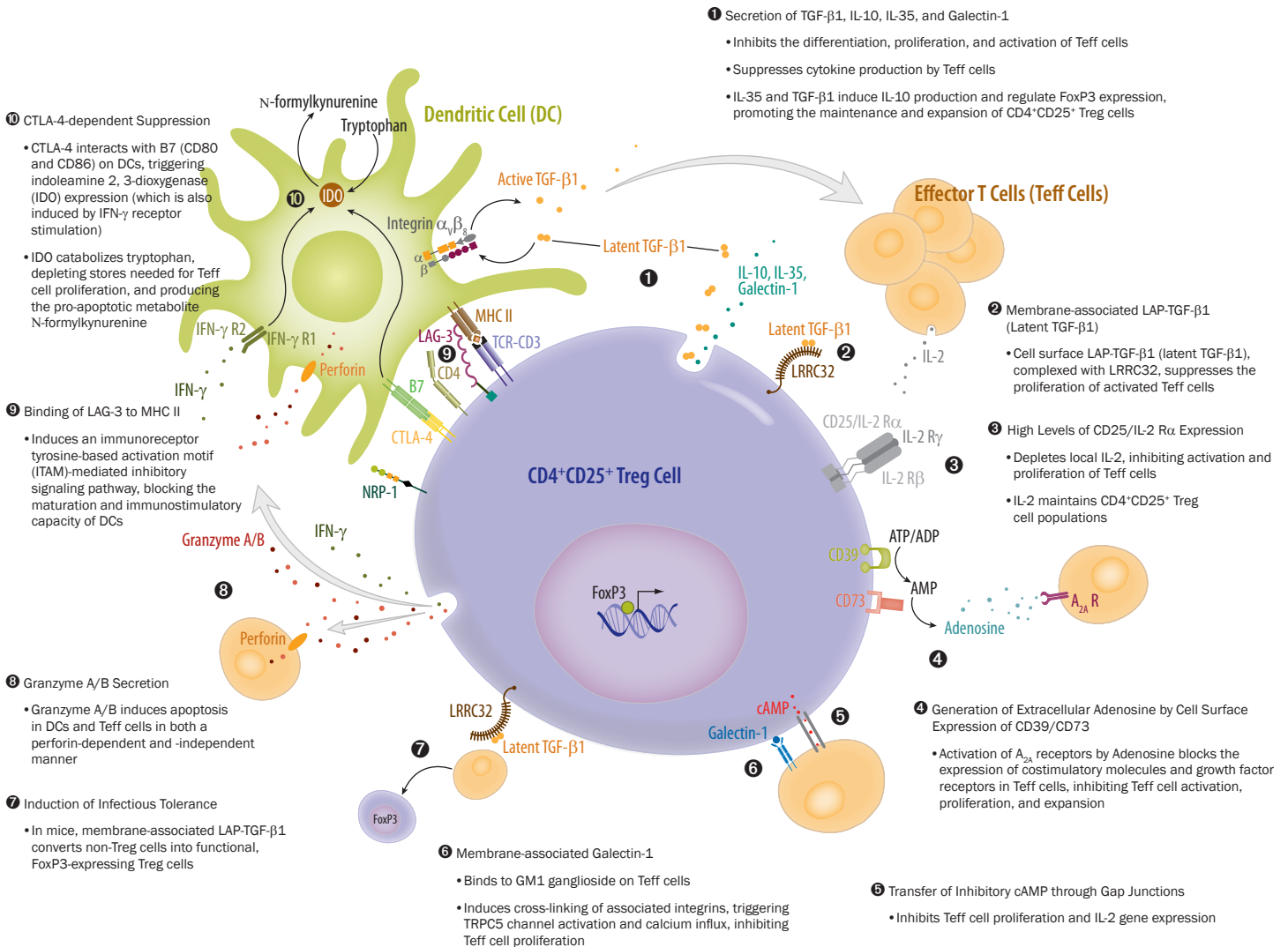


Regulatory T Cells

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.

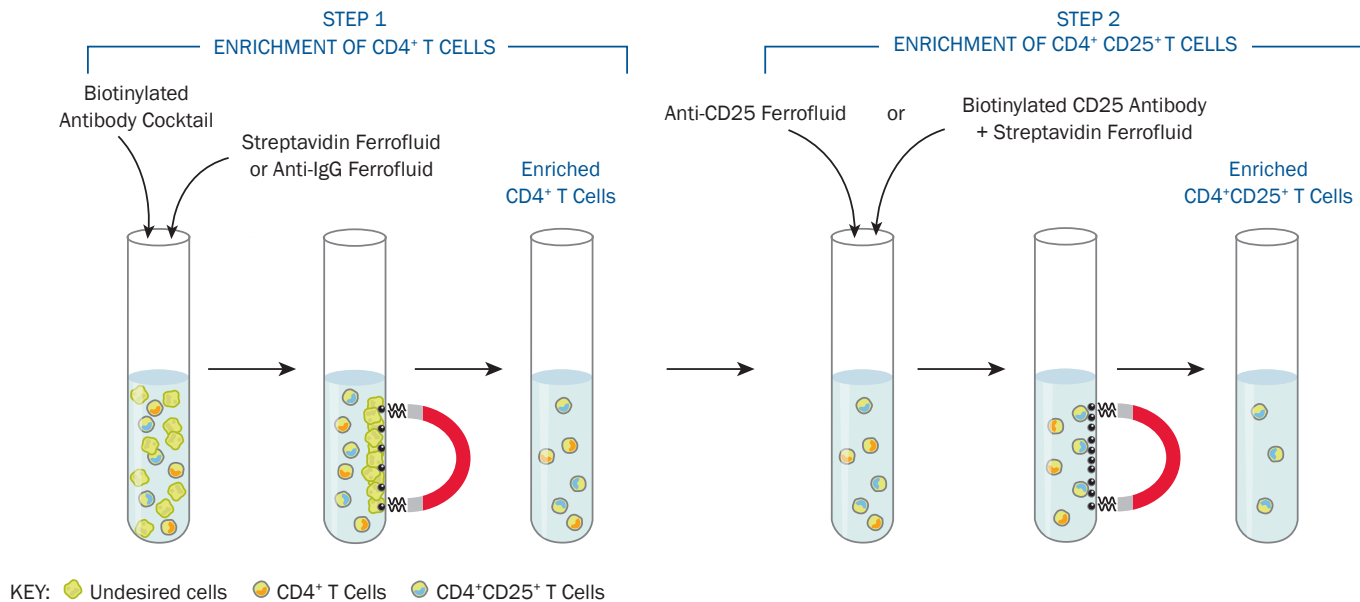
Mechanisms of Regulatory T Cell-mediated Suppression



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

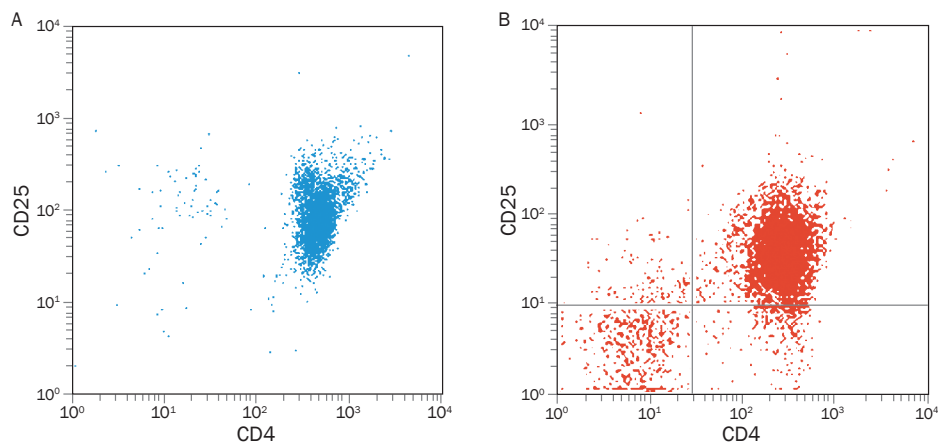
R&D Systems® MagCollect™ Cell Selection Kits are designed to isolate human or mouse CD4⁺CD25⁺ regulatory T cells using a two-step procedure. In the first step, CD4⁺ T cells are isolated by tagging unwanted cells with a biotinylated antibody cocktail followed by the addition of streptavidin ferrofluid. 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 # |
| MagCollect™ Human CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit | MAGH104 |
| MagCollect™ Mouse CD4 ⁺ CD25 ⁺ Regulatory T Cell Isolation Kit | MAGM208 |



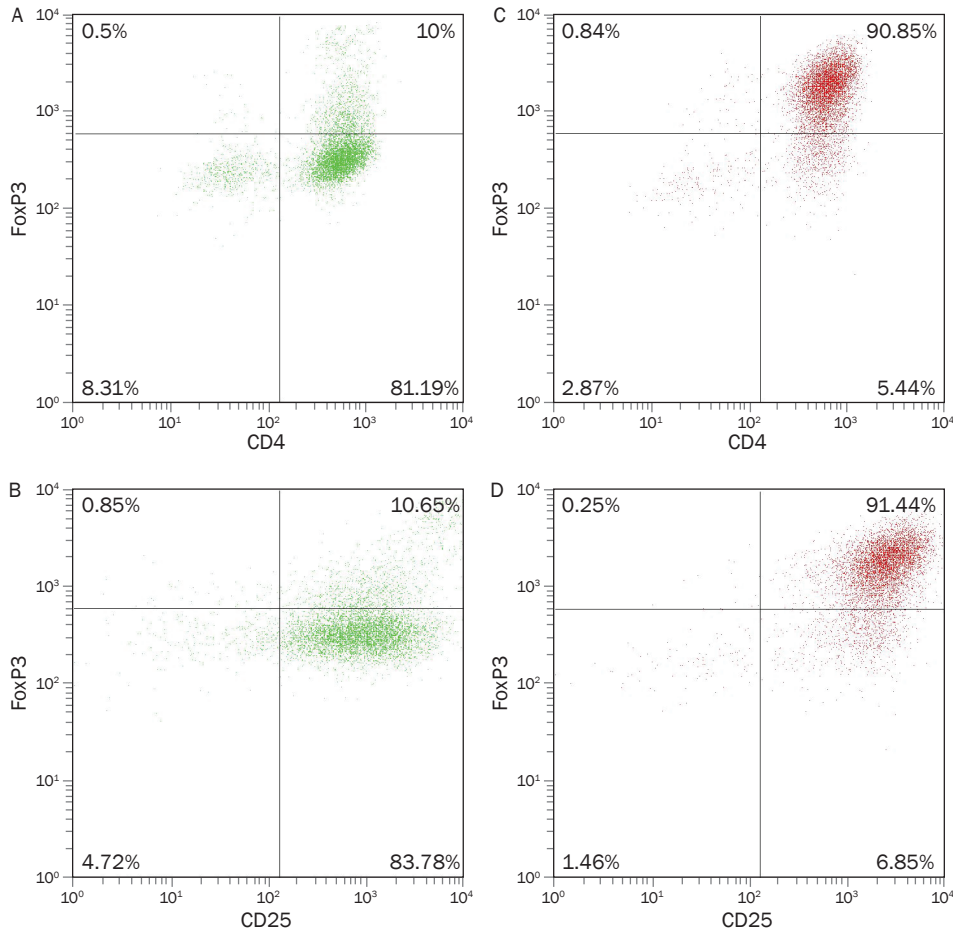
Isolation of CD4⁺CD25⁺ Regulatory T Cells using the MagCollect™ Kits. CD4⁺CD25⁺ regulatory T cells were isolated from (A) human PBMCs using the MagCollect™ Human CD4⁺CD25⁺ Regulatory T Cell Isolation Kit (R&D Systems, Catalog # MAGH104) or (B) mouse splenocytes using the MagCollect™ Mouse CD4⁺CD25⁺ Regulatory 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 <i>In vitro</i> 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 | <i>E. coli</i> | 202-IL |
| | Mouse | <i>E. coli</i> | 402-ML |
| IL-27 | Human | NSO | 2526-IL |
| | Mouse | NSO | 2799-ML |
| IL-33 | Human | <i>E. coli</i> | 3625-IL |
| | Mouse | <i>E. coli</i> | 3626-ML |
| TGF-β1 | Human | CHO | 240-B |
| | | HEK293 | 7754-BH |
| | Mouse | CHO | 7666-MB |
| TGF-β2 | Human | NSO | 302-B2 |
| | Mouse | CHO | 7346-B2 |
| TGF-β3 | Human | Sf21 (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.

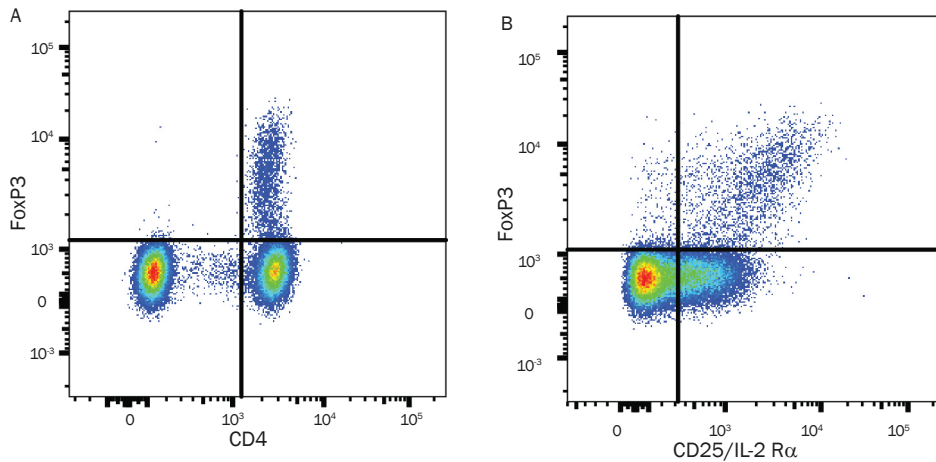
| Antibodies Commonly used to Identify Regulatory T Cells by Flow Cytometry | | | | | | | | | | |
|---|-------------------------|----------|--|-------------|--------------|----------------|------------------|------------------|---|---|
| Molecule | Species | Clone | Fluorochrome-conjugated Antibodies for Flow Cytometry (Catalog #s) | | | | | | | Unconjugated Antibodies (Applications) |
| | | | APC | Fluorescein | PE | PerCP | Alexa Fluor® | | Additional Alexa Fluor® conjugates 350/405/594/647/750 | |
| | | | | | | | 488 | 700 | | |
| 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 R α | 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

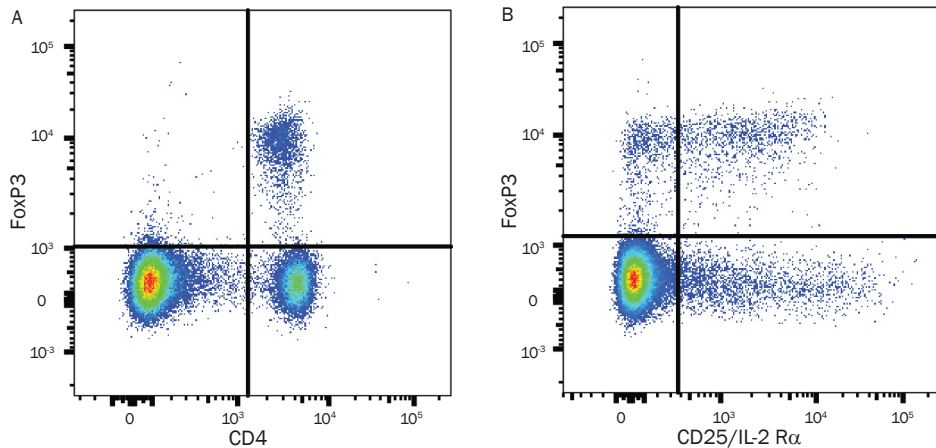
| Regulatory T Cell Multi-Color Flow Cytometry Kits from R&D Systems | |
|--|-------------------------|
| FlowX Human Regulatory T Cell Multi-Color Flow Kit | Catalog # FMC021 |
| Kit Contents | |
| APC-conjugated CD25 (clone 24212) | |
| Fluorescein-conjugated CD4 (clone 11830) | |
| PE-conjugated FoxP3 (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 R α /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



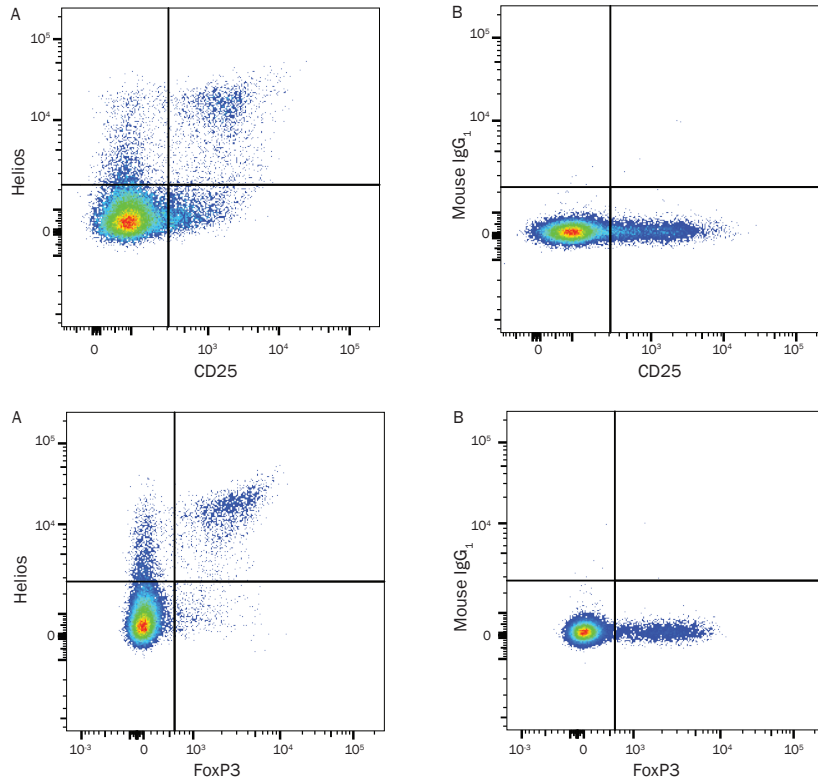
Detection of FoxP3⁺ Regulatory T Cells in Mouse Splenocytes by Flow Cytometry. C57BL/6 mouse splenocytes were surface stained with (A) an Alexa Fluor[®] 488-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (R&D Systems, Catalog # FAB554G) and (B) an APC conjugated Rat Anti-Mouse IL-2 R α /CD25 Monoclonal 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.

LEARN MORE
rndsystems.com/
tregs

LEARN MORE
novusbio.com/
regulatoryimmunology

Analysis of Additional Markers on Regulatory T Cells by Flow Cytometry

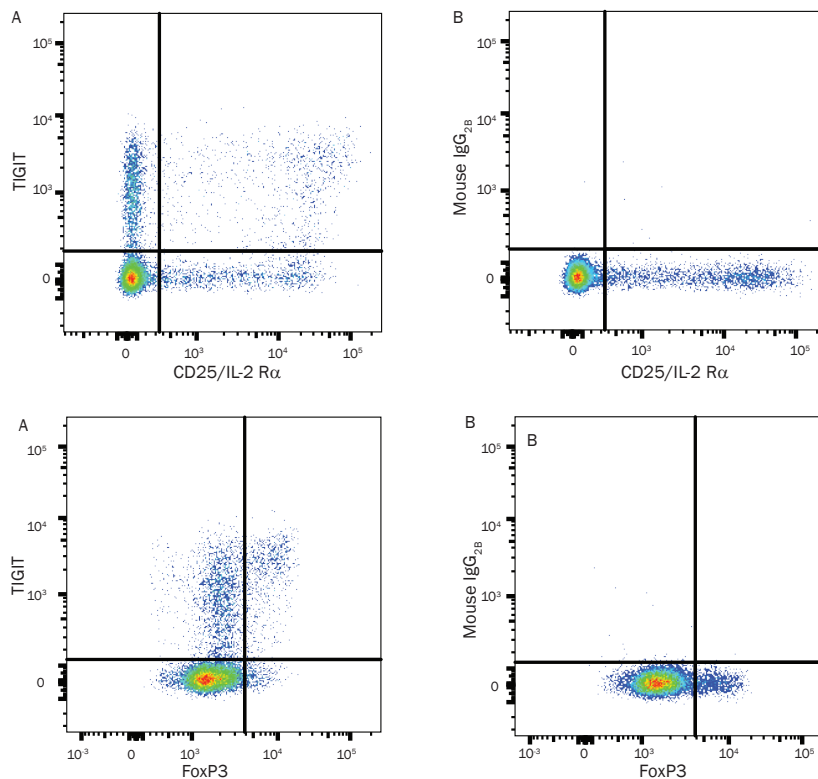
Detection of Helios on Human CD25⁺ or FoxP3⁺ Cells



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 R α 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 IgG₁ 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 IgG₁ 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).

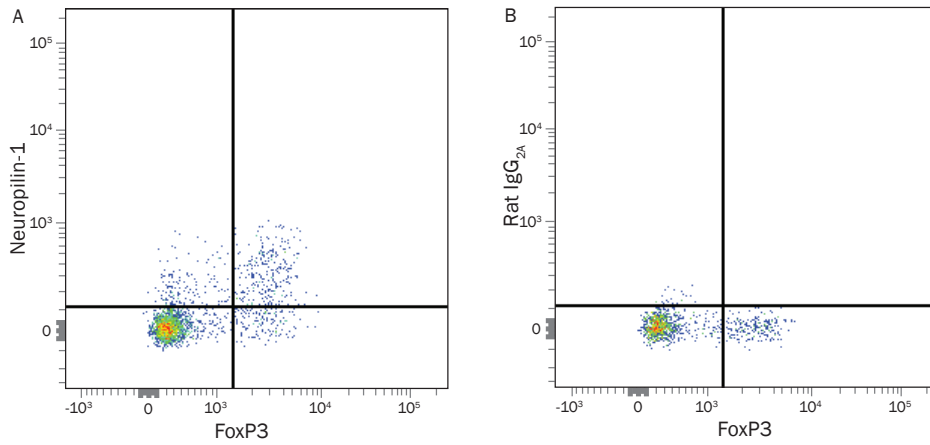
Detection of TIGIT on Human CD25⁺ or FoxP3⁺ Cells



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 R α 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).

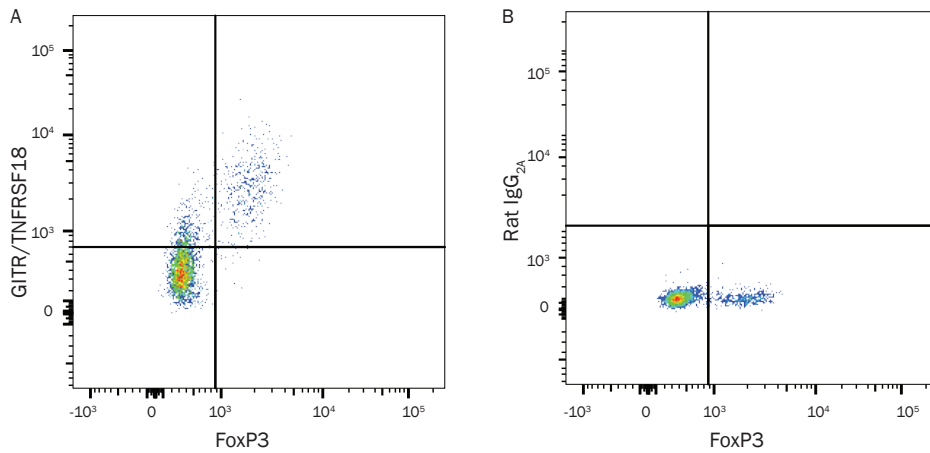
Detection of TIGIT⁺FoxP3⁺ Human Regulatory T Cells by Flow Cytometry. Human peripheral blood mononuclear cells were stained with an Alexa Fluor[®] 488-conjugated Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8214G) and either (A) an APC-conjugated Mouse Anti-Human TIGIT 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® | | Additional Alexa Fluor® conjugates 405/594/647/750 | |
| | | | | | | | 488 | 700 | | |
| 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 | NBP1-43392 | 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 R α /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 Chromatin Immunoprecipitation Depl Depletion E ELISA FA Functional Assay FC Flow Cytometry ■ Indicates an R&D Systems antibody.
 ICC/IF Immunocytochemistry/Immunofluorescence IHC Immunohistochemistry IP Immunoprecipitation IV *In vitro* WB Western Blot ■ Indicates a Novus Biologicals antibody.

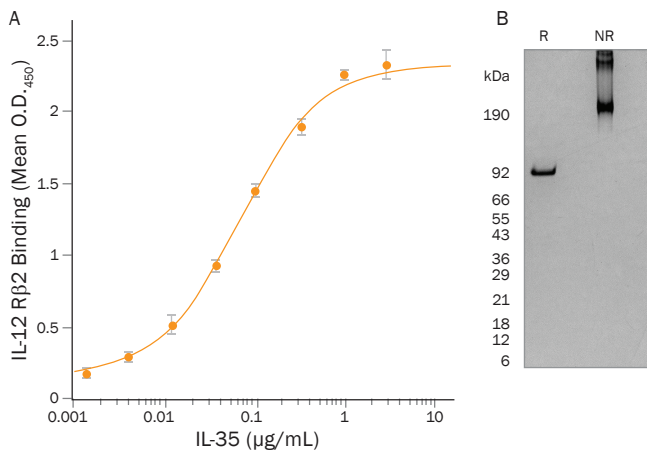
| Antibodies Commonly used to Characterize Regulatory T Cells by Flow Cytometry | | | | | | | | | | |
|---|-------------|------------|--|-------------|---------------|----------------|------------------|------------------|--|--|
| Molecule | Species | Clone | Fluorochrome-conjugated Antibodies for Flow Cytometry (Catalog #s) | | | | | | | Unconjugated Antibodies (Applications) |
| | | | APC | Fluorescein | PE | PerCP | Alexa Fluor® | | Additional Alexa Fluor® conjugates 405/594/647/750 | |
| | | | | | | | 488 | 700 | | |
| IL-12/IL-35 p35 | Human/Mouse | 27537 | IC2191A | IC2191F | IC2191P | IC2191C | | | | MAB1570 (FC, WB) |
| IL-27 R α /WSX-1/TCCR | Human | 191106 | FAB14791A | | FAB14791P | | FAB14791G | | | |
| | Mouse | 263503 | | FAB21091F | FAB21091P | | | FAB21091N | | MAB21091 (FC, WB) |
| Integrin α 2/CD49b | Human | HAS3 | | | FAB1233P | | | | | MAB1233 (FC, ICC/IF, IP) |
| | Mouse | 235033 | FAB1740A | | FAB1740P | | | | | MAB1740 (FC) |
| | Mouse | DX5 | NBP1-28114 | NBP1-28110 | NBP1-28113 | | | | | |
| Integrin α E/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- β 1) | 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/BDC4 | 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- β 1 | 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.



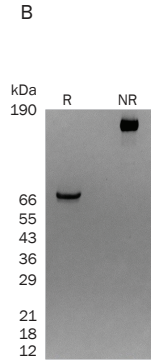
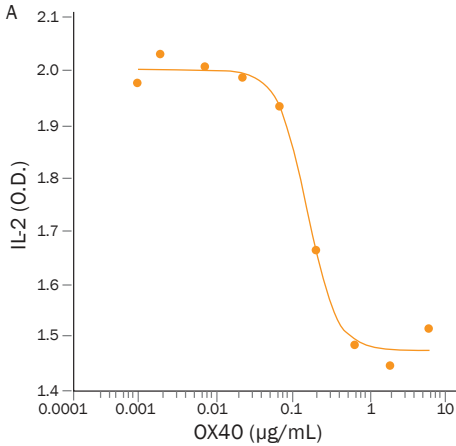
Recombinant Human IL-35 Binds IL-12 Rβ2. (A) Recombinant Human IL-12 Rβ2 Fc Chimera (R&D Systems, Catalog # 1959-B2) was coated onto microplate wells at 5 μg/mL and the indicated concentrations of Recombinant Human IL-35 Fc Chimera (R&D Systems, Catalog # 8608-IL) were added. The concentration of Recombinant 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 | <i>E. coli</i> | 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 | <i>Sf21</i> | 741-B2 |
| CD155/PVR | Human | NS0 | 2530-CD |
| | Mouse | NS0 | 6909-CD |
| CTLA-4 | Human | CHO | 7268-CT |
| | Human | <i>Sf21</i> | 325-CT |
| | Mouse | NS0 | 434-CT |
| DNAM-1/CD226 | Human | NS0 | 666-DN |
| | Mouse | NS0 | 4436-DN |

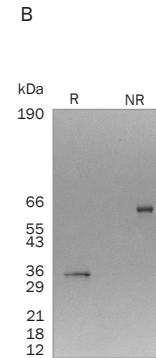
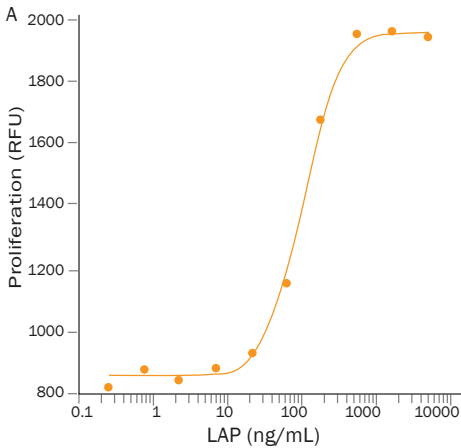
Select Recombinant Proteins from R&D Systems for Functional Characterization of Regulatory T Cell-Expressed Molecules

| Molecule | Species | Source | Catalog # |
|----------------------|---------|----------------------------------|-----------|
| Galectin-1 | Human | <i>E. coli</i> | 1152-GA |
| | Mouse | <i>E. coli</i> | 1245-GA |
| GITR/TNFRSF18 | Human | NS0 | 689-GR |
| | Mouse | NS0 | 524-GR |
| GITR Ligand/TNFSF18 | Human | CHO | 6987-GL |
| | Human | <i>Sf21</i> | 694-GL |
| | Mouse | NS0 | 2177-GL |
| IL-10 | Human | <i>Sf21</i> (baculovirus) | 217-IL |
| | Human | <i>Sf21</i> (stably transfected) | 217-ILB |
| | Human | <i>E. coli</i> | 1064-IL |
| | Mouse | <i>E. coli</i> | 417-ML |
| IL-35 | Human | HEK293 | 8608-IL |
| LAG-3 | Human | NS0 | 2319-L3 |
| | Mouse | NS0 | 3328-L3 |
| LAP (TGF- β 1) | Human | <i>Sf21</i> | 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- β 1 | Human | CHO | 240-B |
| | | HEK293 | 7754-BH |
| | Mouse | CHO | 7666-MB |
| TGF- β 2 | Human | NS0 | 302-B2 |
| | Mouse | CHO | 7346-B2 |
| TGF- β 3 | Human | <i>Sf21</i> (baculovirus) | 243-B3 |
| | Human | CHO | 8420-B3 |

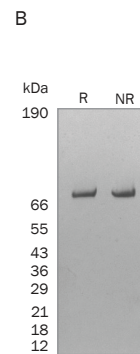
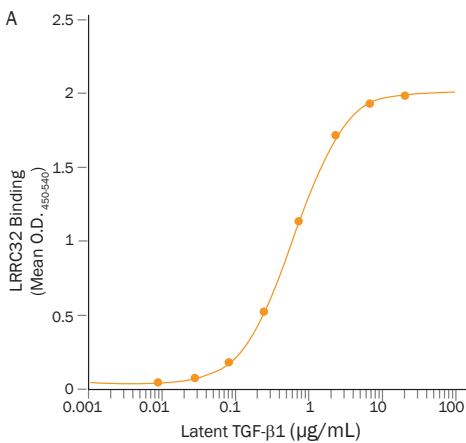
Data for Additional Select Regulatory T Cell-related Recombinant Proteins from R&D Systems



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.

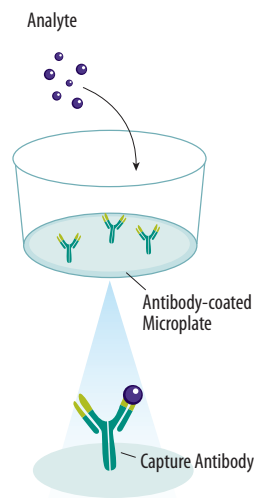
Quantikine® ELISA Kits

Features

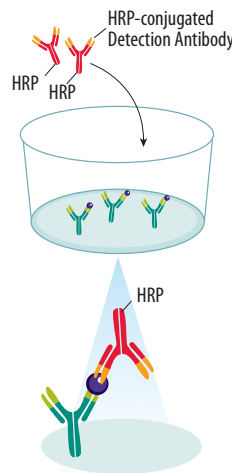
- Complete, ready-to-use kits
- Exhaustively tested for superior quality and reproducibility
- Detailed protocol booklets
- Colorimetric detection

Assay Principle

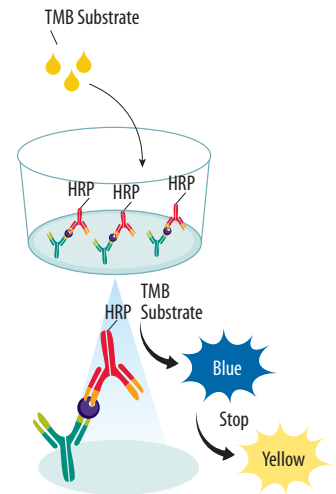
Step 1



Step 2



Step 3



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 |

R&D SYSTEMS

NOVUS
BIOLOGICALS

TOCRIS

protein**simple**

biotechne[®]

Global info@bio-techne.com bio-techne.com/find-us/distributors TEL +1 612 379 2956
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373

bio-techne.com

