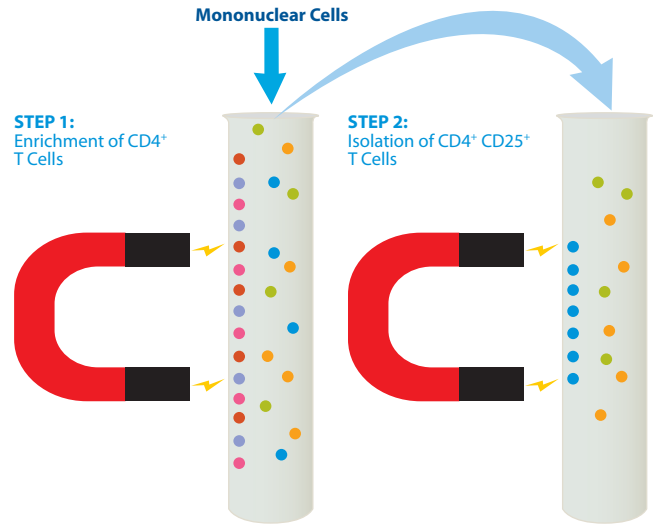
Regulatory T cells (Tregs), generated from different lineages, play important roles in immune homeostasis, autoimmune disease, anti-tumor responses, and transplantation immunology. Populations of Tregs are found in both CD4<sup>+</sup> and CD8<sup>+</sup> T cell lineages. Further characterization of Tregs has identified subsets that are CD4<sup>+</sup>CD25<sup>+</sup>, CD4<sup>+</sup>CD25<sup>-</sup>, and CD8<sup>+</sup>CD28<sup>-</sup>. Studies have concluded that the CD4<sup>+</sup>CD25<sup>+</sup> Treg population constitutes 5 to 10% of peripheral CD4<sup>+</sup> T cells in normal individuals.

Multiple attempts have been made to identify markers that can be utilized to distinguish and/or isolate specific Treg subsets. Candidates such as integrin  $\alpha$ E/CD103 and LAG-3/CD223 are unique to some Treg subsets, while CTLA-4, GITR, and OX40/CD134 are also expressed on activated T cells. Another possible marker, PD-1/CD279 (programmed death-1), is expressed intracellularly in Tregs, but is co-expressed with CD25 on the surface of activated CD4<sup>+</sup> T cells. The nuclear localization of FoxP3, initially thought to be unique to CD4<sup>+</sup> CD25<sup>+</sup> cells, precludes its use for cell isolation purposes. R&D Systems offers a wide range of reagents useful for the characterization, study, and/or isolation of Tregs.

Treg Research Reagents					
MOLECULES	ANTIBODIES	PROTEINS	ELISAs/ASSAYS	ELISpot	Cell Selection
B7-2/CD86	H M R	H M R			
CD3	H M				H M R
CD4	H M Ca F	H			H M R
CD5	H M				
CD8	H M F				H M R
CD25/IL-2 R $\alpha$	H M	H M	H		H M
CD27/TNFRSF7	H M	H M			
CD28	H M	H M			
CD38	H	H			
CD40 Ligand/TNFSF5	H M	H M	H M		
CD45	H M		H M		
CD69	H M				
CTLA-4	H M	H M			
CXCR4	H M				
Fas/TNFRSF6	H M R F	H M R F	H M		
FoxP3	H				
GITR/TNFRSF18	H M	H M	H M		
Granzyme B	H M	H M		M	
ICAM-1/CD54	H M R	H M R	H M R		
IFN- $\gamma$	H M R B Ca C R E F P Pr	H M R B Ca E C R F P Pr	H M R Ca C R F P Pr	H M R Ca C R F P Pr	
IL-2 R $\beta$	H M	H			
IL-4	H M R B Ca C R E F P	H M R B Ca E F C R P Pr	H M R C R F P	H M Ca	
IL-10	H M R Ca C R E F P V	H M R Ca C R E F P V	H M R Ca F P	H M Ca F	
Integrin $\alpha$ E/CD103	M				
LAG-3/CD223	H M	H			
Neuropilin-1	R	R			
OX40/TNFRSF4/CD134	H M	H M			
OX40 Ligand/TNFSF4	H M	H M	H		
PD-1	H M	H M			
RANK/TNFRSF11A	H M	H M			
L-Selectin	H M R	H M R	H M R		
P-Selectin	H M	H M	H M		
TLR4	H M	H			
TRANCE/TNFSF11	H M	H M	M		

Key: H Human M Mouse R Rat B Bovine Ca Canine CR Cotton Rat  
E Equine F Feline P Porcine Pr Primate V Viral

## MagCollect™ Mouse CD4<sup>+</sup>CD25<sup>+</sup> T Cell Isolation Kit (Catalog # MAGM208)



### STEP 1:

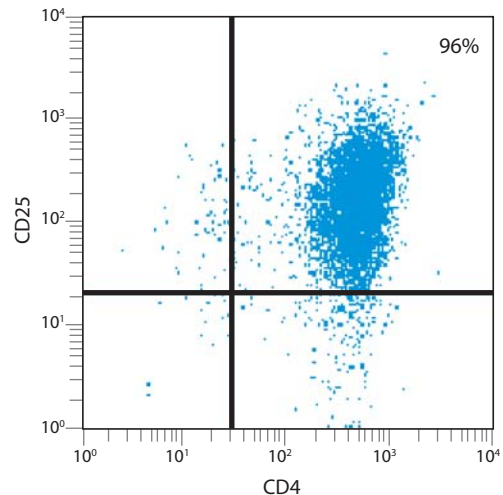
Mononuclear cells are incubated with a cocktail of biotinylated antibodies followed by incubation with streptavidin ferrofluid.

Unwanted cells are magnetically separated using the MagCollect magnet (Catalog # MAG997) and discarded, leaving a CD4<sup>+</sup>-enriched cell population.

### STEP 2:

CD4<sup>+</sup> cells are incubated with a biotinylated CD25 antibody, followed by incubation with streptavidin ferrofluid.

Magnetically tagged CD4<sup>+</sup>CD25<sup>+</sup> T cells (cyan cells) are isolated using the MagCollect magnet.



**Figure 1.** Isolation of mouse CD4<sup>+</sup>CD25<sup>+</sup> T cells from activated splenocytes using R&D Systems MagCollect Mouse CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit (Catalog # MAGM208). Dot plots represent dual staining of all viable cells recovered using the MagCollect Kit and analyzed by flow cytometry. Cells were stained with R&D Systems CD4-Fluorescein antibody (Catalog # FAB554F) and CD25-Phycoerythrin (PE) antibody (Catalog # FAB2438P). Approximately 96% of the cells recovered are CD4<sup>+</sup>CD25<sup>+</sup> cells.