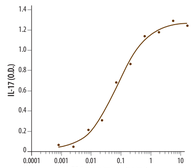
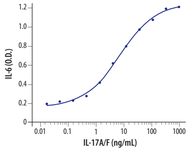
Proteins

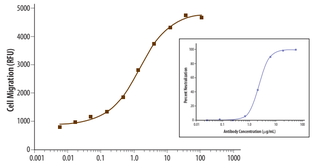
R&D Systems currently offers more than 1,700 proteins from 16 different species. Stringent production and purification guidelines, along with rigorous bioassay testing, ensure quality and minimal lot-to-lot variability.



IL-23 Stimulates IL-17 Secretion by Mouse Splenocytes. Mouse splenocytes were treated with the indicated concentrations of Recombinant Mouse IL-23 (Catalog # 1887-ML). IL-17 secretion was measured using the Mouse IL-17 Quantikine® ELISA Kit (Catalog # M1700).



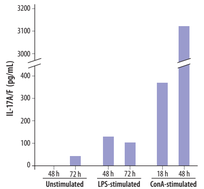
IL-17A/F Stimulates IL-6 Production by Mouse Fibroblasts. NIH-3T3 mouse fibroblasts were treated with the indicated concentrations of Recombinant Human IL-17A/F (Catalog # 5194-IL). Aliquots of the cell culture supernatants were measured using the Mouse IL-6 Quantikine ELISA Kit (Catalog # M6000B).



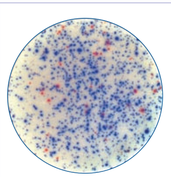
CCL20/MIP-3α-induced Chemotaxis & Antibody Neutralization. BaF3 mouse pro B cells transfected with human CCR6 were placed in the upper compartment of a two level chemotaxis chamber with increasing concentrations of Recombinant Human CCL20/MIP-3α (Catalog # 360-MP) placed in the lower compartment. Cell migration was monitored by staining the cells in the lower chamber with the redox sensitive dye, Resazurin (Catalog # AR002; brown line). The chemotactic effect induced by 10 ng/mL CCL20/MIP-3α was neutralized by pre-incubating the protein with increasing concentrations of Human CCL20/MIP-3α Monoclonal Antibody (Catalog # MAB360) prior to its addition to the chemotaxis chamber (inset).

ELISAs & ELISpot Assays

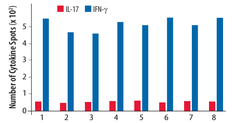
R&D Systems offers over 300 complete Quantikine ELISA Kits and development reagents for the quantification of a wide selection of proteins that occur in a variety of different sample types, including serum, plasma, cell culture supernatants, and more. Complete kits are designed to provide the highest levels of specificity, accuracy, precision, and sensitivity in analyte quantification. Development reagents, including matched antibody pairs and other components required for a customer to develop their own working assay, are also available. In addition, we offer ELISpot Assays, which are highly sensitive microplate-based assays designed to detect absolute numbers of cytokine, chemokine, or protease-secreting cells.



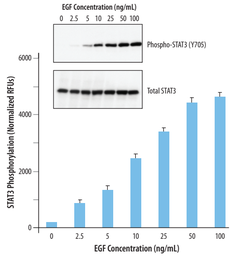
Assessment of the Levels of IL-17A/F in Mouse Spleen Cell Culture Supernatants. Mouse splenocytes were isolated and cultured, either unstimulated or stimulated with lipopolysaccharide (LPS) or Concanavalin A (ConA), for 18, 48, or 72 hours as indicated. Aliquots of the cell culture supernatants were assayed using the Mouse IL-17A/F Heterodimer Quantikine ELISA Kit (Catalog # M17AF0).



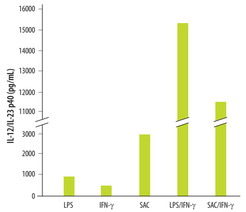
Detection of IL-17 and IFN-γ Secretion by Mouse Splenocytes using the Dual-Color ELISpot Kit. IFN-γ (blue spots) and IL-17 (red spots) were secreted from mouse splenocytes stimulated with PMA/Ca^{2+} ionomycin. Spots of cytokine secretion were visualized using the Mouse IFN-γ/IL-17 Dual-Color ELISpot Kit (Catalog # ELD5007).



Reproducibility in the Number of Cells Releasing Mouse IFN-γ or IL-17 in Multiple Trials. Mouse splenocytes, stimulated with PMA/Ca^{2+} ionomycin, were plated equally into eight wells of a microplate dish and assayed for IFN-γ and IL-17 secretion using the Mouse IFN-γ/IL-17 Dual-Color ELISpot Kit (Catalog # ELD5007). The number of blue spots (IFN-γ) and red spots (IL-17) in each well were counted using an ELISpot reader system and compared to determine the reproducibility of the results.



EGF-induced STAT3 Phosphorylation on Y705 in Human Epithelial Carcinoma Cells. A431 human epithelial carcinoma cells were treated with the indicated concentrations of Recombinant Human EGF (Catalog # 236-EG). STAT3 phosphorylation on Y705 was determined using the Human/Mouse Phospho-STAT3 (Y705) Cell-Based ELISA (Catalog # KCB4607) and normalized to total STAT3 in the same wells (bar graph). Values represent the mean ± the range of duplicate determinations. Detection of STAT3 phosphorylation on Y705 by Western blot is shown for comparison (inset).



Measurement of IL-12/IL-23 p40 Levels using the Quantikine ELISA Kit. Human peripheral blood mononuclear cells were stimulated with lipopolysaccharide (LPS), Recombinant Human IFN-γ (Catalog # 285-IF), 0.0075% *Staphylococcus aureus* Cowan I (SAC), LPS and IFN-γ, or 0.0075% SAC and IFN-γ for 1.5 days. Aliquots of the cell culture supernatants were assayed using the Human IL-12/IL-23 p40 Quantikine ELISA Kit (Catalog # DP400). Aliquots removed from cells that had been treated with SAC, LPS and IFN-γ, or SAC and IFN-γ were diluted prior to the assay.

For more information on Th17-related products, please visit our website at
www.RnDSystems.com/go/Th17

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

MA104_Th17_JUN

PRSRT STD
U.S. POSTAGE
PAID
R&D SYSTEMS

Change Service Requested

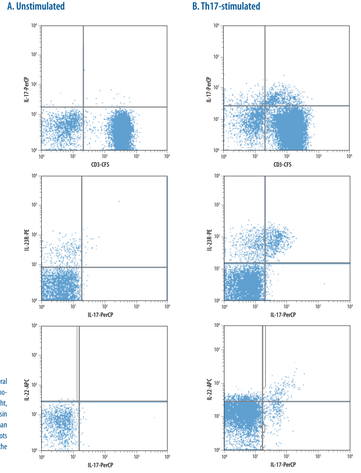
Printed on recyclable paper 10% post consumer waste.

Th17 Multi-Color Flow Cytometry Kits

Multi-Color Flow Cytometry Kits are designed to simplify the identification of specific cell types by flow cytometry. The Human and Mouse Th17 Cell Multi-Color Flow Cytometry Kits provide four different fluorochrome-conjugated antibodies that can be used together for single-step staining of either human or mouse Th17 cells. The kits also contain isotype controls for each antibody, all of the necessary buffers, and detailed protocols. The buffers for these kits are also available individually.

Th17 Multi-Color Flow Cytometry Kits		
CELL TYPE	**ANTIBODIES**	**CATALOG #**
Human Th17	APC-conjugated IL-22	FMC007
	Fluorescein-conjugated CD3	
	PE-conjugated IL-23 R	
	PerCP-conjugated IL-17	
Mouse Th17	APC-conjugated CD4	FMC008
	Fluorescein-conjugated CCR6	
	PE-conjugated IL-22	
	PerCP-conjugated IL-17	

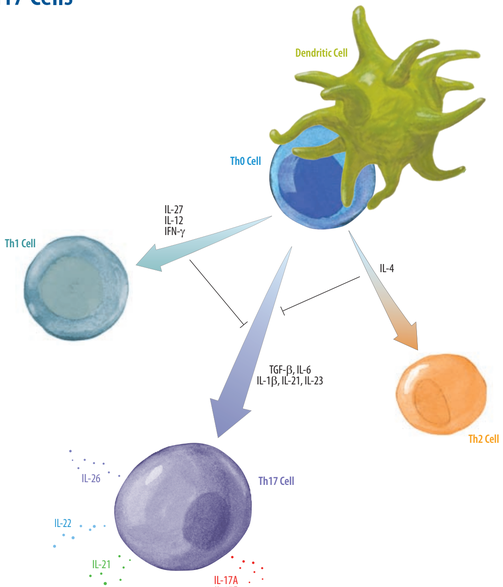
For more information, please visit our website at
www.RnDSystems.com/go/MultiColorFlow



Detection of Human Th17 Cells by Flow Cytometry. Human peripheral blood mononuclear cells, unstimulated (**A**) or stimulated with PMA, ionomycin, Recombinant Human IL-23 (Catalog # 1290-IL) and LPS overnight, followed by 2-4 hours re-stimulation with PMA/ionomycin and monensin (**B**), were stained with the indicated antibodies provided in the Human Th17 Cell Multi-Color Flow Cytometry Kit (Catalog # FMC007). Dot plots shown in the top panel were gated on lymphocytes, and dot plots in the other panels were gated on $CD3^+$ cells.

R&D Systems Tools for Cell Biology Research™

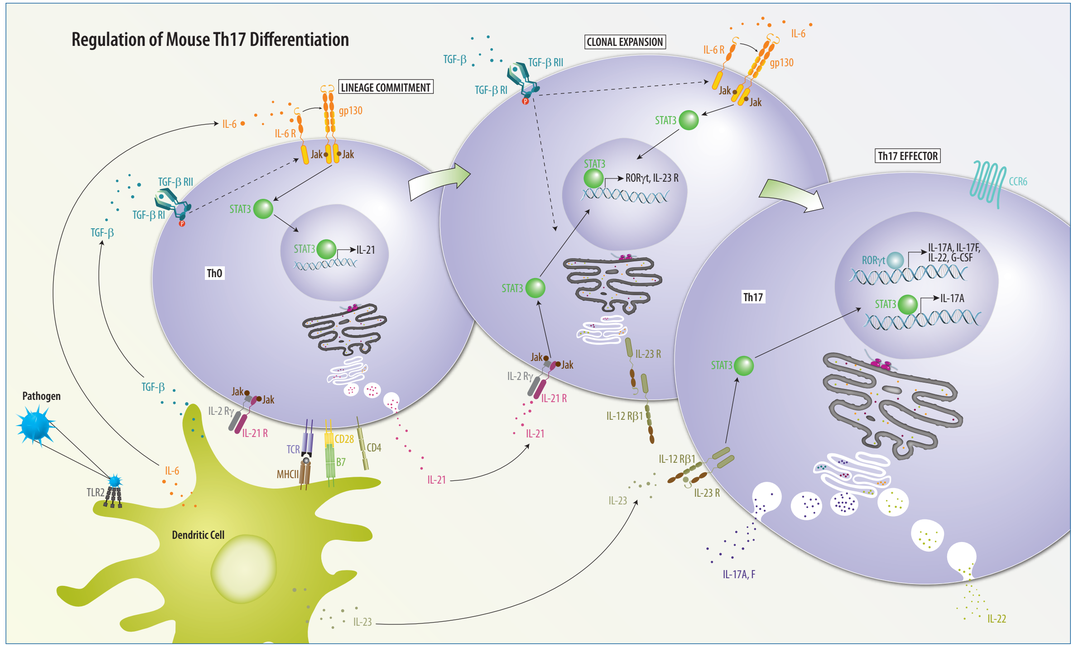
Th17 Cells



Differentiation & Function of Th17 Cells

Interleukin-17 (IL-17)-producing T helper cells (Th17 cells) are a lineage of $CD4^+$ effector T cells that is distinct from the Th1 and Th2 $CD4^+$ lineages. Th17 cells are part of the adaptive immune response mounted against fungal and bacterial infections, but may also contribute to the pathogenesis of autoimmune diseases. In mice, Th17 cells develop from naïve $CD4^+$ T cells in the presence of TGF-β and IL-6. These cytokines induce the STAT3-dependent expression of IL-21, IL-23 R, and the transcription factor, RORγt. IL-21 and IL-23 regulate the establishment and clonal expansion of Th17 cells, while RORγt-induced gene expression leads to the secretion of IL-17A, F and IL-22. Although IL-22 may be involved in promoting tissue protection and regeneration, IL-17, IL-21, and IL-22 are all potent pro-inflammatory mediators. In addition, Th17-related cytokines stimulate chemokine secretion by resident cells leading to the recruitment of neutrophils and macrophages to sites of inflammation. These cells, in turn, produce additional cytokines and proteases that further exacerbate the immune response. In contrast to mouse Th17 differentiation, Th17 polarization in humans is dependent upon IL-1β, IL-6, IL-21, and IL-23, but does not seem to require TGF-β, suggesting that the mechanisms of Th17 differentiation may vary slightly between the two species. One other notable difference is that human Th17 cells have been found to secrete IL-26, an IL-10 family cytokine without a murine homologue.

Cytokines produced by Th17 cells can have both beneficial and pathogenic effects. While these cytokines play a central role in eliminating harmful microbes, persistent secretion of Th17 cytokines promotes chronic inflammation and tissue destruction characteristic of autoimmune diseases. For these reasons, factors involved in Th17 differentiation, or those that inhibit Th17 function, may serve as potential targets for preventing the pathogenesis of a variety of autoimmune conditions, including rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disorders. R&D Systems offers a wide range of research reagents useful for the characterization of Th17 differentiation and Th17-related immune responses.



Th17 Research Reagents Available from R&D Systems

MOLECULE	PROTEINS	ANTIBODIES	ELISA KITS CELL SELECTION KITS	ELISpot KITS *
CCL20/MIP-3α	H M R	H M R	H M R	
CCR2		H		
CCR4		H		
CCR6		H M		
CD3		M	H M R	
CD3ε		H M		
CD4	H	H M Ca F	H M R	
CD28	H M	H M		
CD30/TNFRSF8	H M	H M	M	
CD30 Ligand/TNFSF8	H M	H M	M	
CD39/ENTPD1	H M	H M		
CD40/TNFRSF5	H M	H M	M	
CD40 Ligand/TNFSF5	H M	H M	H M	
Common γ chain/IL-2 Rγ	H M	H M		
CTLA-4	H M	H M	M	
CXCR3		H M		
CXCR4		H M F		
CXCR6		H M		
G-CSF	H M	H M	H M	
GITR/TNFRSF18	H M	H M	H M	
gp130	H M R	H M	H M	
ICOS	H M	H M		
IFN-γ	H M R P B Ca CR E F RM	H M R P B Ca CR E F RM	H M R P B Ca CR E F Pr	H* M* R Ca E F M P Pr
IL-1β/IL-1F2	H M R P Ca CR E F RM	H M R P Ca CR E F	H M R P F	H P
IL-1 RAcP	H	H		
IL-1 RI	H M R	H M	H	
IL-1 RII	H M	H M	H	
IL-2	H M R P B Ca CR E F	H M R P B Ca CR E F	H M R B Ca E F	H* M* R Ca E F
IL-2 Rα	H M R	H M R	H M	
IL-4	H M R P B Ca CR E F RM	H M R P B Ca CR E F	H M R P CR E F	H* M* E
IL-4 Rα	H M	H M		
IL-6	H M R P Ca CR E F	H M R P Ca CR E F	H M R P Ca F	H M R
IL-6 Rα	H M	H M		
IL-7 Rα/CD127	H M R	H M R	M	
IL-10	H M R P Ca CR E F GP V	H M R P Ca CR E F V	H M R P Ca E F	H* M* Ca F
IL-12	H M R P Ca F RM	H M R P Ca	H M	
IL-12/IL-23 p40	H Ca F	H M R Ca P F	H M Ca F P	
IL-12 Rβ1	H M	H M		
IL-12 Rβ2	H	H M		
IL-17/IL-17A	H M Ca	H M	H M	H* M*
IL-17A/F Heterodimer	H M		H M	

MOLECULE	PROTEINS	ANTIBODIES	ELISA KITS CELL SELECTION KITS	ELISpot KITS *
IL-17F	H M R	H M	H M	
IL-17 R	H M	H M	H	
IL-17 RC	H M	H M		
IL-18 Rα/IL-1 R5	H	H M		
IL-21	M Ca	M	M	
IL-21 R	H M	H M		
IL-22	H M R	H M	H M R	
IL-23	H M R	M	H M	
IL-23 p19		H M		
IL-23 R	H M	H M		
IL-26/AK155	H	H		
IL-27	H M	H M	H M	
Jak1		H M R		
Jak2		M R		
Jak3		H		
RORγt		H M		
SCF	H M Ca F	H M Ca F	H M Ca F	
STAT1		H M	H M	
STAT3		H M R	H M	
TGF-β		Ms		
TGF-β1	H P	H Ms	H M R P Ca	
TGF-β1,2,3		Ms		
TGF-β1.2	H	Ms		
TGF-β1/1.2		Ms		
TGF-β2	H P	Ms	H	
TGF-β2/1.2		Ms		
TGF-β3	H	Ms	H	
TGF-β RI/ALK5	M	H M		
TGF-β RII	H M	H M	H	
TIM-3	H M	H M		
TRANCE/RANK L/TNSF11	H M	H M	M	

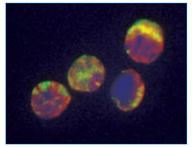
* Dual-Color ELISpot Kits are also available for these molecules. Please refer to the product table below.

MOLECULE 1	MOLECULE 2	DUAL-COLOR ELISpot KITS
IFN-γ	IL-2	H M
	IL-4	H M
	IL-10	H
	IL-17	H M
IL-2	IL-4	H M
	IL-10	H M
IL-4	IL-10	M
	IL-17	H M

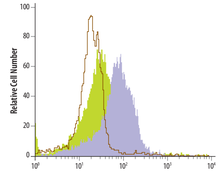
KEY: **H:** Human **M:** Mouse **R:** Rat **B:** Bovine **Ca:** Canine **CR:** Cotton Rat **E:** Equine **F:** Feline **GP:** Guinea Pig **Ms:** Multi-species **P:** Porcine **Pr:** Primate **RM:** Rhesus Macaque **V:** Viral

Antibodies

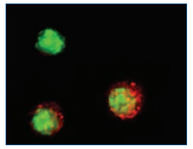
We offer an extensive selection of antigen affinity-purified polyclonal antibodies, monoclonal antibodies, and fluorochrome-conjugated antibodies that detect cytokines, chemokines, cell surface receptors, kinases, transcription factors, and a multitude of other factors. R&D Systems antibodies are generated in several different host species and are quality tested for a broad range of applications, including flow cytometry, Western blot, neutralization, blockage of receptor-ligand interactions, immunohistochemistry/immunocytochemistry, and ELISA.



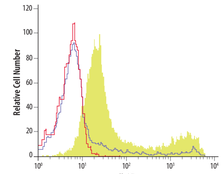
Detection of IL-22 and IL-23 R in $CD4^+$ Human PBMCs. $CD4^+$ human peripheral blood mononuclear cells (PBMCs) were activated with PMA/Ca^{2+} ionomycin. IL-22 was detected using Human IL-22 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF782) followed by NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001; red). IL-23 R was detected using Human IL-23 R Monoclonal Antibody (Catalog # MAB14001) followed by NorthernLights 637-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # NL008; pseudocolored green). Nuclei were counterstained with DAPI (blue).



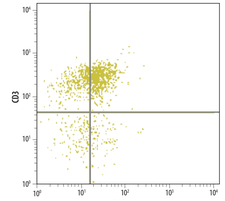
Detection of IL-23 R by Flow Cytometry. $CD4^+$ human peripheral blood mononuclear cells, untreated (light green histogram) or activated with PMA/Ca^{2+} ionomycin under Th17-inducing conditions (purple histogram), were stained with PE-conjugated Human IL-23 R Monoclonal Antibody (Catalog # FAB14001P) or PE-conjugated Mouse IgG_{2B} Isotype Control Antibody (Catalog # IC0041P; open histogram).



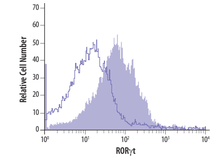
Detection of IL-21 in Mouse Splenocytes. Mouse splenocytes were stimulated with Concanavalin A. IL-21 was detected using Mouse IL-21 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF594) followed by staining with NorthernLights 557-conjugated Anti-Goat IgG Secondary Antibody (Catalog # NL001; red). Cells were counterstained green.



Detection of IL-27 by Flow Cytometry. Human peripheral blood mononuclear cells, untreated (open histogram - purple line) or activated with PHA and Recombinant Human IL-2 (Catalog # 202-IL; filled histogram), were stained with APC-conjugated Human IL-27 Monoclonal Antibody (Catalog # IC25261A) or APC-conjugated Mouse IgG_{2B} Isotype Control Antibody (Catalog # IC003A; open histogram – red line).



$CD3^+$ / RORγt$^+$ Cells Identified in PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were treated with PMA/Ca^{2+} ionomycin, lipopolysaccharide, and Recombinant Human IL-23 (Catalog # 1290-IL). Cells were stained with APC-conjugated Human CD3ε Monoclonal Antibody (Catalog # FAB100A) and PE-conjugated Human/Mouse RORγt/RORC2/NR1F3 Monoclonal Antibody (Catalog # IC6006P). Quadrant markers were set based on staining with PE-conjugated Mouse IgG_{2B} Isotype Control Antibody (Catalog # IC0041P).



Detection of RORγt in Mouse Thymocytes by Flow Cytometry. $CD4^+$/$CD8^+$ mouse thymocytes were stained with PE-conjugated Human/Mouse RORγt/RORC2/NR1F3 Monoclonal Antibody (Catalog # IC6006P; filled histogram) or PE-conjugated Mouse IgG_{2B} Isotype Control Antibody (Catalog # IC0041P; open histogram).