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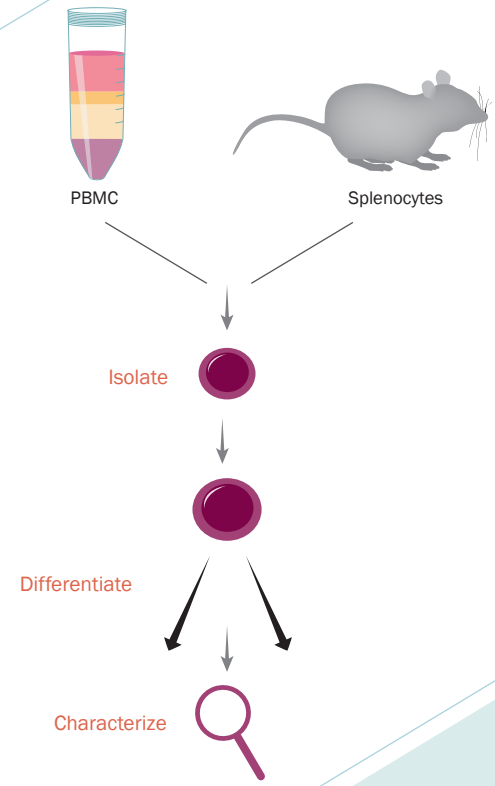
CD4+ T Cell Subset Characterization



CD4+ T Cells

T lymphocytes in the CD4+ lineage carry out a wide range of partially overlapping functions during immune responses. CD4+ T cells are critically involved in recruiting and activating other immune cells, dampening ongoing immune responses, and maintaining immunologic memory. When dysregulated, these cells can affect the severity of pathogenic infection, chronic inflammation, allergy, and autoimmunity.

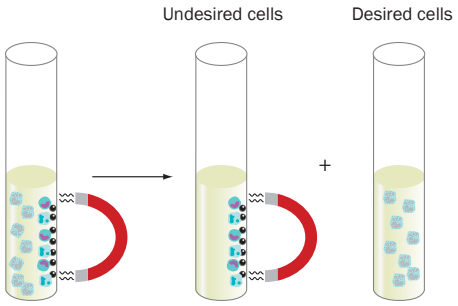
CD4+ T cells develop in the thymus and differentiate into subsets of more specialized T lymphocytes. These subsets express characteristic combinations of transcription factors, cell surface proteins, and secreted molecules. Some cells exhibit phenotypic and functional plasticity by shifting from one subset to another. In addition, the recent description of CD4+ T cells at intermediate stages of differentiation may blur the current subset classification.

Prominent Characteristics of CD4+ T Cell Subsets

	Functional	Molecular
Th1	Th1 cells protect against intracellular bacterial and viral infections and contribute to disease pathogenesis in autoimmunity.	• Develop in response to IL-12 and IFN-γ • Require transcription factors STAT4 and T-bet • Secrete IFN-γ, TNF-α, IL-2
Th2	Th2 cells protect against intestinal helminths and extracellular bacteria, support B cell-dependent humoral immune responses, and contribute to the development of allergic inflammation.	• Develop in response to IL-4 and IL-2 • Require transcription factors STAT6 and GATA3 • Secrete IL-4, IL-5, IL-9, IL-13, IL-17E
Th17	Th17 cells protect against extracellular bacteria and fungi, oppose some functions of regulatory T cells, and mediate autoimmune and inflammatory disease pathogenesis.	• Develop in response to TGF-β and IL-6 • Expand and survive in the presence of IL-21 and IL-23 • Require transcription factor RORγt • Secrete IL-17A, IL-17F, IL-21, IL-22, IL-26, TNF-α, IL-6, IL-9
Treg	Regulatory T cells (Treg) limit the development and progression of immune responses, oppose some functions of Th17 cells, and suppress the development of autoimmunity.	• Develop in response to TGF-β and IL-2 • Require transcription factor FoxP3 • Secrete TGF-β, IL-9, IL-10, IL-35
Th9	Th9 cells protect against helminth infection and contribute to inflammation, allergic pathogenicity, and anti-tumor immunity.	• Develop in response to IL-2 • Require transcription factors BATF, GATA-3, IRF1, IRF4, PU.1, STAT6 • Secrete IL-9, IL-10, IL-21
Th22	Th22 cells support mucosal immunity to microbial infection.	• Require transcription factors AHR and T-bet • Secrete IL-22, IL-10, IL-13, TNF-α
Tfh	Follicular helper T cells (Tfh) provide support for germinal center development and B cell responses.	• Require transcription factors Bcl6, IRF4, STAT4 • Secrete IL-4, IL-6, IL-21
Tfr	T follicular regulatory cells (Tfr) limit the development of germinal center reactions.	• Require transcription factors Bcl-6 and FoxP3 • Secrete IL-10

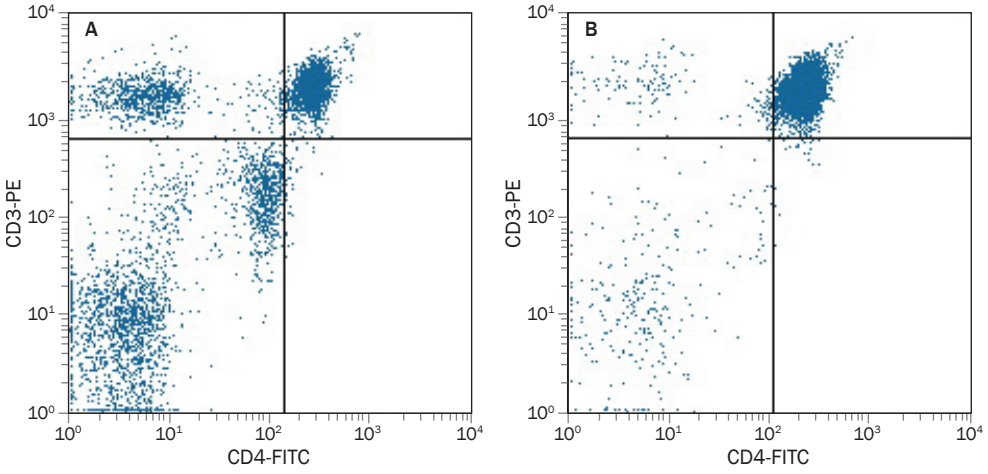
Isolation of CD4+ T Cells from Whole Blood or Spleen

Peripheral blood mononuclear cells (PBMCs) can be recovered from whole blood by centrifugation on a density medium such as Ficoll-Paque™. Erythrocytes are then cleared from the PBMCs by selective lysis. Alternatively, splenocytes can be recovered from excised spleens by gentle physical disruption. Either PBMCs or splenocytes are suitable starting samples for the isolation of CD4+ T cells. Enriched populations of CD4+ T cells can be prepared using the straightforward protocols of MagCellect™ kits. In these protocols, antibody-tagged cells bind to nanoparticles in a ferrofluid and are then subjected to a magnetic field.



MagCellect Cell Isolation Kits

T Cell Type	Catalog #
Human CD4+ Cells	MAGH102
Mouse CD4+ Cells	MAGM202
Rat CD4+ Cells	MAGR302
Mouse Naïve CD4+ Cells	MAGM205
Human Naïve CD4+ Cells	MAGH115
Mouse CD4+ CD25+ Regulatory T Cells	MAGM208
Human CD4+ CD25+ Regulatory T Cells	MAGH104
Rat CD4+ CD25+ Regulatory T Cells	MAGR304



Enrichment of CD4+ T Cells from Human PBMC Using the MagCellect Human CD4+ T Cell Isolation Kit (Catalog # MAGH102). Human peripheral blood mononuclear cells (PBMCs) before (**A**) and after (**B**) isolation of CD4+ T cells. Cells were stained with PE-conjugated Mouse Anti-Human CD3ε Monoclonal Antibody (Catalog # FAB100P) and FITC-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791F).

Differentiation of CD4+ T Cell Populations

The development of CD4+ T cell subsets is induced by distinct extracellular signals and is controlled by distinct transcription factors. CD4+ T cell subsets produce characteristic combinations of cytokines which enable them to exert diverse functions. The enrichment of desired CD4+ T cell subsets facilitates investigations into these subsets by reducing functional interference by other cell types.

Recombinant Cytokines

R&D Systems is the world's premier source of recombinant proteins. Extensive quality control produces industry leading bioactivity and lot-to-lot consistency that instills confidence in results and ensures reproducibility. Please see the back page for a sampling of our recombinant cytokines for CD4+ T cell differentiation.

CellXVivo™ Cell Differentiation and Expansion Kits

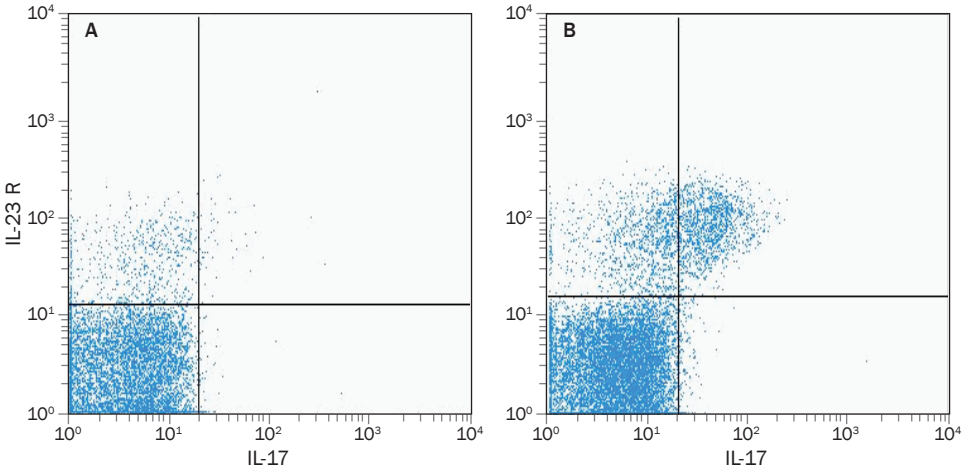
In addition to a wide range of high quality cytokines, we offer lymphocyte differentiation and expansion kits. These kits contain optimized concentrations of cytokine cocktails and validated, straightforward protocols.

CellXVivo Cell Differentiation Kits

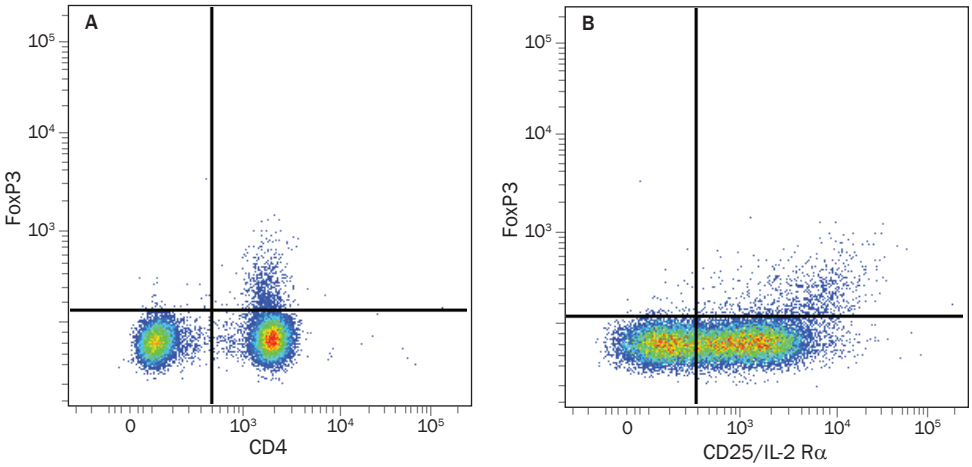
T Cell Subset	Catalog #
Human Th1 Cells	CDK001
Human Th2 Cells	CDK002
Human Th17 Cells	CDK003

Characterization of CD4$^+$ T Cell Subsets by Flow Cytometry

Following cell differentiation, flow cytometry can be used to verify the expression of established markers of CD4$^+$ T cell subsets. Flow cytometry can detect cell surface markers as well as intracellular molecules in permeabilized cells. Intracellular markers include transcription factors that control CD4$^+$ T cell differentiation and signature cytokines as they traffic through secretory organelles.



Staining of Th17-Activated PBMCs Using the Human Th17 Cell Multi-Color Flow Cytometry Kit. Human PBMCs were unstimulated (**A**) or stimulated (**B**) with 50 ng/mL PMA, 200 ng/mL ionomycin, 10 ng/mL Recombinant Human IL-23 (Catalog # 1290-IL), and 500 ng/mL LPS overnight and then incubated with PMA, ionomycin, and 3 µM monesin for 2-4 hours. The cells were stained simultaneously with fluorochrome-conjugated antibodies to IL-17 and IL-23 R provided in the kit (Catalog # FMC007). Dot plots were gated on CD3$^+$ cells.



Staining of CD4$^+$CD25$^+$ T Cells Using the FlowX™ Human Regulatory T Cell Multi-Color Flow Cytometry Kit. Human PBMCs were surface stained with (**A**) Fluorescein-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791F) and (**B**) PE-conjugated Mouse Anti-Human IL-2 Rα/CD25 Monoclonal Antibody (Catalog # FAB1020P), followed by intracellular staining using APC-conjugated Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody (Catalog # IC8214A). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). Quadrants were set based on lymphocytes.

Multi-Color Flow Cytometry Kits

T Cell Subset	Catalog #
Human Th1 Cells	FMC009
Mouse Th1 Cells	FMC010
Human Th2 Cells	FMC011
Mouse Th2 Cells	FMC012
FlowX Human Regulatory T Cells	FMC021
FlowX Mouse Regulatory T Cells	FMC022
Rat Regulatory T Cells	FMC015
Human Th17 Cells	FMC007

Transcription Factor Antibodies

Transcription Factor	Species	Catalog #	Cell Subset
AHR	Mouse	IC6697A, P, G	• Th22
BATF	Human	IC8054A	• Th9
FoxP3	Human	IC8214A, G, N, P	• Treg • Tfr
	Mouse	IC8214A, G, N, P	
GATA-3	Human	IC2605F, P	• Th2 • Th9
PU.1/Spi-1	Human	IC5870F, P	• Th2 • Th9
RORγt	Human	IC6006A, P, C	• Th17
	Mouse	IC6006A, P, C	
STAT6	Human	IC2167A, P	• Th2 • Th9
T-bet	Human	IC5385F, C	• Th1 • Th22

A Allophycocyanin, **C** PerCP, **F** Fluorescein, **G** Alexa Fluor® 488, **N** Alexa Fluor 700, **P** Phycoerythrin

Please see the back page for a larger sampling of our flurochrome-conjugated antibodies for Flow Cytometry.

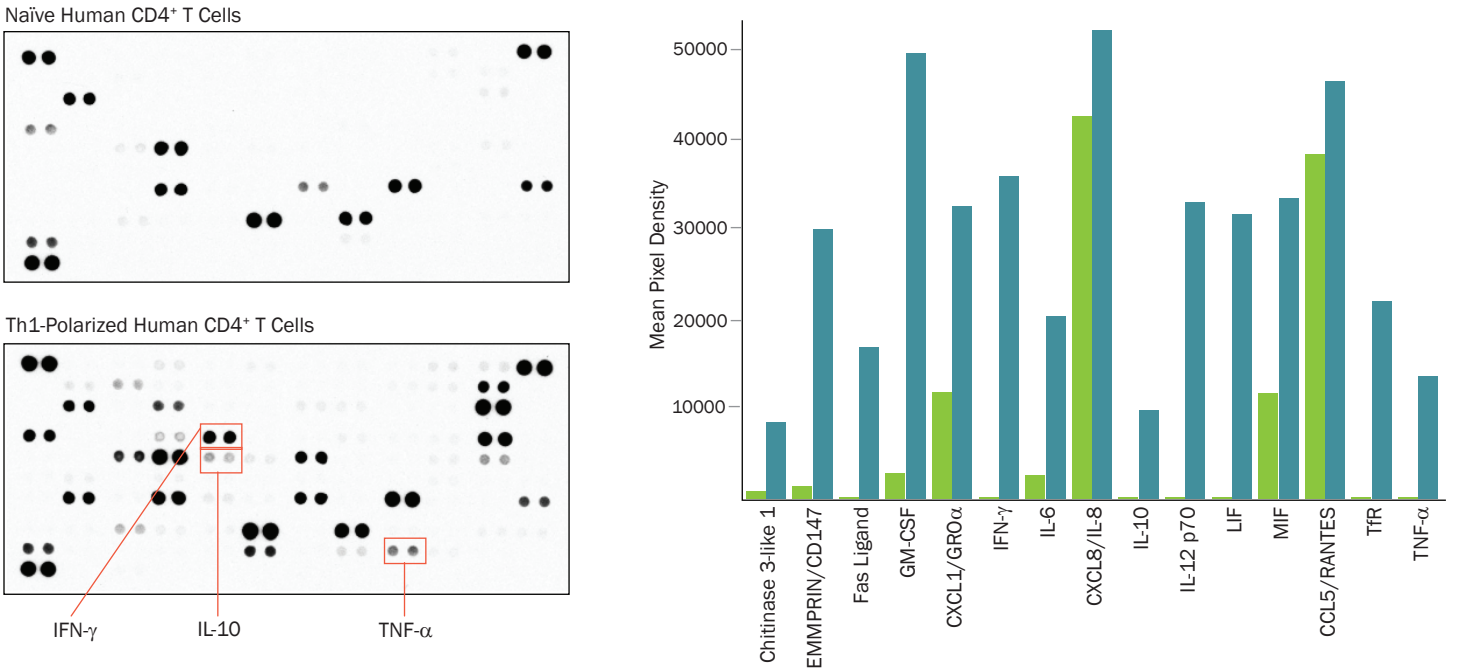
• cell surface proteins • intracellular cytokines

Characterization of Cytokine Secretion by Multiplexing

The culture medium from CD4+ T cell differentiation procedures should be tested to confirm that the cells are secreting cytokines relevant to the desired cell subset. Multi-analyte detection techniques enable efficient screening for many cytokines simultaneously. Both Proteome Profiler™ Antibody Arrays and Luminex®-based Flow Cytometry Assays are optimized for maximum specificity and sensitivity of analyte detection.

Proteome Profiler Antibody Arrays

Proteome Profiler Antibody Arrays allow for the measurement of up to 111 proteins in a single sample. These arrays do not require specialized equipment and eliminate the need for multiple Western blot experiments. Antibody array kit contains buffers, detection antibodies, and membranes spotted in duplicate with high-quality capture antibodies. The arrays utilize chemiluminescence for detection, and membranes can be assessed for protein levels in the same manner as traditional Western blots. Select arrays are also suitable for use with the LI-COR® detection system.



Detection of Cytokines Secreted by Th1-Polarized Human CD4+ T Cells Using the Proteome Profiler XL Cytokine Array. Naïve T cells were isolated from human PBMCs using the MagCellect Human Naïve CD4+ T Cell Isolation Kit (Catalog # MAGH115). These cells were either untreated or treated with CellXVivo Human Th1 Cell Differentiation Kit (Catalog # CDK001) reagents. Array images show the detection of secreted cytokines. The densitometric profile of other cytokines before (green bars) and after (blue bars) Th1 polarization is shown in the histogram.

Proteome Profiler Antibody Arrays

Cytokine Array	Analytes	Catalog #	LI-COR	Chemiluminescence
Human Cytokine Array Kit, Panel A	36	ARY005	✓	✓
Mouse Cytokine Array Kit, Panel A	40	ARY006	✓	✓
Rat Cytokine Array Kit	29	ARY008	✓	✓
Proteome Profiler Mouse XL Cytokine Array Kit	111	ARY028	✓	✓
Proteome Profiler Human XL Cytokine Array Kit	102	ARY022	✓	✓

Characterization of Cytokine Secretion by Multiplexing (continued)

Luminex

Luminex Screening and Performance Assays utilize color-coded polystyrene or superparamagnetic beads coated with analyte-specific antibodies. Beads recognizing different target analytes are mixed together and incubated with the sample. Captured analytes are subsequently detected using a cocktail of biotinylated detection antibodies and a streptavidin-phycoerythrin conjugate.

User-selected combinations of analytes can be assembled with our Luminex Assay Online Ordering Tool. The ordering tool walks you step-by-step through choices of screening or performance assays, polystyrene or magnetic bead formats, species, and target analytes of interest.

Luminex Screening Assays

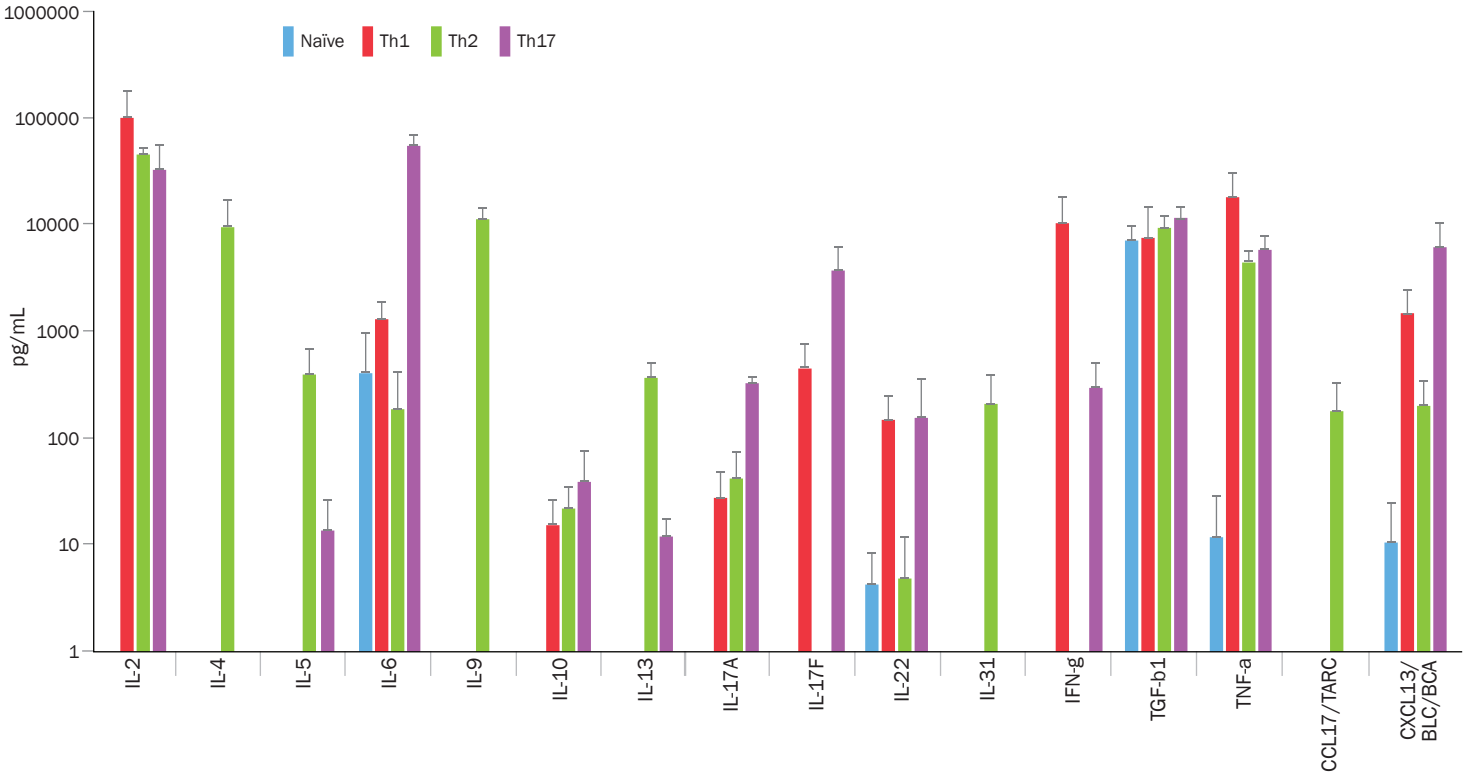
Luminex Screening Assays are the most flexible bead-based multiplex assays that we offer. They allow up to 100 user-defined target analytes to be simultaneously profiled using cell culture supernates, serum, or plasma samples.

Luminex Performance Assays

Luminex Performance Assays are the most accurate and precise bead-based multiplex assays for simultaneously measuring up to 22 analytes in qualified complex matrices.

Polystyrene beads are designed for use with the Luminex 100™, Luminex 200, or Bio-Rad® Bio-Plex® dual-laser analyzers.

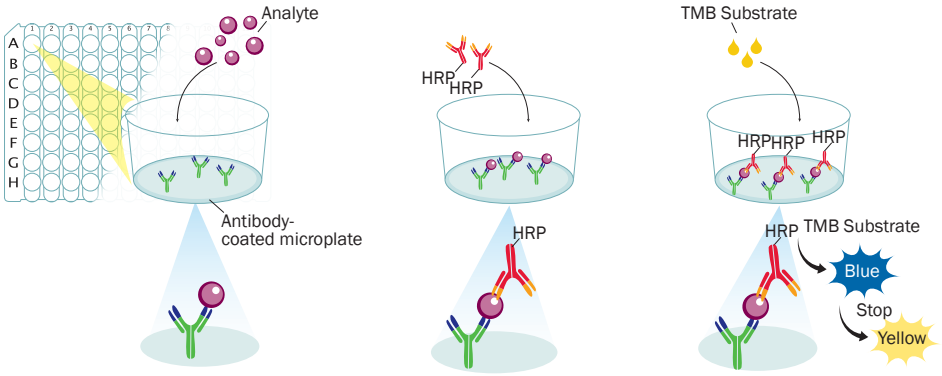
Magnetic beads are compatible with Luminex MAGPIX®, Luminex 100, Luminex 200, and Bio-Rad Bio-Plex analyzers.



Detection of Cytokines Secreted by Polarized Human CD4⁺ T Cells Using Luminex Screening Assays. Naïve T cells were isolated from PBMCs of three donors using the MagCellect Human Naïve CD4⁺ T Cell Isolation Kit (Catalog # MAGH115). For Th1 differentiation, cells were treated with CellXVivo Human Th1 Cell Differentiation Kit (Catalog # CDK001) reagents. For Th2 differentiation, cells were treated with Mouse Anti-Human CD3ε Monoclonal Antibody (Catalog # MAB100), Recombinant Human IL-2 (Catalog # 202-IL), Recombinant Human IL-4 (Catalog # 204-IL), Mouse Anti-Human IFN-γ Monoclonal Antibody (Catalog # MAB285), and Mouse Anti-Human CD28 Antibody. For Th17 differentiation, cells were treated with Mouse Anti-Human CD3ε Monoclonal Antibody (Catalog # MAB100), Recombinant Human IL-2 (Catalog # 202-IL), Recombinant Human IL-23 (Catalog # 1290-IL), Recombinant Human IL-1β (Catalog # 201-LB), and Mouse Anti-Human CD28 Antibody. Analytes were measured in a single Luminex Screening Assay with polystyrene beads (Catalog # LXSAH). TGF-β levels were determined using the TGF-β 1,-2,-3 Luminex Performance Assay. Assays were read using a Luminex LX100/200 instrument calibrated for polystyrene beads. Data represent averaged values from the three donors.

Quantitation of Cytokine Secretion by ELISA

R&D Systems offers a wide variety of sandwich ELISA kits that are designed to provide high levels of specificity, accuracy, precision, and sensitivity in analyte quantification. These kits enable the precise quantitation of inividual cytokine concentrations in a biological sample. They are available in a range of formats including colorimetric, fluorescent, and chemiluminescent for measuring intracellular and extracellular proteins. Cytokines shown in the table below are secreted by the $CD4^+$ T cell subsets in the right hand column.



Quantikine® ELISA Kits have been exhaustively tested for superior quality and reproducibility. Kit performance relies heavily on the selection of high quality antibody pairs and rigorous in-house testing throughout the development process. This includes component and kit stability, sensitivity, linearity, recovery, intra- and inter-assay precision, as well as cross-reactivity and interference testing with related analytes to confirm assay specificity. This stringent validation testing is used to optimize assay performance and verify that each kit will provide reproducible results both well-to-well and lot-to-lot.

DuoSet® ELISA Development Kits contain the basic components required to develop an immunoassay. They offer an economical alternative to Quantikine ELISA Kits.

ELISA Kits

		Catalog #				
Analyte	**Species**	**Quantikine**	**Quantikine High Sensitivity**	**QuantiGlo®**	**DuoSet**	**Cell Subset**
IFN-γ	Human	DIF50			DY285	• Th1
	Mouse	MIF00			DY485	
	Rat	RIF00				
IL-2	Human	D2050			DY202	• Th1
	Mouse	M2000			DY402	
	Rat	R2000			DY502	
IL-4	Human	D4050			DY204	• Th2 • Tfh
	Mouse	M4000B			DY404	
	Rat	R4000			DY504	
IL-5	Human	D5000B			DY205	• Th2
	Mouse	M5000			DY405	
IL-6	Human	D6050	HS600B	Q6000B	DY206	• Th17 • Tfh
	Mouse	M6000B			DY406	
	Rat	R6000B			DY506	
IL-9	Mouse				DY409	• Th2 • Th9 • Th17 • Treg • Th1 • Th2 • Treg • Th9 • Th22 • Tfr
IL-10	Human	D1000B	HS100C		DY217	
	Mouse	M1000B			DY417	
	Rat	SR1000			DY522	
IL-13	Human	D1300B			DY213	• Th2 • Th22
	Mouse	M1300CB			DY413	
IL-17A	Human	D1700			DY317	• Th17
	Mouse	M1700			DY421	
IL-17E	Mouse				DY1399	• Th2
IL-17F	Human				DY1335	• Th17
	Mouse				DY2057	
	Rat				DY4437	
IL-21	Mouse				DY594	• Th17 • Tfh
IL-22	Human	D2200			DY782	• Th17 • Th22
	Mouse	M2200			DY582	
	Rat	M2200				

Differentiation Cytokines

Cytokine	Species	Catalog #	Subset Induction
IL-2	Human	202-IL, 1081-IL, AFL202	• Th2 • Treg • Th9 • Tfh
	Mouse	402-ML, 1150-ML	
	Rat	502-RL	
IL-4	Human	204-IL, 6507-IL, 204-GMP	• Th2 • Th9
	Mouse	404-ML	
	Rat	504-RL	
IL-6	Human	206-IL, 7270-IL, 206-GMP	• Th2 • Th22 • Th17 • Tfh • Th9
	Mouse	406-ML	
	Rat	506-RL	
IL-12	Human	219-IL	• Th1 • Tfh
	Mouse	419-ML	
	Rat	1760-RL	
IL-23	Human	1290-IL	• Th17 • Tfh
	Mouse	1887-ML	
	Rat	3136-RL	
IL-27	Human	2526-IL	• Th1 • Tfh
	Mouse	2799-ML	
TGF-β1	Human	240-B, 100-B, 7754-BH, 240-GMP	• Th17 • Treg • Th9
	Mouse	7666-MB	

Cytokine Antibodies

Cytokine	Species	Catalog #	Cell Subset
IFN-γ	Human	IC285A, F, P, C, G	• Th1
	Mouse	IC485A, F, P, C, N	
IL-2	Human	IC202F, P	• Th1
	Mouse	IC402F, P	
IL-4	Human	IC304A, F, P, C	• Th2 • Tfh
IL-5	Human	IC605F, P	• Th2
	Mouse	IC405P	
IL-6	Human	IC206F, P	• Th17 • Tfh
	Mouse	IC406F, P	
IL-9	Human	IC209A	• Th2 • Th17 • Treg • Th9
IL-10	Human	IC2172A, F, P, C	• Th2 • Treg • Th22 • Tfr • Th9
IL-13	Human	IC2131A, F, P	• Th2 • Th22
IL-17A	Human	IC3171A, P, C, G, N	• Th17
	Mouse	IC421F, P, C	
IL-17E	Human	IC1285A, F, P	• Th2
	Mouse	IC13991P	
IL-17F	Human	IC13351A, F, P	• Th17
	Mouse	IC2057A, F, P, C	
IL-21	Mouse	IC594A, P, C, G	• Th17 • Th9 • Tfh
IL-22	Human	IC7821A, F, P, C	• Th17 • Th22
	Mouse	IC582A, F, P, C	
IL-26/AK155 (human only)	Human	IC13751A, P	• Th17
TNF-α	Human	IC210F, P	• Th1 • Th17 • Th22
	Mouse	IC410F, P	
TGF-β1	Human	IC240A, F, P, N	• Treg

Cell Surface Molecule Antibodies

Cell Surface Molecule	Species	Catalog #	Cell Subset
CCR4	Human	FAB1567A, F, P, C	• Th2 • Th22 • Th17
CCR6	Human	FAB195A, F, P, C	• Th1 • Th9 • Th17 • Th22
	Mouse	FAB590A, G, N	
CCR10	Human	FAB3478A, P, C, G	• Th22
	Mouse	FAB2815A, P, C, N	
CD25/IL-2 Rα	Human	FAB1020A, P, G	• Treg
	Mouse	FAB2438A, P, C, N	
Common γ Chain	Human	FAB2842A, F, P	• Th2 • Th17
	Mouse	FAB7842A	
CXCR5	Human	FAB190A, F, P, C, N	• Tfh • Tfr
	Mouse	FAB6198A, F, P, C	
GITR	Human	FAB689A, F, P, G, N	• Treg • Tfr
	Mouse	FAB5241A, F, P	
gp130	Human	FAB228A, F, P, C, N	• Th1 • Th17
	Mouse	FAB4681A, P	
ICOS	Human	FAB6975A, P	• Tfh • Tfr
	Mouse	FAB168A, P	
IFN-γ R1	Human	FAB673F, P	• Th1
	Mouse	FAB1029P	
IFN-γ R2	Human	FAB773A, F	• Th1
IL-1 RI	Human	FAB269A, F, P, N	• Th17
	Mouse	FAB7712F, P	
IL-1 RAcP/IL-1 R3	Human	FAB6876A, P, C, G	• Th17
IL-4 Rα	Human	FAB230A, F, P, C, N	• Th2
	Mouse	FAB530F, P	
IL-6 Rα	Human	FAB227A, F, P	• Th17
	Mouse	FAB8180A, F, P, G	
IL-12 Rβ1	Human	FAB839A, F, P, C, N	• Th1 • Th17
	Mouse	FAB1998F, P	
IL-12 Rβ2	Human	FAB1959A, P, C, G	• Th1
	Mouse	FAB1959A, P, C, G	
IL-17 RB	Human	FAB1207A, P, C	• Th9
	Mouse	FAB10402A, P, G	
IL-18 R/IL-1 R5	Human	FAB840A, P, G	• Th1
	Mouse	FAB1216A, F, N	
IL-18 Rβ /IL-1 R7	Human	FAB118F, P	• Th1
IL-21 R	Human	FAB9911A, F, P	• Th17
	Mouse	FAB5961P	
IL-23 R	Human	FAB14001A, F, P, C, N	• Th17
	Mouse	FAB16861F, P, N	
IL-27 Rα/WSX-1/TCCR	Human	FAB14791A, P, G	• Th1
	Mouse	FAB21091F, P, N	
Integrin αE/CD103	Mouse	FAB1990A, P, G	• Treg • Tfr
PD-1	Human	FAB7115P, G	• Tfh • Tfr
	Mouse	FAB1021F, P, G	
TGF-β RI	Mouse	FAB5871A, P	• Th17
TGF-β RII	Human	FAB2411A, F, P, C, N	• Th17
	Mouse	FAB532A, F, P, C, N	

A Allophycocyanin, **C** PerCP, **F** Fluorescein, **G** Alexa Fluor® 488, **N** Alexa Fluor 700, **P** Phycoerythrin

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