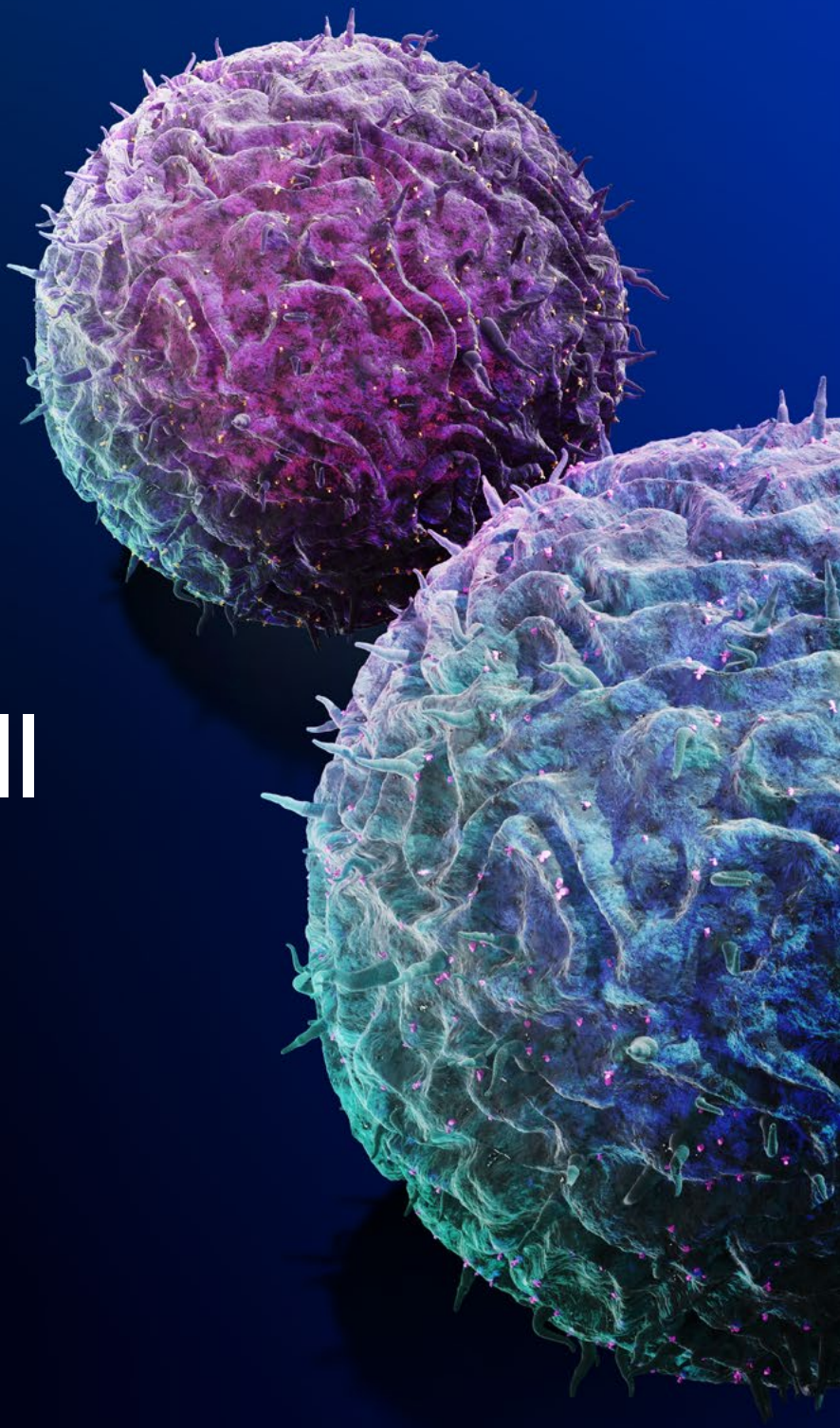
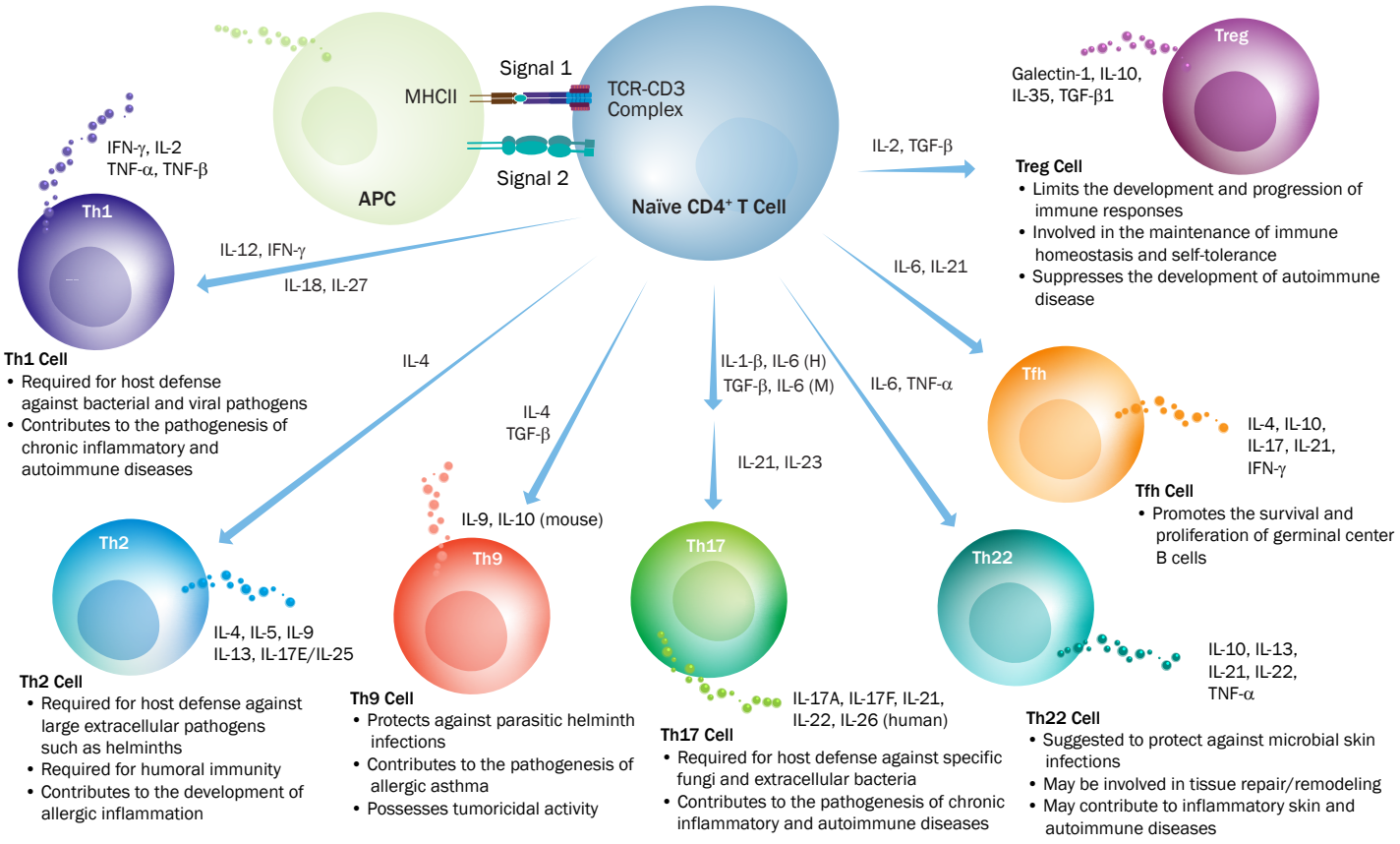
bio-techne®

$CD4^+$ T Cell Subsets



CD4⁺ T Cell Subsets

CD4⁺ T cells play a central role in directing adaptive immune responses against invading microbial pathogens. Naïve CD4⁺ T cells differentiate into more specialized subsets following T cell activation, which requires recognition of the antigenic peptide/major histocompatibility complex (MHC) by the T cell receptor (Signal 1), along with antigen-independent co-stimulation (Signal 2). Differentiation into a specific CD4⁺ T cell subset depends on the antigen, the strength of the TCR signal, and cytokines present in the surrounding extracellular environment. The different CD4⁺ T helper cell subsets that have been identified include T helper 1 (Th1), Th2, Th9, Th17, and Th22 cells, along with follicular helper T (Tfh) cells, and regulatory T cells (Tregs). Each of these subsets expresses unique combinations of cell surface receptors, transcription factors, and secreted cytokines. While Tfh cells are primarily involved in promoting the survival and proliferation of germinal center B cells and supporting germinal center development, the Th1, Th2, Th9, Th17, and Th22 subsets have all been found to be involved in host defense against specific microbial pathogens. The defined array of cytokines secreted by these effector T cell subsets contributes to both elimination of the foreign pathogen and activation of other immune cell types. The activities of these cells are balanced in part by a unique subpopulation of CD4⁺ T cells known as regulatory T cells (Tregs). Tregs specialize in maintaining immune homeostasis and self-tolerance, dampening inflammation, and preventing the development of autoimmune disease. This balance between pro-inflammatory and anti-inflammatory signals is critically important as exaggerated or inappropriate T cell responses have been found to be associated with allergic responses, inflammatory disorders, and autoimmune diseases.



Isolation of CD4+ T cells

MagCellect™ Cell Selection Kits & Reagents

R&D Systems® MagCellect CD4+ T Cell Selection Kits are designed to isolate a specific population of CD4+ T cells from a mononuclear cell suspension. The protocols utilize either a negative selection principle for the isolation of CD4+ T cells, naïve CD4+ T cells, or memory CD4+ T cells, or a two-step procedure that combines both negative and positive selection for the isolation of CD4+CD25+ regulatory T cells. Features of the kits include:

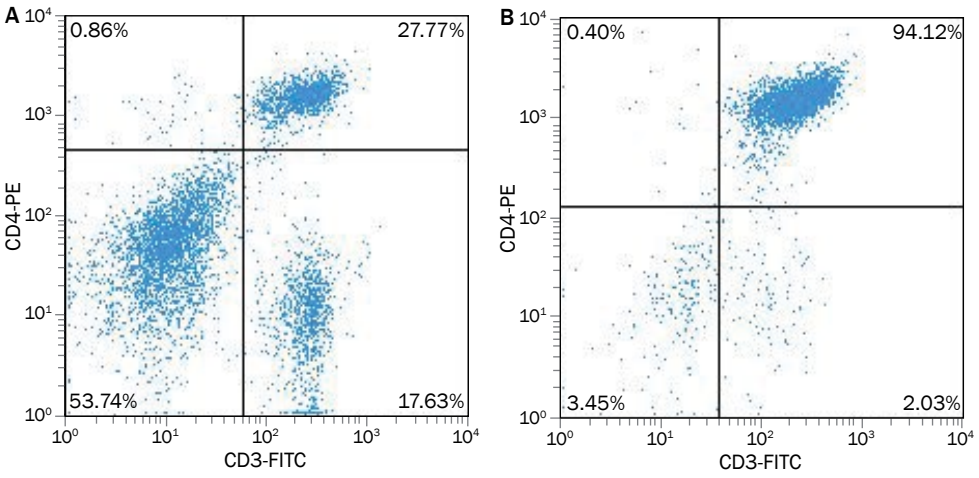
- Beads with a high binding capacity
- Small beads in ferrofluid that have no magnetic memory and will not induce cell damage
- Cells can be separated to a very high purity in minutes

MagCellect Human CD4+ T Cell Isolation Kits	Catalog #
Human Naïve CD4+ T Cell Isolation Kit	MAGH115
Human CD4+ T Cell Isolation Kit	MAGH102
Human CD4+CD25+ Regulatory T Cell Isolation Kit	MAGH104
Human Memory CD4+ T Cell Isolation Kit	MAGH116

MagCellect Mouse CD4+ T Cell Isolation Kits	Catalog #
Mouse Naïve CD4+ T Cell Isolation Kit	MAGM205
Mouse CD4+ T Cell Isolation Kit	MAGM202
Mouse CD4+CD25+ Regulatory T Cell Isolation Kit	MAGM208
Mouse Memory CD4+ T Cell Isolation Kit	MAGM206

MagCellect Rat CD4+ T Cell Isolation Kits	Catalog #
Rat CD4+ T Cell Isolation Kit	MAGR302B

MagCellect Reagents	Catalog #
MagCellect Magnet	MAG997
MagCellect Streptavidin Ferrofluid	MAG999B



Enrichment of Mouse CD4+ T Cells using the MagCellect Mouse CD4+ T Cell Isolation Kit. Mouse splenocytes before (**A**) and after (**B**) isolation of CD4+ T cells using the MagCellect Mouse CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGM202). Dot plots reflect double-staining of all viable cells with a Fluorescein-conjugated Rat Anti-Mouse CD3 Monoclonal Antibody (R&D Systems, Catalog # FAB4841F) and a PE-conjugated Rat Anti-Mouse CD4 Monoclonal Antibody (R&D Systems, Catalog # FAB554P).

CD4+ T Cell Enrichment Columns

In addition to our MagCellect Cell Selection Kits, Bio-Techne also offers human and mouse CD4+ T cell enrichment columns from our R&D Systems brand that are designed to enrich for a specific CD4+ T cell population from a mononuclear cell suspension. Key benefits of the columns include:

- Yields a highly pure (84-97%) population of T cells
- Separates cells based on gravitational flow
- Negative selection reduces sources of experimental variation

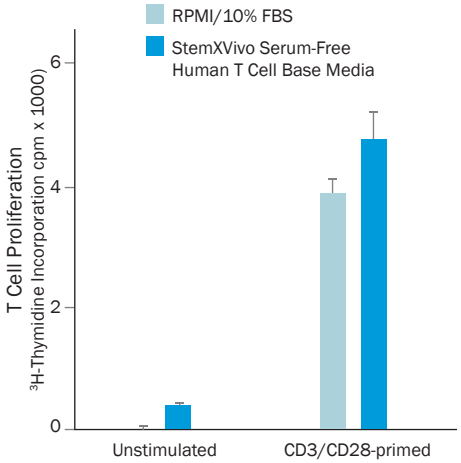
Human T Cell Enrichment Columns	Catalog #
Human CD4+ T Cell Enrichment Column	HCD4C-1000
Human CD4+ T Cell Enrichment Column, Mini	HCD43
Human CD4+/CD45RA- Memory T Cell Enrichment Column	HCD42
Human CD4+/CD45RO- Naïve T Cell Enrichment Column	HCD41

Mouse T Cell Enrichment Columns	Catalog #
Mouse CD4+ T Cell Enrichment Column	MCD4C-1000
Mouse CD4+ T Cell Enrichment Column, Mini	MCD43

Optimized Media for the Culture of Human T Cells

Following isolation, the maintenance and expansion of T cells is highly dependent on media and cytokines. Bio-Techne offers R&D Systems Human StemXVivo® Serum-Free T Cell Base Media for the culture and expansion of human T cells. This media supports T cell expansion better than RPMI containing 10% fetal bovine serum (FBS) and has a high lot-to-lot consistency, which decreases variation from one experiment to the next.

Specialized Media	
Product	**Catalog #**
StemXVivo Serum-Free Human T Cell Base Media	CCM010



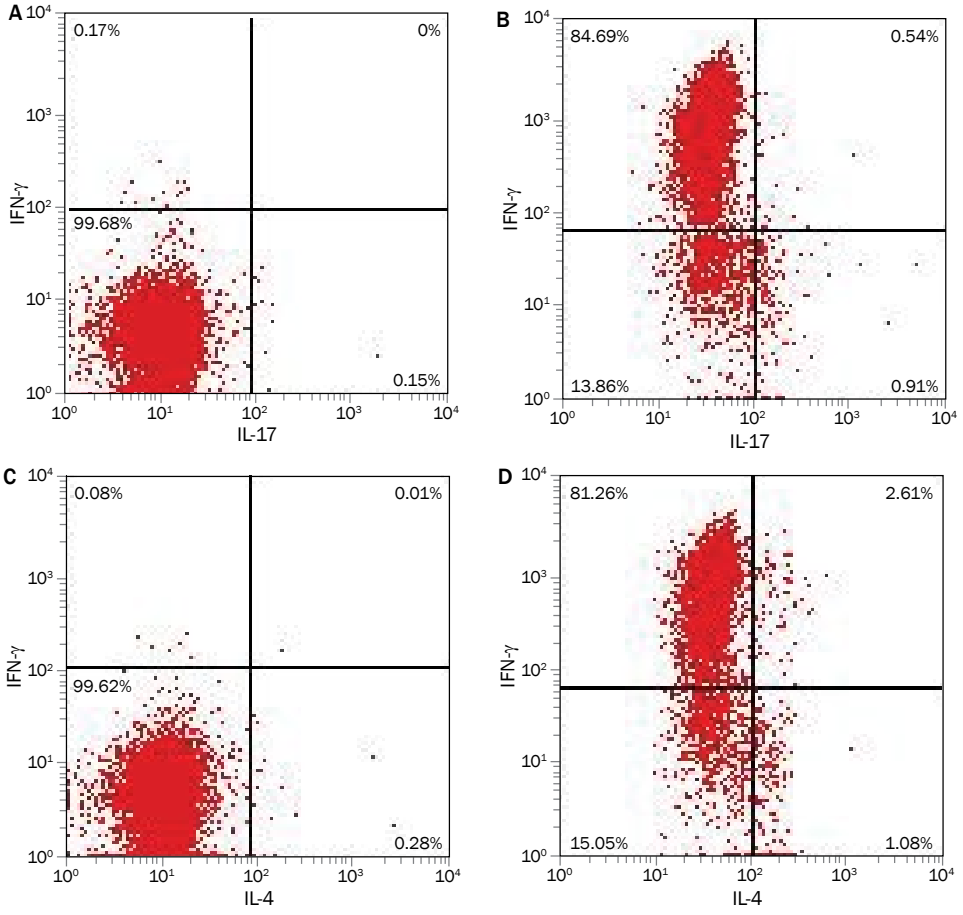
Proliferative Response of Cultured CD3/CD28-Primed T Cells in Human StemXVivo® Serum-Free T Cell Base Media. 1 x 10^5 purified CD3+ T cells were cultured for five days in StemXVivo Serum-Free T Cell Base Media (Catalog # CCM010) or with RPMI supplemented with 10% Fetal Bovine Serum (RPMI/10% FBS). The purified CD3+ T cells were cultured on 96 well microplates coated with Mouse Anti-Human CD3 epsilon (R&D Systems; Catalog # MAB100) and Goat Anti-Human CD28 Antigen Affinity-purified Polyclonal Antibody. [^{3}H]-thymidine was added for the final 18 hours. Cells were harvested and the incorporation of [^{3}H]-thymidine was measured using a beta-scintillation counter. Results are presented as the mean ± the standard deviation of samples run in triplicate.

Differentiation of Naïve CD4$^+$ T Cells into Specific T Helper Cell Subsets

CellXVivo™ Immune Cell Differentiation and Expansion Kits

R&D Systems CellXVivo Immune Cell Differentiation and Expansion Kits contain optimized combinations of the highest quality proteins and antibodies, along with simple, reproducible protocols for differentiating immune cells *ex vivo*. Kits are available for the differentiation of human or mouse Th1 cells, Th2 cells, Th17 cells, or regulatory T cells. Benefits of the kits include:

- Conveniently packages all reagents necessary for immune cell differentiation or expansion
- Guaranteed to produce consistent, reliable results
- Cost-effective and efficient



Intracellular Cytokine Staining of Mouse Th1 Cells Following Differentiation Using the CellXVivo Mouse Th1 Cell Differentiation Kit. Mouse naïve CD4$^+$ T cells without (**A**, **C**) or with (**B**, **D**) a 5-day differentiation using the reagents provided in the CellXVivo Mouse Th1 Differentiation Kit (R&D Systems, Catalog # CDK018) were stimulated with Cell Activation Cocktail (Tocris, Catalog # 5476) and stained with a PE-conjugated Rat Anti-Mouse IFN-γ Monoclonal Antibody (R&D Systems, Catalog # IC485P) and either an (**A**, **B**) APC-conjugated Rat Anti-Mouse IL-17 Monoclonal Antibody (Novus Biologicals, Catalog # NBP1-72027) or an (**C**, **D**) APC-conjugated Rabbit Anti-Mouse IL-4 Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-52656) and detected by flow cytometry. Quadrants were set based on staining with the appropriate isotype controls.

Human CellXVivo T Cell Differentiation Kits	Catalog #
Human Th1 Cell Differentiation Kit	CDK001
Human Th2 Cell Differentiation Kit	CDK002
Human Th17 Cell Differentiation Kit	CDK003C
Human Treg Cell Differentiation Kit	CDK006

Mouse CellXVivo T Cell Differentiation Kits	Catalog #
Mouse Th1 Cell Differentiation Kit	CDK018
Mouse Th2 Cell Differentiation Kit	CDK019
Mouse Th17 Cell Differentiation Kit	CDK017
Mouse Treg Cell Differentiation Kit	CDK007

Recombinant Proteins and Antibodies for T Cell Activation, Differentiation, and Expansion

In addition to our CellXVivo Immune Cell Differentiation and Expansion Kits, Bio-Techne also offers individually packaged R&D Systems recombinant proteins and antibodies for activating, differentiating, and expanding different $CD4^+$ T helper cell subsets. In addition to the standard recombinant proteins shown in the product tables below, our catalog also includes, Animal-Free™ and Animal Component-Free Process recombinant proteins, GMP-grade recombinant proteins, and custom protein development services, which are detailed in the sections that follow.

T Cell Activation Antibodies

Molecule	Species	Clone	Catalog # (Applications)
CD3	Human	UCHT1	MAB100 (CFA, FC, ICC/IF, IP)
	Mouse	145-2C11	MAB484 (Depl., FA, FC, IP, WB)
CD28	Human	37407	MAB342 (FA, WB)
	Human	Polyclonal	AF-342-PB (FA, FC, ICC/IF, IHC)
	Mouse	794716	MAB4832 (FA, FC)

Product	Description	Catalog #
Cell Activation Cocktail	Contains Monensin sodium salt (1.5 mM), Phorbol 12-myristate 13-acetate (0.0405 mM) and Ionomycin calcium salt (0.67 mM). Cocktail is provided as 1 ml of 500X concentrate in ethanol.	5476

Recombinant Cytokines

Molecule	Species	Source	Catalog #
IFN-γ	Human	*E. coli*	285-IF
	Human	HEK293	10067-IF
	Mouse	*E. coli*	485-MI
	Rat	*E. coli*	585-IF
IL-1β/IL-1F2	Human	*E. coli*	201-LB
	Mouse	*E. coli*	401-ML
	Rat	*E. coli*	501-RL
IL-2	Human	*E. coli*	202-IL
	Mouse	*E. coli*	402-ML
	Rat	*E. coli*	502-RL
IL-4	Human	*E. coli*	204-IL
	Human	CHO	6507-IL
	Mouse	*E. coli*	404-ML
	Rat	*E. coli*	504-RL
IL-6	Human	*E. coli*	206-IL
	Human	HEK293	7270-IL
	Mouse	*E. coli*	406-ML
	Rat	*E. coli*	506-RL
IL-7	Human	*E. coli*	207-IL
	Mouse	*E. coli*	407-ML

Recombinant Cytokines

Molecule	Species	Source	Catalog #
IL-12	Human	*Sf*21 (baculovirus)	219-IL
	Human	HEK293	10018-IL
	Mouse	*Sf*21 (baculovirus)	419-ML
	Mouse	NS0	10051-ML
IL-15	Human	*E. coli*	247-ILB
	Mouse	*E. coli*	447-ML
IL-21	Human	*E. coli*	8879-IL
	Mouse	*E. coli*	594-ML
IL-23	Human	*Sf*21 (baculovirus)	1290-IL
	Mouse	*Sf*21 (stably transfected)	1887-ML
IL-27	Human	NS0	2526-IL
	Mouse	NS0	2799-ML
TGF-β1	Human	HEK293	7754-BH
	Human	CHO	240-B
	Human	Platelets	100-B
	Mouse	CHO	7666-MB
TNF-α	Human	*E. coli*	210-TA
	Mouse	*E. coli*	410-MT
	Mouse	*E. coli*	410-TRNC
	Rat	*E. coli*	510-RT

Antibodies

Molecule	Species	Clone	Catalog # (Applications)
IFN-γ	Human	25718	MAB285 (B/N, FC, ICC/IF)
	Human	25718R	MAB285R (B/N, ICC/IF)
	Human	K3.53	MAB2852 (B/N, Ecap, FC, WB)
	Human	Polyclonal	AF-285-NA (B/N, ICC/IF, WB)
	Human	Polyclonal	AB-285-NA (B/N, WB)
	Mouse	37895	MAB485 (B/N, FC, WB)
	Mouse	37895R	MAB485R (B/N, FC)
	Mouse	H22	MAB4851 (B/N, ICC/IF)
	Mouse	Polyclonal	AF-485-NA (B/N, WB)
	Mouse/Rat	Polyclonal	AF-585-NA (B/N, ICC/IF, WB)
	Rat	88915	MAB585 (B/N, WB)

Antibodies

Molecule	Species	Clone	Catalog # (Applications)
IL-4	Human	34019	MAB204 (B/N, WB)
	Human	3007	MAB304 (B/N, ICC/IF, WB)
	Human	Polyclonal	AF-204-NA (B/N, WB)
	Mouse	30340	MAB404 (B/N, Ecap, WB)
	Mouse	Polyclonal	AF-404-NA (B/N, WB)
	Mouse	30340R	MAB404R (B/N, Ecap)
	Rat	Polyclonal	AF-504-NA (B/N, WB)
	Rat	154101	MAB5041 (B/N, WB)
IL-12	Human	Polyclonal	AF-219-NA (B/N, ICC/IF, WB)
	Mouse	Polyclonal	AF-419-NA (B/N, WB)

Antibody Application Key: **B/N** Blocking/Neutralization **Ecap** ELISA Capture **FC** Flow Cytometry **ICC/IF** Immunocytochemistry/Immunofluorescence **IHC** Immunohistochemistry **WB** Western Blot

Animal-Free™ and Animal Component-Free Process (ACFP) Recombinant Proteins

Animal-free proteins are particularly important for researchers concerned with experimental variables caused by trace animal components or mammalian pathogens. To support developing research and bioproduction demands, Bio-techne offers R&D Systems-branded Animal-Free recombinant proteins, which are manufactured in a laboratory exclusively dedicated to the production and purification of proteins under animal-free conditions. We also offer R&D Systems Animal Component-Free Process (ACFP) recombinant proteins that are expressed in an animal-free certified *Sf* 9 insect cell line using dedicated animal-free raw materials and labware. Features of our Animal-Free proteins include:

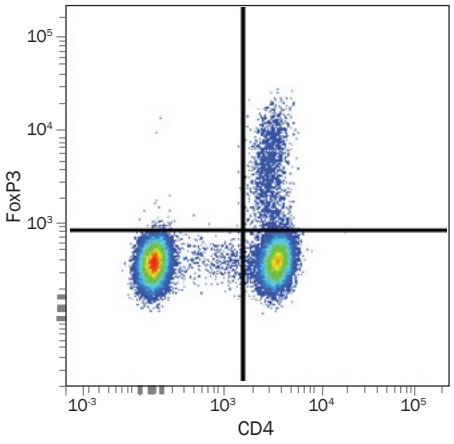
- Endotoxin levels < 1.0 EU per 1 µg of product
- Tested to ensure no impairment of biological activity
- Additional documentation that can be provided for *ex vivo* research or bioproduction

Preclinical Animal-Free RUO Proteins	
Protein (Human; Source: *E. coli*)	**Animal-Free RUO Protein (Catalog #)**
Activin A	AFL338
BDNF	BT-BDNF-AFL
Betacellulin	BT-BTC-AFL
BMP-4	AFL314E
CD40 Ligand/TNFSF5 (a.a. 108-261)	AFL617
EGF	AFL236
FGF acidic/FGF1	AFL232
FGF basic/FGF2/bFGF	BT-FGFB-AFL
Flt-3 Ligand/FLT3L	BT-FT3L-AFL
G-CSF	AFL214
GM-CSF	AFL215
IFN-γ	AFL285
IGF-I/IGF-1	AFL291
IL-1β/IL-1F2	AFL201
IL-2	BT-002-AFL

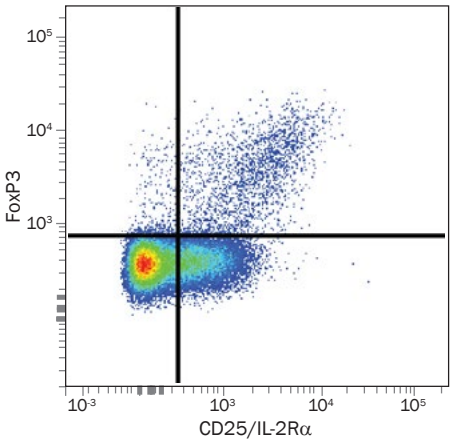
Preclinical Animal-Free RUO Proteins	
Protein (Human; Source: *E. coli*)	**Animal-Free RUO Protein (Catalog #)**
IL-3	AFL203
IL-4	BT-004-AFL
IL-6	AFL206
IL-7	BT-007-AFL
IL-10	AFL1064
IL-15	BT-015-AFL
IL-21	BT-021-AFL
M-CSF	AFL216
PDGF-AA	AFL221
PDGF-BB	AFL220
SCF/c-kit Ligand	BT-SCF-AFL
Sonic Hedgehog/Shh	AFL1845
TNF-α	AFL210
VEGF	BT-VEGF-AFL

Identification and Characterization of CD4+ T Cell Subsets by Flow Cytometry

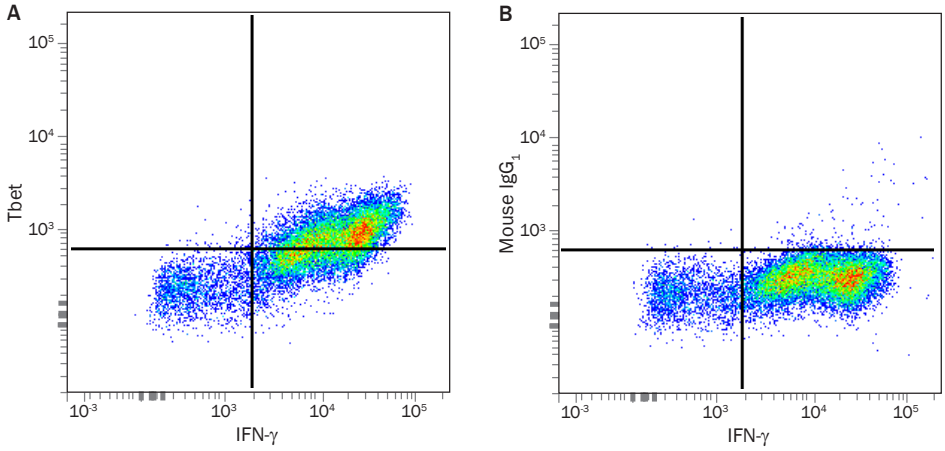
Following T cell differentiation, the different CD4+ T cell subsets can be identified by analyzing the expression of cell surface receptors, transcription factors, and secreted cytokines. Bio-Techne offers a wide selection of fluorochrome-conjugated R&D Systems and Novus Biologicals antibodies qualified for flow cytometry that can be used to detect different T cell markers. Hundreds of world-renowned unique clones are available from the R&D Systems brand, many of which have been used to establish CD nomenclature through HLDA workshops. In addition, Bio-Techne also offers antibodies from the Novus Biologicals brand, which includes an expansive collection of some of the most highly referenced antibody clones on the market.



Detection of FoxP3 in CD4+ Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were stained with a PE-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8970P) and a Fluorescein-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (R&D Systems, Catalog # FAB3791F). Quadrant markers were set based on control antibody staining (R&D Systems, Catalog # IC002P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012).



Detection of FoxP3 in CD25+ Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were stained with a PE-conjugated Mouse Anti-Human/Mouse/Rat FoxP3 Monoclonal Antibody (R&D Systems, Catalog # IC8970P) and an APC-conjugated Mouse Anti-Human CD25/IL-2 Rα Monoclonal Antibody (R&D Systems, Catalog # FAB1020A). Quadrant markers were set based on control antibody staining (R&D Systems, Catalog # IC002P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012).



Detection of T-bet/TBX21 in CD4+ Human PBMCs Stimulated to Induce Th1 Cells by Flow Cytometry. CD4+ human peripheral blood mononuclear cells (PBMCs) were treated with 20 ng/mL Anti-Human IL-4 Polyclonal Antibody (R&D Systems, Catalog # AB-204-NA) and 10 ng/mL Recombinant Human IL-12 (R&D Systems, Catalog # 219-IL) for 5 days to induce Th1 cell development. Cells were stained with a PE-conjugated Mouse Anti-Human IFN-γ Monoclonal Antibody (R&D Systems, Catalog # IC285P) and either (**A**) an Alexa Fluor 488-conjugated Mouse Anti-Human T-bet/TBX21 Monoclonal Antibody (R&D Systems, Catalog # IC53851G) or (**B**) an Alexa Fluor 488-conjugated Mouse IgG Isotype Control (R&D Systems, Catalog # IC002G). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012).

Flow Cytometry Antibodies Commonly Used to Identify Different CD4+ T Cell Subsets

Cell Surface Markers					
Molecule	**Species**	**Clone**	**Fluorochrome-conjugated Antibodies Catalog # - Fluorochrome**	**Unlabeled Antibodies Catalog # (Applications)**	**Recombinant Monoclonal Antibodies Catalog # (Applications)**
CCR1	Human	53504*	FAB145-N, P, R, S, T, U, V	MAB145 (CyTOF-ready, FC)	MAB145R (CyTOF-ready, FC)
	Mouse	643854	FAB5986-A, F, G, N, P, R, S, T, U, V	MAB5986 (CyTOF-ready, FC, WB)	MAB5986R (CyTOF-ready, FC)
CCR3	Human	61828*	FAB155-A, C, F, G, P, R, S, T, U, V	MAB155 (B/N, CyTOF-ready, FC, IHC)	
	Mouse	83101	FAB729-C, F, G, P, R, S, T, U, V	MAB729 (FC)	
CCR4	Human/Rat	205410*	FAB1567-A, C, F, G, N, P, R, S, T, U, V	MAB1567 (CyTOF-reported, FC)	MAB1567R (CyTOF-ready, FC)
CCR5	Human/Mouse/Rat	CTC5	FAB1802-A, F, G, N, P, R, S, T, U, V	MAB1802 (CyTOF-ready, FC, WB)	MAB1802R (CyTOF-ready, FC)
CCR6	Human	53103*	FAB195-A, C, F, G, N, P, R, S, T, U, V	MAB195 (CyTOF-ready, FC, IHC)	MAB195R (CyTOF-ready, FC, IHC)
	Human/Mouse	140706	FAB590-A, G, P, R, S, T, U, V	MAB590 (CyTOF-ready, FC, IHC)	MAB590R (CyTOF-ready, FC, IHC)
CCR8	Human/Rat	191704*	FAB1429-A, C, P	MAB1429 (B/N)	
	Mouse	1055C	FAB8324-A, G, N, P, R, S, T, U, V		MAB8324 (FC)
CCR10	Human	314305	FAB3478-A, G, N, P, R, S, T, U, V	MAB3478 (CyTOF-reported, FC)	MAB3478R (CyTOF-ready, FC)
	Mouse	248918	FAB2815-A, C, G, N, P, R, S, T, U, V	MAB2815 (CyTOF-ready, FC)	MAB2815R (CyTOF-ready, FC)
CXCR3	Human	49801	FAB160-A, C, F, G, N, P, R, S, T, U, V	MAB160 (B/N, CyTOF-ready, FC, IHC)	MAB160R (B/N, CyTOF-ready, FC, IHC)
	Mouse	220803	FAB1685-A, G, N, P, R, S, T, U, V	MAB1685 (CyTOF-ready, FC)	MAB1685R (CyTOF-ready, FC)
CXCR4	Human	12G5	FAB170-A, F, G, N, P, R, S, T, U, V	MAB170 (B/N, CyTOF-reported, FC)	MAB170R (B/N, FC)
	Human	44716	FAB172-G, N, R, S, T, U, V	MAB172 (B/N, CyTOF-ready, FC, IHC)	
	Mouse	247506	FAB21651-A, C, F, G, N, P, R, S, T, U, V	MAB21651 (B/N, CyTOF-ready, FC, ICC/IF, IHC)	MAB21651R (CyTOF-ready, FC, ICC, IHC)
CXCR5	Human	51505*	FAB190-A, C, F, G, P, R, S, T, U, V	MAB190 (B/N, CyTOF-ready, FC, ICC/IF, IHC)	MAB190R (IHC)
	Mouse	614641	FAB6198-A, G, N, R, S, T, U, V	MAB6198 (CyTOF-reported, FC, ICC/IF)	
CD3	Human	UCHT1*	FAB100-A, C, F, G, N, P, S, T, U, V	MAB100 (CyTOF-reported, FA, FC, ICC/IF, IP)	MAB100R (FA, FC, ICC)
	Mouse	17A2	FAB4841-A, C, F, G, N, P, R, S, T, U, V	MAB4841 (CyTOF-ready, FA, FC, ICC/IF, IHC, IP)	
	Mouse	145-2C11	FAB484-G, N, R, S, T, U, V	MAB484 (CyTOF-ready, Depl., FA, FC, IP, WB)	
CD4	Human	34930	FAB379-G, N, R, S, T, U, V	MAB379 (CyTOF-ready, FC, ICC)	
	Human**	RPA-T4	NBP2-27216	NBP2-25199 (B/N, FC, IHC)	
	Mouse	GK1.5	FAB554-A, C, F, G, N, P, R, S, T, U, V	MAB554 (CyTOF-ready, Depl., FA, FC, IHC, IP)	
CD25/IL-2 Rα	Human	24212	FAB1020-A, G, N, P, R, S, T, U, V	MAB1020 (CyTOF-ready, FC, WB)	
	Mouse	280406	FAB2438-A, G, N, P, R, S, T, U, V	MAB2438 (CyTOF-ready, FC)	
	Mouse**	PC61	NBP2-27425	NBP2-27425 (CyTOF-ready, FC)	
CD39/ENTPD1	Human	498403	FAB4397-A, G, N, R, S, T, U, V	MAB4397 (CyTOF-ready, FC)	
	Mouse	495826	FAB4398-A, G, N, R, S, T, U, V	MAB4398 (CyTOF-ready, FC, IP, WB)	
CD73/5'-Nucleotidase	Human	606112	FAB5795-A, G, N, P, R, S, T, U, V	MAB5795 (CyTOF-ready, FC)	
	Mouse	496406	FAB4488-F, G, N, R, S, T, U, V	MAB4488 (CyTOF-ready, FC)	
GITR	Human	110416	FAB689-A, F, G, N, P, R, S, T, U, V	MAB689 (B/N, CyTOF-ready, Ecap, FC, WB)	
	Mouse	108619	FAB5241-A, G, N, R, S, T, U, V	MAB5241 (CyTOF-ready, FC, WB)	
IFN-γ R1/CD119	Human	92101	FAB673-F, P, FAB6731-G, N, R, S, T, U, V	MAB6731 (B/N, CyTOF-ready, FC, WB)	

Fluorochrome and Application Key on page 12

Cell Surface Markers					
Molecule	**Species**	**Clone**	**Fluorochrome-conjugated Antibodies Catalog # - Fluorochrome**	**Unlabeled Antibodies Catalog # (Applications)**	**Recombinant Monoclonal Antibodies Catalog # (Applications)**
IFN-γ R2	Human	Polyclonal	FAB773-A, F	AF773 (B/N, CyTOF-ready, FC, WB)	
	Mouse	M0B47	FAB773-G, N, R, S, T, U, V	MAB773 (B/N, CyTOF-ready, FC, WB)	
IL-1 RI	Human	732229	FAB2692-G, N, R, S, T, U, V	MAB2692 (CyTOF-ready, FC)	
	Mouse	129304	FAB7712-F, G, N, P, R, S, T, U, V	MAB7712 (CyTOF-ready, FC, WB)	
IL-4 Rα	Human	25463	FAB230-A, G, N, P, R, S, T, U, V	MAB230 (B/N, CyTOF-ready, FC, ICC/IF, IHC, WB)	
	Mouse	Polyclonal	FAB530-P	AF530 (CyTOF-ready, FC, WB)	
IL-6 Rα	Human	17506	FAB227-A, F, G, N, P, R, S, T, U, V	MAB227 (B/N, CyTOF-ready, Ecap, FC, WB)	MAB227R (B/N, CyTOF-ready, Ecap, FC, WB)
	Mouse	255821	FAB1830-G, N, R, S, T, U, V	MAB1830 (CyTOF-ready, FC, ICC/IF)	
IL-12 Rβ1	Human	69310	FAB839-A, F, G, N, P, R, S, T, U, V	MAB839 (CyTOF-ready, FC)	
	Mouse	Polyclonal	FAB1998-P		
IL-12 Rβ2	Human/Mouse	305719	FAB1959-A, C, G, P		
ICOS	Human	669222	FAB6975-A, G, N, P, R, S, T, U, V	MAB6975 (CyTOF-ready, FC)	
	Mouse	1001423	FAB1681-G, N, R, S, T, U, V	MAB1681 (CyTOF-ready, FC)	
IL-17 RB	Human	170220	FAB1207-A, C, G, N, P, R, S, T, U, V	MAB1207 (CyTOF-ready, FC, WB)	
	Mouse	752101	FAB10402-A, G, N, P, R, S, T, U, V	MAB10402 (CyTOF-ready, FC)	
IL-18 Rα/IL-1 R5	Human	70625	FAB840-A, G, N, P, R, S, T, U, V	MAB840 (B/N, CyTOF-ready, FC, ICC, IHC)	
	Mouse	112614	FAB1216-A, F, G, R, S, T, U, V	MAB1216 (CyTOF-ready, FC, WB)	
IL-18 Rβ/IL-1 R7	Human	132029	FAB118-F, G, N, P, R, S, T, U, V	MAB118 (CyTOF-ready, FC, WB)	
IL-21 R	Human	152512*	FAB9911-A, G, N, P, R, S, T, U, V	MAB9911 (CyTOF-ready, FC)	
	Mouse	Polyclonal		AF596 (CyTOF-ready, FC, ICC/IF, WB)	
IL-23 R	Human	218213	FAB14001-A, C, F, N, P	MAB14001 (WB)	
	Mouse	753317	FAB16861-A, C, G, N, P, R, S, T, U, V	MAB16861 (CyTOF-ready, FC)	
IL-27 Rα/WSX-1	Human	191106	FAB14791-A, G, P	MAB1479 (WB)	
	Mouse	263503	FAB21091-F, G, P, R, S, T, U, V	MAB21091 (CyTOF-ready, FC, WB)	
LAG-3	Human	874501	FAB23193-A, G, N, P, R, S, T, U, V	MAB23193 (CyTOF-reported, FC, WB)	
	Mouse	631501	FAB33281-N, P, R, S, T, U, V	MAB33281 (CyTOF-ready, FC)	
LRRC32/GARP	Human	855151	FAB6055-N, R, S, T, U, V	MAB6055 (CyTOF-ready, FC)	
	Mouse	725226	FAB62291-R, S, T, U, V	MAB62291 (CyTOF-ready, FC)	
Neprilysin/CD10	Human	212504	FAB1182-G, N, R, S, T, U, V	MAB1182 (CyTOF-ready, FC, WB)	
	Human/Mouse/ Rat**	SN75-07		NBP2-67717 (FC, ICC/IF, IHC, WB)	
Neuropilin-1/BDCA-4	Human	446921	FAB3870-A, F, G, P, R, S, T, U, V	MAB3870 (CyTOF-ready, FC)	
	Mouse/Rat	761704	FAB59941-G, N, R, S, T, U, V	MAB59941 (B/N, CyTOF-ready, FC)	
OX40	Human	443318	FAB3388-A, F, G, N, P, R, S, T, U, V	MAB3388 (CyTOF-ready, FC, WB)	
	Human	977974	FAB10543-G, N, R, S, T, U, V	MAB10543 (B/N, CyTOF-ready, FC)	
	Human	977960	FAB10542-G, N, R, S, T, U, V	MAB10542 (CyTOF-ready, FC)	
	Mouse	Polyclonal	FAB1256-P		
PD-1	Human	913429	FAB10861-G, N, R, S, T, U, V		MAB10861 (B/N, CyTOF-ready, FC, WB)
	Mouse	766104	FAB7738-G, N, P, R, S, T, U, V	MAB7738 (CyTOF-ready, FC)	
	Mouse	996221	FAB77381-G, N, R, S, T, U, V	MAB77381 (CyTOF-ready, FC, WB)	
ST2/IL-33 R	Human	2154E	FAB10118-F, G, N, R, S, T, U, V		MAB10118 (E, FC)
	Mouse	245707	FAB10041-A, G, N, P, R, S, T, U, V	MAB10041 (B/N, CyTOF-ready, Ecap, FC)	
TGF-β RI	Mouse	141231	FAB5871-A, P	MAB5871 (WB)	

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Cell Surface Markers					
Molecule	**Species**	**Clone**	**Fluorochrome-conjugated Antibodies Catalog # - Fluorochrome**	**Unlabeled Antibodies Catalog # (Applications)**	**Recombinant Monoclonal Antibodies Catalog # (Applications)**
TGF-β RII	Human	25508	FAB241-A, C, F, N, P		
	Human	Polyclonal	FAB2411-A, F, N, P	AF-241-NA (CyTOF-ready, FC, IHC, WB)	
	Mouse	Polyclonal	FAB532-A, C, F, N, P	AF532 (CyTOF-ready, FC, WB)	
TNF RI	Human	16803	FAB225-A, C, G, N, P, R, S, T, U, V	MAB225 (B/N, CyTOF-ready, FC, WB)	
	Mouse	Polyclonal		AF-425-PB (CyTOF-ready, FA, FC, IHC, WB)	
TSLP R	Human	147036		MAB981 (CyTOF-ready, FC, WB)	
	Mouse	Polyclonal	FAB5461-A, F, P	AF546 (B/N, CyTOF-ready, FC, WB)	

Transcription Factors					
Molecule	**Species**	**Clone**	**Fluorochrome-conjugated Antibodies Catalog # - Fluorochrome**	**Unlabeled Antibodies Catalog # (Applications)**	**Recombinant Monoclonal Antibodies Catalog # (Applications)**
AHR	Human	Polyclonal		AF6185 (CyTOF-ready, FC, ICC/IF, SW, WB)	
	Mouse	Polyclonal	IC6697-G		
Batf	Human	687706	IC8054-A, G, N, R, S, T, U, V	MAB8054 (CyTOF-ready, FC, WB)	
FoxP3	Human/Mouse	1054C	IC8214-A, G, N, P, S, T, U, V		MAB8214 (FC, ICC/IF, IHC)
	Human/Mouse/Rat	376209	IC8970-P, R		MAB8970 (CyTOF-ready, FC)
GATA-3	Human	634919	IC63301-A, T	MAB26052 (FC, WB)	
	Human	291106	IC26051-P	MAB26051 (ICC/IF, WB)	
PU.1/Spi-1	Human	732322	IC5870-G, N, R, S, T, U, V	MAB5870 (CyTOF-ready, FC, WB)	
RORγt	Human	1181A	IC9125-A, G, N, R, S, T, U, V		MAB61093 (FC)
	Human/Mouse	600380	IC6006-A, C, P		
STAT1	Human	246523	IC1490-A, G, N, R, S, T, U, V	MAB1490 (CyTOF-ready, FC, ICC/IF)	
STAT3	Human/Mouse/Rat	232209	IC1799-F, G, N, P, R, S, T, U, V	MAB1799 (FC, ICC/IF, IP, KO, WB)	
STAT5a	Human	251610	IC21741-F, G, N, R, S, T, U, V	MAB21741 (CyTOF-ready, FC, ICC/IF)	
STAT5b	Human	389215	IC1584-G, N, R, S, T, U, V	MAB1584 (CyTOF-ready, FC, KO, WB)	
STAT6	Human	253906	IC2167-A, G, N, R, S, T, U, V	MAB2167 (CyTOF-ready, FC, ICC/IF, WB)	
T-bet	Human	525803	IC5385-C, F, G, N, R, S, T, U, V	MAB5385 (FC, ICC/IF, WB)	
	Human	525831	IC53851-G, N, R, S, T, U, V	MAB53851 (CyTOF-ready, FC)	

Secreted Cytokines					
Molecule	**Species**	**Clone**	**Fluorochrome-conjugated Antibodies Catalog # - Fluorochrome**	**Unlabeled Antibodies Catalog # (Applications)**	**Recombinant Monoclonal Antibodies Catalog # (Applications)**
CCL7/MCP-3	Human	36320	IC282-G, N, R, S, T, U, V	MAB282 (B/N, CyTOF-ready, Ecap, FC, WB)	
CCL17	Human	54015	IC3641-G, N, P, R, S, T, U, V	MAB3641 (CyTOF-ready, FC, WB)	
CCL20/MIP-3α	Mouse	114906	IC760-N	MAB760 (B/N, WB)	
CCL22	Human	57203	IC3361-G, N, P, R, S, T, U, V	MAB3361 (CyTOF-ready, FC, WB)	
CXCL13	Human	53610	IC801-A, G, N, P, R, S, T, U, V	MAB801 (B/N, CyTOF-ready, Ecap, FC, WB)	
IFN-γ	Human	25723	IC285-A, C, F, G, N, P, R, S, T, U, V	MAB2851 (B/N, CyTOF-ready, FC, ICC/IF)	MAB285R (B/N, ICC)
	Mouse	37895	IC485-A, F, G, N, P, R, S, T, U, V	MAB485 (B/N, CyTOF-ready, FC, WB)	MAB485R (B/N, FC)
IL-2	Human	5334	IC202-F, G, N, P, R, S, T, U, V	MAB202 (B/N, CyTOF-ready, FC, ICC/IF)	

Fluorochrome and Application Key on page 12

Secreted Cytokines					
Molecule	**Species**	**Clone**	**Fluorochrome-conjugated Antibodies Catalog # - Fluorochrome**	**Unlabeled Antibodies Catalog # (Applications)**	**Recombinant Monoclonal Antibodies Catalog # (Applications)**
IL-4	Human	3007	IC204-F, P		
IL-5	Human	9906	IC605-F, G, N, P, R, S, T, U, V	MAB605 (CyTOF-ready, FC, ICC/IF, WB)	
	Human/Mouse	TRFK5	IC405-G, N, R, S, T, U, V	MAB405 (B/N, CyTOF-reported, Ecap, FC)	
IL-9	Human	623153	IC209-A, G, N, R, S, T, U, V	MAB209 (CyTOF-ready, FC)	
	Mouse	222604	IC409-G, N, R, S, T, U, V	MAB409 (CyTOF-ready, FC, WB)	
IL-10	Human	2050B	IC9210-G		MAB9210 (B/N, FC)
IL-13	Human	32116	FAB213-G, N, R, S, T, U, V	MAB213 (B/N, CyTOF-ready, Ecap, FC)	
	Human	31606	FAB2131-G, N, R, S, T, U, V	MAB2131 (B/N, CyTOF-ready, FC, WB)	
IL-17A	Human	41802	IC3171-A, C, G, N, P, R, S, T, U, V	MAB3171 (CyTOF-ready, FC, IHC, IP, WB)	MAB3171R (FC, WB)
	Human/Primate	41809	IC317-A, G, N, P	MAB317 (B/N, Ecap, IHC, IP)	
	Mouse	881309	IC7211-G, N, P, R, S, T, U, V	MAB7211 (CyTOF-ready, FC)	
	Mouse	Polyclonal	IC421-C, F	AF-421-NA (B/N, CyTOF-ready, FC, WB)	
IL-17E/IL-25	Human	182203	IC1258-A, G, N, P, R, S, T, U, V	MAB1258 (CyTOF-ready, FC, WB)	
	Mouse	207702	IC13991G, N, P, R, S, T, U, V	MAB13991 (CyTOF-ready, Ecap, FC)	
IL-17F	Human	197301	IC13351-G, N, R, S, T, U, V	MAB13351 (CyTOF-ready, FC, ICC/IF)	
	Mouse	316016	IC2057G, N, R, S, T, U, V	MAB2057 (CyTOF-ready, FC, WB)	
	Mouse	1058A	IC20571G, N, P, R, S, T, U, V		MAB20571 (FC)
IL-21	Mouse	149204	IC594-A, N, P, R, S, T, U, V	MAB594 (CyTOF-ready, FC, WB)	
IL-22	Human	142928	IC7821-A, P		
	Mouse	140301	IC582-G, N, P, R, S, T, U, V	MAB582 (CyTOF-ready, FC, WB)	
IL-26/AK155	Human	510414	IC13751-A, G, N, P, R, S, T, U, V	MAB13751 (CyTOF-ready, FC)	
TGF-β1	Human	9016	IC240-A, F, G, N, P, R, S, T, U, V	MAB240 (B/N, CyTOF-ready, Ecap, FC, ICC/IF, IHC, WB)	
TGF-β1, 2, 3	Human	1D11	IC1835-A, N, P	MAB1835 (B/N, Ecap, ICC/IF, IHC, WB)	
TNF-α - Membrane Form	Human	6401	FAB210-F, P	MAB2101 (B/N)	
TNF-α	Human	6402	IC210-F, P		
	Mouse	MP6-XT22	IC410-F, P	MAB4101 (B/N)	

* Clone was used by HLDA to establish CD designation.

Fluorochrome Key:
A Allophycocyanin, **C** PerCP, **F** Fluorescein, **G** Alexa Fluor®488, **N** Alexa Fluor 700, **P** Phycoerythrin, **R** Alexa Fluor 647, **S** Alexa Fluor 750, **T** Alexa Fluor 594, **U** Alexa Fluor 350, **V** Alexa Fluor 405

** Indicates a Novus Biologicals Antibody

Application Key:
B/N Blocking Neutralization **CyTOF-ready/CyTOF-reported** Mass Cytometry **Depl.** Cell Depletion **Ecap** ELISA Capture **FA** Functional Assay **FC** Flow Cytometry **ICC/IF** Immunocytochemistry/Immunofluorescence **IHC** Immunohistochemistry **IP** Immunoprecipitation **KO** Knockout **WB** Western Blot

Multi-Color Flow Cytometry Kits

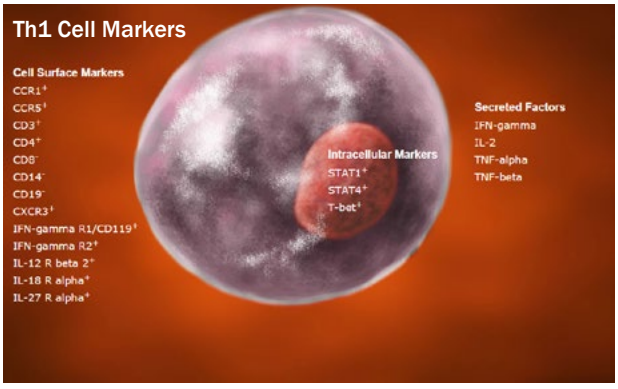
In addition to individually packaged antibodies, Bio-Techne also offers R&D Systems Multi-Color Flow Cytometry Kits to simplify the identification of different T helper cell subsets. These kits include up to four different fluorochrome-conjugated antibodies that can be used together for simultaneous, single-step staining of Th1 cells, Th2 cells, Th17 cells, or regulatory T cells. Additionally, the kits also contain the necessary buffers and detailed protocols.

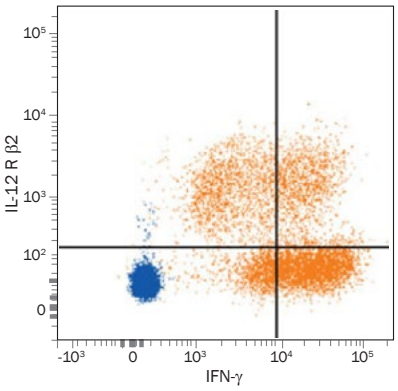
- A cost-effective option for rapid and reliable cell identification
- Optimized to include robust combinations of fluorochrome-conjugated antibodies
- Efficient protocols for the simultaneous detection of multiple cell markers

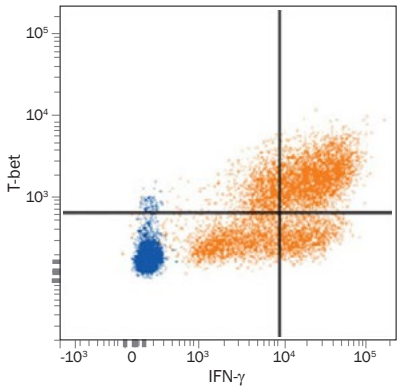
Multi-Color Flow Cytometry Kits for CD4$^+$ T Cell Subsets

Product	Kit Contents	Catalog #
FlowX Human Regulatory T Cell Multi-Color Flow Cytometry Kit	• APC-conjugated CD25 (clone 24212) • Fluorescein-conjugated CD4 (clone 11830) • PE-conjugated FoxP3 (clone 1054C)	FMC021
FlowX Human Th1 Cell Multi-Color Flow Cytometry Kit	• Alexa Fluor® 488-conjugated T-bet (clone 525831) • Alexa Fluor 594-conjugated TIM-3 (clone 344823) • Alexa Fluor 700-conjugated CD4 (clone 11830) • PE-conjugated IL-12 Rβ2 (clone 305719) • PerCP-conjugated IFN-γ (clone 25723)	FMC009B
FlowX Human Th2 Cell Multi-Color Flow Cytometry Kit	• Alexa Fluor 488-conjugated IL-10 (clone 2050B) • Alexa Fluor 594-conjugated GATA-3 (clone 634919) • Alexa Fluor 647-conjugated Phospho-STAT6 (clone 1248D) • Alexa Fluor 700-conjugated CD4 (clone 11830) • PE-conjugated CCR3 (clone 61828)	FMC011B
FlowX Human Th17 Cell Multi-Color Flow Cytometry Kit	• Alexa Fluor® 488-conjugated RORγt (clone 1181A) • Alexa Fluor 594-conjugated CD4 (clone 11830) • Alexa Fluor 700-conjugated IL-17A (clone 41809) • APC-conjugated CXCR6 (clone 56811) • PE-conjugated RORα (clone 784652)	FMC007B
FlowX Mouse Regulatory T Cell Multi-Color Flow Cytometry Kit	• APC-conjugated CD25 (clone 280406) • Fluorescein-conjugated CD4 (clone GK1.5) • PE-conjugated FoxP3 (clone 1054C)	FMC022
Mouse Th1 Cell Multi-Color Flow Cytometry Kit	• APC-conjugated IL-12 Rβ2 (clone 305719) • Fluorescein-conjugated IFN-γ (clone 37895) • PE-conjugated CD4 (clone GK1.5) • PerCP-conjugated T-bet (clone 525803)	FMC010
Mouse Th2 Cell Multi-Color Flow Cytometry Kit	• APC-conjugated STAT6 (clone 253906) • Fluorescein-conjugated IL-4 R (Goat IgG) • PE-conjugated IL-5 (clone TRFK5) • PerCP-conjugated CD4 (clone GK1.5)	FMC012

Explore Our Cell Markers Interactive Resource Tool







To learn more about the markers that are most commonly used in the published literature to identify different CD4$^+$ T cell subsets, explore our Cell Markers Interactive Resource Tool | bio-techne.com/research-areas/cell-biology/cell-markers

Characterization of Cytokine Secretion by Single Analyte ELISA

Different T helper cell subsets can also be characterized by their cytokine secretion profiles. Bio-Techne offers R&D Systems Quantikine® ELISA Kits and the more flexible DuoSet® ELISA Development Systems for single analyte quantification. Quantikine ELISA Kits are complete, fully validated, ready-to-run ELISAs that are designed to measure proteins in complex sample types. These kits undergo rigorous validation testing to ensure that they provide the highest levels of specificity, accuracy, sensitivity, and reproducibility in analyte quantification. DuoSet ELISA Development Systems offer an economical alternative to Quantikine Kits by providing all of the components necessary for a customer to develop their own working assay.

Single Analyte ELISA Kits			
Analyte	**Species**	**Quantikine Kit Catalog #**	**DuoSet ELISA Development System Catalog #**
IFN-γ	Human	DIF50	DY285B
	Mouse	MIF00	DY485
IL-2	Human*	D2050	DY202
	Mouse	M2000	DY402
IL-4	Human*	D4050	DY204
	Mouse	M4000B	DY404
IL-5	Human	D5000B	DY205
	Mouse	M5000	DY405
IL-6	Human*	D6050	DY206
	Mouse	M6000B	DY406
IL-9	Human		DY209
	Mouse		DY409
IL-10	Human*	D1000B	DY217B
	Mouse	M1000B	DY417
IL-13	Human	D1300B	DY213
	Mouse	M1300CB (Culture media)	DY413
IL-17/IL-17A	Human*	D1700	DY317
	Mouse	M1700	DY421
IL-17A/F Heterodimer	Human		DY5194
	Mouse	M17AF0	DY5390
IL-17E/IL-25	Human		DY1258
	Mouse		DY1399
IL-17F	Human		DY1335B
	Mouse	M17F0	DY2057
IL-21	Human		DY8879
	Mouse		DY594
IL-22	Human	D2200	DY782
	Mouse	M2200	DY582
TGF-β1	Human	DB100B	DY240
	Mouse	MB100B	DY1679
TNF-α	Human*	DTA00D	DY210
	Mouse*	MTA00B	DY410

* Quantikine High Sensitivity ELISA Kits are also available for these analytes and species.

Characterization of Cytokine Secretion by Multiplexing

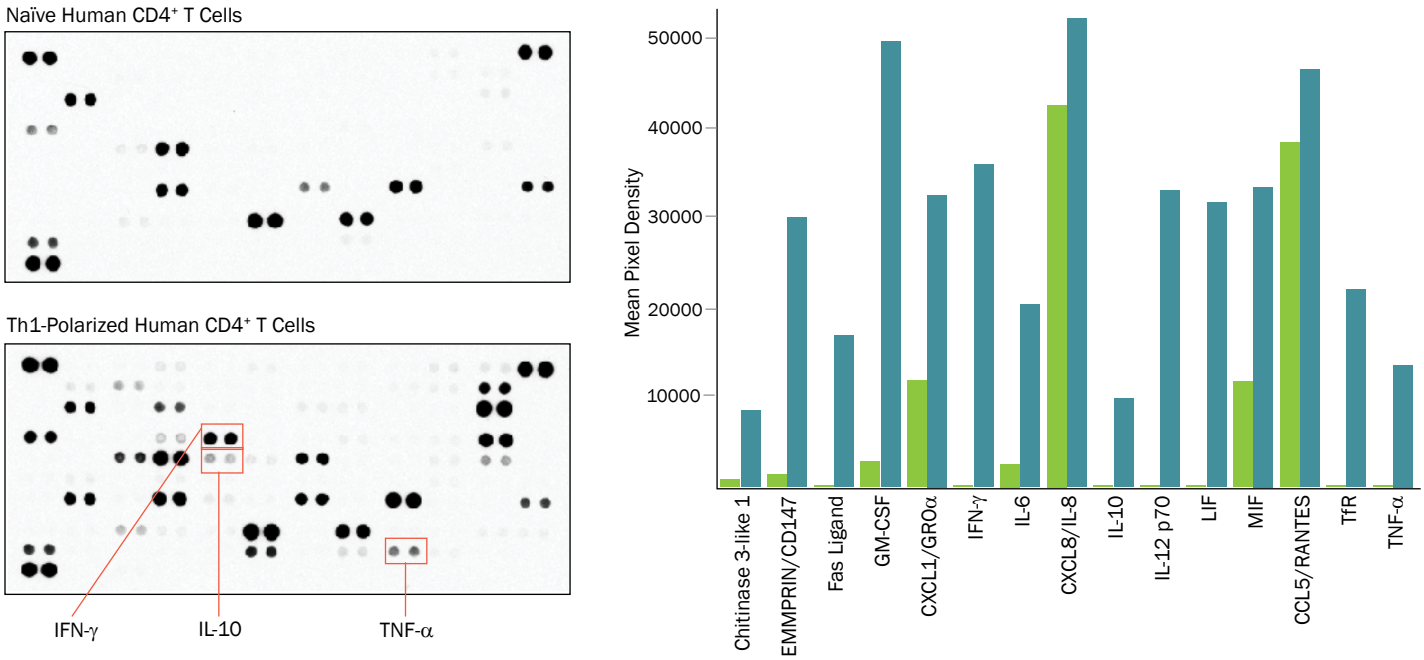
Cytokine Multiplex Assay Kits allow researchers to simultaneously detect the levels of multiple cytokines in a single sample. For multianalyte cytokine detection, Bio-Techne offers both membrane-based Proteome Profiler™ Cytokine Antibody Arrays and bead-based Luminex® Assays from our R&D Systems brand.

R&D Systems® Proteome Profiler™ Antibody Arrays

Proteome Profiler Antibody Arrays allow for the measurement of up to 119 proteins in a single sample. They require no specialized equipment and eliminate the need for multiple Western blot experiments. Antibody array kits contain buffers, detection antibodies, and membranes spotted in duplicate with high-quality capture antibodies. They utilize chemiluminescence for detection, and membranes can be assessed for protein levels in the same manner as traditional Western blots. The analytes included in our Human and Mouse Cytokine and Chemokine Antibody Arrays are shown below.

Proteome Profiler Antibody Arrays	Analytes	Catalog #
Human Cytokine Antibody Array	• C5a • CCL1 • CCL2 • CCL5 • CXCL1 • CXCL10 • CXCL11 • CXCL12 • CD40 Ligand • G-CSF • GM-CSF • ICAM-1 • IFN-γ • IL-1α • IL-1β • IL-1ra • IL-2 • IL-4 • IL-5 • IL-6 • IL-8 • IL-10 • IL-12 p70 • IL-13 • IL-16 • IL-17 • IL-17E • IL-18 • IL-21 • IL-27 • IL-32α • MIF • MIP-1α/MIP-1β • Serpin E1 • TNF-α • TREM-1	ARY005B
Human Chemokine Antibody Array	• CCL1 • CCL2 • CCL3/CCL4 • CCL5 • CCL7 • CCL14 • CCL15 • CCL17 • CCL18 • CCL19 • CCL20 • CCL21 • CCL22 • CCL26 • CCL28 • Chemerin • CX3CL1 • CXCL1 • CXCL4 • CXCL5 • CXCL7 • CXCL8 • CXCL9 • CXCL10 • CXCL11 • CXCL12 • CXCL16 • CXCL17 • IL-16 • Midkine • XCL1	ARY017
Mouse Cytokine Antibody Array	• C5a • CCL1 • CCL2 • CCL3 • CCL4 • CCL5 • CCL11 • CCL12 • CCL17 • CXCL1 • CXCL2 • CXCL9 • CXCL10 • CXCL11 • CXCL12 • CXCL13 • G-CSF • GM-CSF • ICAM-1 • IFN-γ • IL-1α • IL-1β • IL-1ra • IL-2 • IL-3 • IL-4 • IL-5 • IL-6 • IL-7 • IL-10 • IL-12 p70 • IL-13 • IL-16 • IL-17 • IL-23 • IL-27 • M-CSF • TIMP-1 • TNF-α • TREM-1	ARY006
Mouse Chemokine Antibody Array	• CCL2 • CCL3/CCL4 • CCL5 • CCL6 • CCL8 • CCL9/10 • CCL11 • CCL12 • CCL21 • CCL22 • CCL27 • CCL28 • Chemerin • Complement Component C5 • CX3CL1 • CXCL1 • CXCL2 • CXCL9 • CXCL10 • CXCL11 • CXCL12 • CXCL13 • CXCL16 • IL-16 • LIX	ARY020

For a complete listing of Proteome Profiler Antibody Arrays | bio-techne.com/reagents/proteome-profiler-antibody-arrays



Detection of Cytokines Secreted by Th1-Polarized Human CD4+ T Cells Using the Proteome Profiler Human XL Cytokine Array. Naïve T cells were isolated from human peripheral blood mononuclear cells using the MagCellect Human Naïve CD4+ T Cell Isolation Kit (R&D Systems, Catalog # MAGH115). These cells were either left untreated or treated for 5 days using the reagents provided in the CellXVivo Human Th1 Cell Differentiation Kit (R&D Systems, Catalog # CDK001). The relative levels of secreted cytokines detected in the cell culture supernate (spotted in duplicate) before (top membrane) and after (bottom membrane) Th1 polarization were then assessed using the Proteome Profiler Human XL Cytokine Antibody Array (R&D Systems Catalog # ARY022B). The histogram shows the average densitometric profiles of cytokines detected before (green bars) and after (blue bars) Th1 polarization.

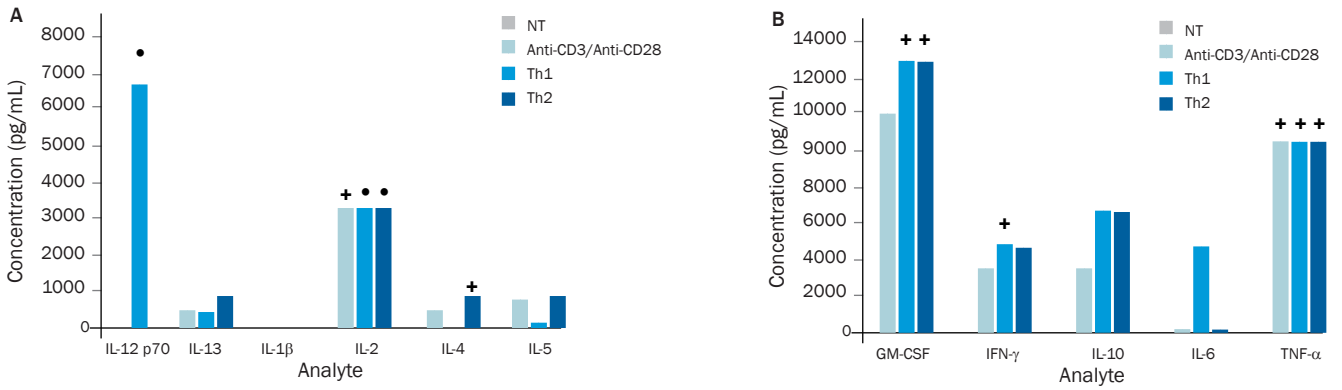
Learn more | bio-techne.com/reagents/proteome-profiler-antibody-arrays

R&D Systems® Luminex® Assays

Luminex Assays and Luminex High Performance Assays are bead-based multiplex assays for simultaneously detecting and quantifying multiple target analytes in qualified complex sample types. With a menu of more than 450 analytes, Luminex Assays are our most flexible bead-based assays, allowing researchers to select up to 50 target analytes to simultaneously profile in cell culture supernates, serum, or plasma samples. Luminex High Performance Assays are our most accurate and precise bead-based multiplex assays for simultaneously measuring up to 45 analytes in qualified complex matrices. The pre-configured analyte panels are optimized and tested together to ensure maximum performance and accuracy for the entire panel. This includes validation testing that is similar to the testing performed on our single analyte Quantikine ELISA Kits. Additionally, we offer High Performance High Sensitivity Luminex Assays for low abundance analytes and a High Performance Discovery Panel, which offers 45 human analytes that can either be customized into a premixed or user-mixed assay or ordered from a selection of 7 fixed panels that includes a Th1/Th2 11-plex assay, a Th9/Th17/Th22 17-plex assay, a cytokine 14-plex assay, a chemokine 8-plex assay, and the cytokine 45 plex-assay. The analytes included in these assays are listed below.

Magnetic Luminex Performance Discovery Panels	Analytes	Catalog #
Human Fixed Chemokine Discovery 8-plex	• CCL2/MCP-1 • CCL3/MIP-1α • CCL4/MIP-1β • CCL5/RANTES • CCL11/Eotaxin • CXCL1/GROα • CXCL10/IP-10 • IL-8/CXCL8	LKTM012
Human Fixed Cytokine Discovery 45-plex	• CCL2/MCP-1 • CCL3/MIP-1α • CCL4/MIP-1β • CCL5/RANTES • CCL11/Eotaxin • CCL19/MIP-3β • CCL20/MIP-3α • CD40 Ligand • Fractalkine/CX3CL1 • CXCL1/GROα • CXCL2/GROβ • CXCL10/IP-10 • EGF • FGF basic • Flt-3 Ligand • G-CSF • GM-CSF • Granzyme B • IFN-α • IFN-β • IFN-γ • IL-1α • IL-1β • IL-1ra • IL-2 • IL-3 • IL-4 • IL-5 • IL-6 • IL-7 • IL-8/CXCL8 • IL-10 • IL-12 p70 • IL-13 • IL-15 • IL-17/IL-17A • IL-17E/IL-25 • IL-33 • PD-L1/B7-H1 • PDGF-AA • PDGF-AB/BB • TGF-α • TNF-α • TRAIL • VEGF	LKTM014
Human Fixed Cytokine Discovery 14-plex	• IFN-α • IFN-γ • IL-1α • IL-1β • IL-1ra • IL-2 • IL-3 • IL-4 • IL-6 • IL-7 • IL-10 • IL-15 • IL-33 • VEGF	LKTM011
Human Fixed Th1/Th2 Discovery 11-plex	• GM-CSF • IFN-γ • IL-1β • IL-2 • IL-4 • IL-5 • IL-6 • IL-10 • IL-12 p70 • IL-13 • TNF-α	LKTM008
Human Fixed Th9/Th17/Th22 Discovery 17-plex	• CCL20 • CD40 Ligand • GM-CSF • IFN-γ • IL-1β • IL-2 • IL-4 • IL-5 • IL-6 • IL-10 • IL-12 p70 • IL-13 • IL-15 • IL-17/IL-17A • IL-17E/IL-25 • IL-33 • TNF-α	LKTM009

To see a complete listing of analytes available for Luminex Assays and the other Luminex High Performance Assays | bio-techne.com/reagents/luminex-assays



Quantitation of Cytokines in Cell Culture Supernates from Activated Th1 and Th2 Cells. $CD4^+$ T cells were isolated from peripheral blood mononuclear cells (PBMCs) using the MagCellect Human $CD4^+$ T Cell Isolation Kit (R&D Systems, Catalog # MAGH102). Cells were either untreated (NT), treated with Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL), Recombinant Human IL-12 (R&D Systems, Catalog # 219-IL), and Mouse Anti-Human IL-4 Monoclonal Antibody (R&D Systems, Catalog # MAB304) and activated with immobilized Mouse Anti-Human CD3ε Monoclonal Antibody (R&D Systems, Catalog # MAB100) and soluble Mouse Anti-Human CD28 Monoclonal Antibody (R&D Systems, Catalog # MAB342) to induce Th1 differentiation, or treated with Recombinant Human IL-2 (R&D Systems, Catalog # 202-IL) and activated with Phytohemagglutinin-L (PHA) to induce Th2 differentiation. All stimulated cells were then treated with PMA (Tocris, Catalog # 1201; 10 ng/mL) and Ionomycin calcium salt (Tocris, Catalog # 1704; 500 ng/mL) for 24 hours after activation. Cell culture supernates were analyzed using the Magnetic Luminex Performance Human Fixed Th1/Th2 Discovery 11-plex Panel (R&D Systems, Catalog # LKTM008).
+ = Values above the limits of the standard curve
• = Stimulating cytokine

Learn more | bio-techne.com/reagents/luminex-assays

Simple Plex™ Assays

Simple Plex assays are fully automated, single or multianalyte assays run on Ella. Ella has three Simple Plex cartridges, either a 32x4 or a 16x4 cartridge for multiplexing, or a 72x1 cartridge for single analyte assays. The table below shows the number of analytes and samples that can be assessed on each cartridge. Simple Plex assays are powered by R&D Systems reagents and are correlated against Quantikine assays. The standard Simple Plex menu offers a large selection of human, mouse, and rat analytes for researchers to choose from. Additionally, we now offer a 48-Digoxigenin (48-Dig) Simple Plex cartridge that gives researchers the freedom to choose the analytes that they want to detect in a Simple Plex assay. This new 48-Dig cartridge expands your assay options beyond the preset menu, offering researchers the opportunity to build their own sandwich assays on Ella.

- Single and multianalyte assay formats are available
- Unprecedented reproducibility
- Wide dynamic range and sensitivity
- Hands-free, fast time to results

Cartridge	Number of Analytes	Number of Samples	Number of Answers*
72x1	1	72	72
16x4	4	16	64
32x4	4	32	128

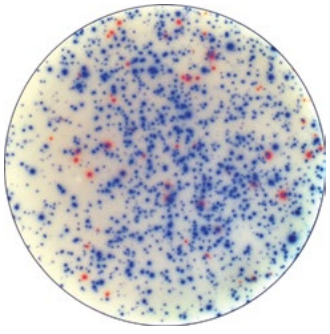
*Less any controls

Learn more | bio-techne.com/instruments/simple-plex

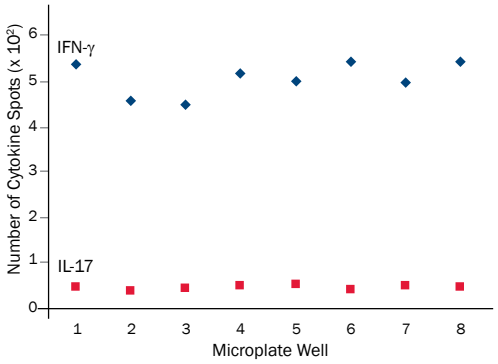
Characterization of Cytokine Secretion Using ELISpot and FluoroSpot Kits

Bio-Techne also offers microplate-based ELISpot Kits, FluoroSpot Kits, and ELISpot Development Modules for detecting cells secreting specific cytokines. Complete ELISpot Kits are ready-to-run and require no further development or refinement. These assays are highly sensitive and can quantitate actively secreting cells even when cell frequencies fall below 1 in 100,000. As an alternative to our complete kits, we also offer Development Modules, which provide a flexible, do-it-yourself format for ELISpot development. Features of the kits include:

- Offer up to 20% greater sensitivity than the competition – measure responses with frequencies below 1 in 100,000 cells
- Brighter, crisper spots with less background noise
- Wide dynamic range of quantifiable spots: up to 1000 spots per well
- Large kit selection including single analyte and dual-color ELISpot Kits



Detection of IFN-γ and IL-17 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 (R&D Systems, 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 (R&D Systems, 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.

For a complete listing of our FluoroSpot and ELISpot Kits and Development Modules
bio-techne.com/reagents/elispot-kits

GMP-grade Recombinant Proteins

R&D Systems GMP-grade recombinant proteins are manufactured under guidelines that allow for their use as ancillary materials in cell therapy or for further manufacturing processes. They come with extensive documentation and traceability, as well as additional quality control testing. Each GMP-grade protein is tested for performance using cell-based bioassays including proliferation, induced cytokine secretion, cytotoxicity, and others. Whenever possible, our GMP proteins are made in an entirely animal-free process using *E. coli* as the source, although there are some instances when a protein requires production in a eukaryotic system to maintain activity. If this is the case, it is always clearly indicated on the R&D Systems website if a specific GMP-grade protein was not produced in an animal-free process.

Protein	GMP	GMP Source
IFN-gamma	285-GMP (DMF on file)	*E.coli*
IL-1 beta	201-GMP	*E.coli*
IL-2	BT-002-GMP (DMF on file)	*E.coli*
IL-3	203-GMP	*E.coli*
IL-4	BT-004-GMP (DMF on file)	*E.coli*
IL-6	206-GMP (DMF on file)	*E.coli*

Protein	GMP	GMP Source
IL-7	BT-007-GMP (DMF on file)	*E.coli*
IL-10	1064-GMP (DMF on file)	*E.coli*
IL-15	BT-015-GMP (DMF on file)	*E.coli*
IL-21	BT-021-GMP	*E.coli*
TNF-alpha	210-GMP	*E.coli*

Learn more | bio-techne.com/gmp-products/gmp-proteins

Custom Services

If you still don't see what you need, let us know. Bio-Techne offers expert custom cell culture, protein, antibody, ELISA, and Luminex services to ensure that we can meet your research needs.

Learn more | bio-techne.com/services/custom-services

Notes

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