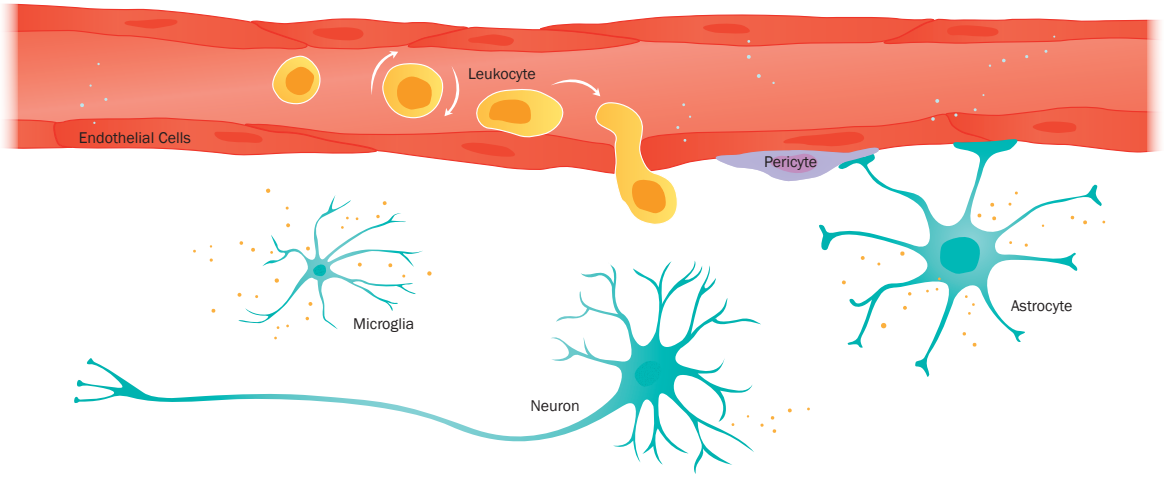
Neuroscience
Immunology

Blood-Brain Barrier
and Immune Cell Transmigration



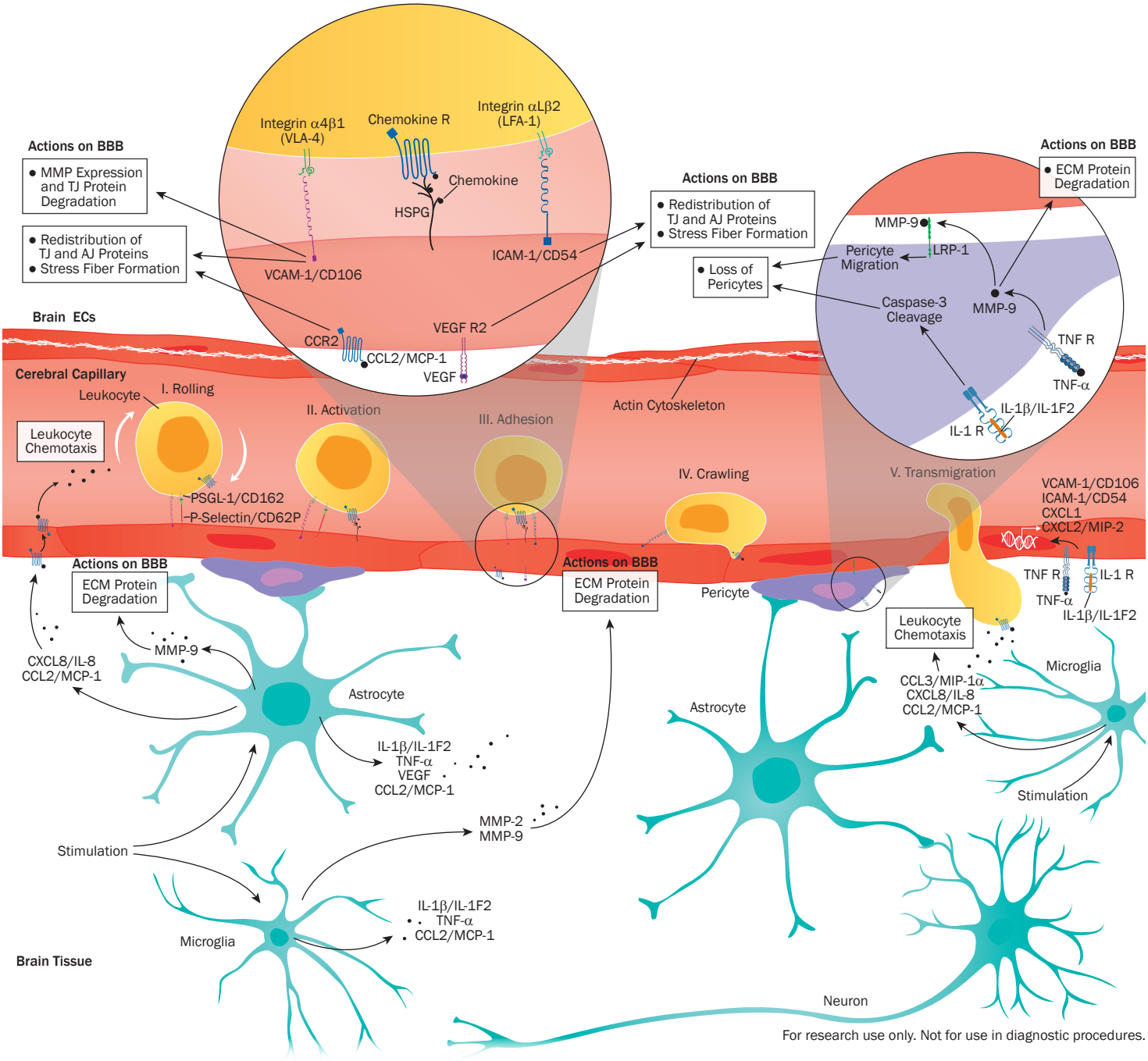
R&D systems™
a bio-techne brand

Immune Cell Transmigration Across the Blood-Brain Barrier

The blood-brain barrier (BBB) is a highly specialized, multi-cellular structure that functions as a selective diffusion barrier between the peripheral circulation and the central nervous system (CNS). It is composed of specialized endothelial cells (ECs) that are linked by complex tight junctions (TJs) and adherens junctions (AJs) and is surrounded by astrocytes and pericytes. Under normal conditions, the specialized structure of the BBB hinders paracellular transport of most hydrophilic compounds across the cerebral endothelium and restricts migration of blood-borne cells into the CNS. As a result, resident immune cells, such as microglia, are the initial responders to pathogens or tissue damage. However, prolonged tissue insult triggers inflammatory conditions that cause the BBB to lose its restrictive features, resulting in the subsequent infiltration of peripheral immune cells.

Reactive microglia, astrocytes, and pericytes, as well as ECs, release numerous molecules that promote invasion of peripheral immune cells into CNS. Secreted inflammatory mediators, including CXCL8, CCL2/MCP-1, TNF-α, IL-1β/IL-1F2, recruit immune cells and stimulate the expression of adhesion molecules on ECs that participate in integrin-mediated leukocyte tethering, rolling, and activation. These molecules also trigger the dynamic reorganization of junctional complexes between ECs and EC retraction, thereby promoting the formation of paracellular gaps. Matrix metalloproteases (MMPs) are also released and degrade proteins present in the extracellular matrix and may contribute to the loss of pericytes.

Bio-Techne offers an extensive collection of R&D Systems products for researching neuroinflammation including antibodies for the detection of specific cell types and immunoassays for the measurement of cytokine levels.



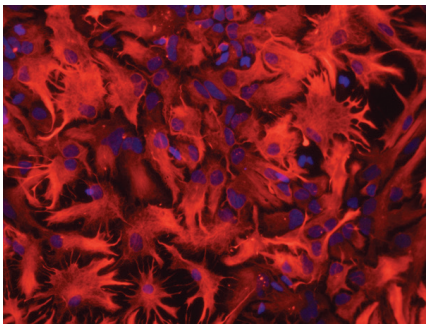
Detect and Identify Cells

A variety of cell types are involved in mediating inflammatory responses in neural tissue. Detection and identification of these different cells is essential when investigating neuroinflammation. Bio-Techne offers a wide range of high-performance R&D Systems antibodies that can facilitate your research.

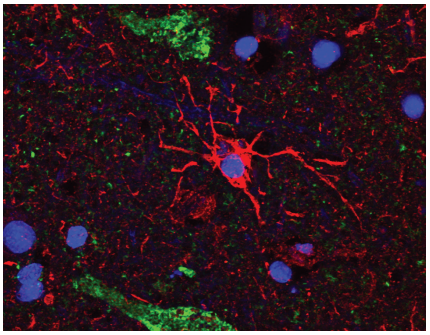
High-Performance Antibodies

Our extensive antibody portfolio is comprised of over 13,000 high-performance antibodies. Greater than 95% of these antibodies are developed and validated onsite allowing us to ensure that R&D Systems brand antibodies are of the highest quality.

- **Guaranteed Performance** Rigorous lot specific QC testing supports all applications listed on our datasheets to ensure superior performance.
- **Exclusive Clones** Hundreds of world-renowned, unique clones, many of which have been used by HLDA to establish CD nomenclature.
- **Complete Transparency** Interpretable antibody reactivity starts with fully characterized antigens. Unlike our competitors, we provide detailed antigen descriptions on our datasheets.



GFAP in Rat Astrocytes. Glial Fibrillary Acidic Protein (GFAP) was detected in immersion-fixed rat astrocytes using a Sheep Anti-Human GFAP Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF2594). The cells were stained using the NorthernLights™ (NL) 557-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # NL010; red) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm.



Integrin αM/CD11b in Human Brain. Integrin αM/CD11b was detected in immersion-fixed paraffin-embedded tissue sections of human brain (cerebral cortex) using a Mouse Anti-Human/Equine Integrin αM/CD11b Monoclonal Antibody (Clone 238446; Catalog # MAB16991). The tissue was subjected to antigen retrieval using the Antigen Retrieval Reagent-Basic (Catalog # CTS013) and stained using the NL557-Conjugated Donkey Anti-Mouse IgG Secondary Antibody (Catalog # NL007; red). Nuclei were counterstained with DAPI (blue). Specific staining was localized to the cytoplasm of microglia (red color). The tissue was double-stained with a Sheep Anti-Human/Mouse/Rat Neurogranin Antigen Affinity-Purified Polyclonal Antibody (Catalog # AF7947) and an Alexa Fluor® 488-conjugated donkey anti-sheep IgG secondary antibody (green).

Neurons	Antibodies (Applications)
β-III Tubulin	**MS** (FC, ICC, WB)
Enolase 2/Neuron-Specific Enolase	**H** (IHC, IP, WB) **M** (IHC, WB)
Synaptophysin	**H** (ICC, IHC, WB) **R** (ICC, IHC, WB)
Tyrosine Hydroxylase	**H** (ICC, IHC, WB) **M** (ICC, IF, IHC, WB) **R** (ICC, IF, IHC, WB) **Pr** (IF, IHC, WB)
Astrocytes	
GFAP	**H** (ICC, WB) **R** (ICC, WB)
S100B	**H** (IHC, WB)
Microglia	
AIF-1/Iba1	**H** (IHC)
CD68/SR-D1	**H** (FC, ICC, WB)
Integrin αM/CD11b	**H** (FC, ICC, IHC, WB) **M** (CD, FC, ICC, IHC, IP) **Ca** (FC, WB) **E** (FC, ICC)
Pericytes	
PDGF Rβ	**H** (B/N, FC, IHC, IP, WB) **M** (IHC, WB)
α-Smooth Muscle Actin	**H** (FC, ICC, IHC)
B Cells	
CD19	**H** (FC) **M** (FC) **R** (FC, IHC, WB)
MS4A1/CD20	**H** (FC)
Dendritic Cells	
CD11c	**H** (FC, IP, WB) **M** (FC, WB)
CD83	**H** (FC, ICC, WB) **M** (B/N, FC, ICC, WB)
DC-SIGN/CD209	**H** (B/N, FC, IHC, ICC, WB) **M** (FC, WB)
Monocytes/Macrophages	
CD14	**H** (B/N, E, FC, IHC, WB) **M** (FC, WB) **E** (FC, WB) **P** (FC, WB)
CD45	**H** (FC, ICC) **M** (FA, FC, ICC, IHC, IP, WB)
F4/80/EMR1	**M** (FC, ICC)
Integrin αM/CD11b	**H** (FC, ICC, IHC, WB) **M** (CD, FC, ICC, IHC, IP) **Ca** (FC, WB) **E** (FC, ICC)
Natural Killer Cells	
B3GAT1	**R** (ICC)
NCAM-1/CD56	**H** (E, FC, ICC, IHC, WB) **M** (FC, WB) **R** (WB)
NKp46/NCR1	**H** (FA, FC, ICC, WB) **M** (FA, FC, WB)
Neutrophils	
Gr-1/Ly-6G	**M** (CD, ICC, IHC, IP, FC)
Integrin αM/CD11b	**H** (FC, ICC, IHC, WB) **M** (CD, FC, ICC, IHC, IP) **Ca** (FC, WB) **E** (FC, ICC)
Myeloperoxidase	**H** (ICC, IHC, WB) **M** (ICC, IHC, WB)
T Cells	
CD3 (pan T cell marker)	**H** (FA, FC, ICC, IP) **M** (CD, FA, FC, ICC, IHC, IP)
CD4 (helper T cell marker)	**H** (B/N, FC, ICC, IHC, WB) **M** (CD, FA, FC, ICC, IHC, IP, WB) **R** (FC, WB) **Ca** (FC, ICC, WB) **CR** (FC) **F** (FC, ICC, WB)
CD8α (cytotoxic T cell marker)	**H** (FC, ICC) **M** (CD, FA, FC, ICC, IP) **Ca** (ICC) **CR** (FC) **F** (ICC, WB)
CD25/IL-2 Rα (regulatory T cell marker)	**H** (B/N, E, FC, ICC, IHC, WB) **M** (B/N, E, FC, IHC, WB) **R** (FC, ICC, WB) **Ca** (FC)
FoxP3 (regulatory T cell marker)	**H** (ChIP, FC, ICC, IHC, WB) **M** (FC) **R** (FC)

Species Key: **H** Human **M** Mouse **R** Rat **Ca** Canine **CR** Cotton Rat **E** Equine **F** Feline **MS** Multi-species **P** Porcine **Pr** Primate

Application Key: **B/N** Blocking/Neutralization **CD** Cell Depletion **ChIP** Chromatin Immunoprecipitation **E** ELISA **FA** Functional Assay **FC** Flow Cytometry **ICC** Immunocytochemistry **IF** Immunofluorescence **IHC** Immunohistochemistry **IP** Immunoprecipitation **WB** Western blot

Survey Cytokine Secretion

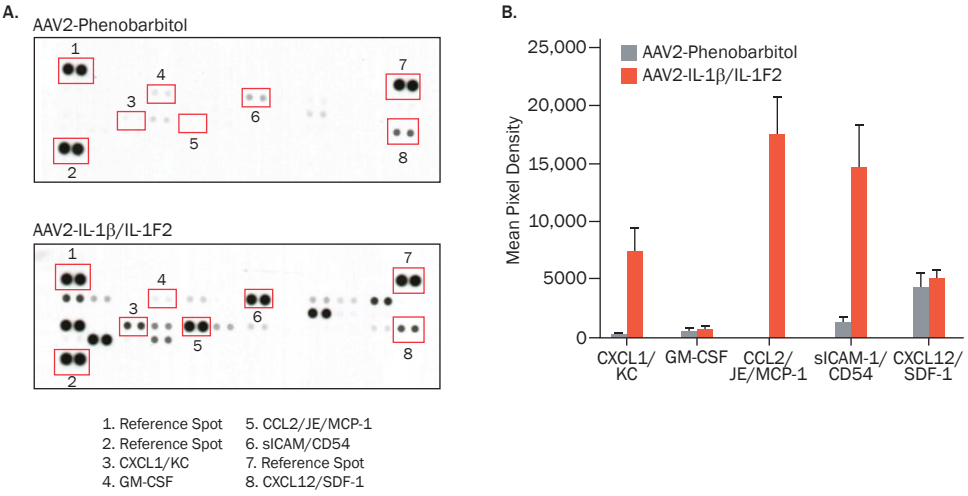
Unexpected results can be missed when only a subset of proteins is analyzed. Uncover a more complete view of a process by investigating the effects of your experimental conditions on the behavior of multiple cytokines at once. Bio-Techne offers several highly efficient, qualitative tools for simultaneously measuring the levels of multiple proteins in a single sample.

Proteome Profiler™ Antibody Arrays

Analyze the expression levels of up to 102 cytokines and growth factors in a single sample with R&D Systems Proteome Profiler membrane-based antibody arrays. Broad in scope, the data generated from the arrays can uncover unexpected cellular responses. These assays are ideal for surveying which cytokines and growth factors are present in cell culture supernatants and tissue homogenates.

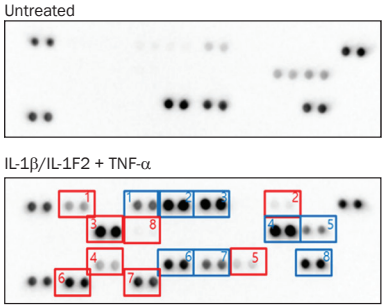
- **Cost-Effective Screening Method** Each array offers a quick and inexpensive analysis of many analytes simultaneously, in less time than it takes to perform a Western blot.
- **No Specialized Equipment Needed** They are designed to utilize the same data collection equipment used for Western blots.
- **Wide Selection** Choose from over 25 arrays for the detection of both intracellular and extracellular factors from a wide variety of sample types.
- **User-Friendly Design** Each array is stamped with an identification number for easy record keeping and contains reference spots in three corners for orientation purposes.
- **Multiple Detection Methods Available** We offer arrays that can utilize either chemiluminescence or LI-COR® infrared fluorescence detection*.

Selected Kits/Description
Human Cytokine Antibody Array (Catalog # ARY005) Detects 36 different cytokines, chemokines, and acute phase proteins.
Human XL Cytokine Antibody Array (Catalog # ARY022) Detects 102 different cytokines, chemokines, and acute phase proteins.
Human Chemokine Antibody Array (Catalog # ARY017) Detects 31 different chemokines.
Mouse Cytokine Antibody Array (Catalog # ARY006) Detects 40 different cytokines, chemokines, and acute phase proteins.
Mouse Chemokine Antibody Array (Catalog # ARY020) Detects 25 different chemokines.
Rat Cytokine Antibody Array (Catalog # ARY008) Detects 29 different cytokines and chemokines.



1. Reference Spot
2. Reference Spot
3. CXCL1/KC
4. GM-CSF
5. CCL2/JE/MCP-1
6. sICAM/CD54
7. Reference Spot
8. CXCL12/SDF-1

Cytokine Expression Induced by IL-1β/IL-1F2 in Mouse Brain. C57BL/6 mice received a bilateral intrahippocampal injection of adeno-associated virus (AAV2) vector expressing either a single chain antibody to phenobarbital (for a control) or IL-1β/IL-1F2. After 4 weeks, brains of the mice were collected, and cytokine expression in brain homogenates was analyzed using the Proteome Profiler Mouse Cytokine Antibody Array (Catalog # ARY006). Representative arrays (**A**) and histogram profiles (**B**) for select analytes from control (gray bars) and IL-1β/IL-1F2 treated (orange bars) mice. Data were generated by analysis of the mean pixel density of individual antibody spots using image software analysis. *Data courtesy of Dr. Jonathan Cherry, University of Rochester Medical Center, Rochester, NY.*



Cytokines that were Newly Expressed

1. C5/C5a (Complement Component 5/5a)
2. sICAM-1 (CD54)
3. IL-1β (IL-1F2)
4. IP-10 (CXCL10)
5. MIP-1α (CCL3)
6. RANTES (CCL5)
7. TNF-α (TNFSF1A)
8. IL-1ra (IL-1F3)

Cytokines with Changes in Expression Levels

1. G-CSF (CSFβ, CSF-3)
2. GM-CSF (CSFα, CSF-2)
3. GROα (CXCL1)
4. IL-6
5. IL-8 (CXCL8)
6. MCP-1 (CCL2)
7. MIF (GIF, DER6)
8. Serpin E1 (PAI-1)

Cytokine Profile of Cultured Human Astrocytes. Human primary astrocyte cell cultures were treated with recombinant human IL-1β/IL-1F2 and TNF-α for 24 hours or remained untreated. Conditioned media from the cultures were harvested and assayed for the presence of 36 cytokines using the Proteome Profiler Human Cytokine Antibody Array (Catalog # ARY005). Cytokines that were newly expressed (red boxes) or whose expression changed (blue boxes) following treatment are highlighted. *Image from Choi, S.S. et al. (2014) PLoS One* ***9****:e92325.*

* Select arrays only.

Luminex® Bead-Based Assays

We offer two bead-based multiplex immunoassay formats for detecting protein analytes in biological fluids. These R&D Systems assays utilize Luminex xMAP® microparticle technology allowing users to better tailor assay selection to their individual research needs. Additionally, our Luminex assays are specifically designed to optimize the benefits and overcome the challenges of multiplexing.

R&D Systems Luminex Screening Assays

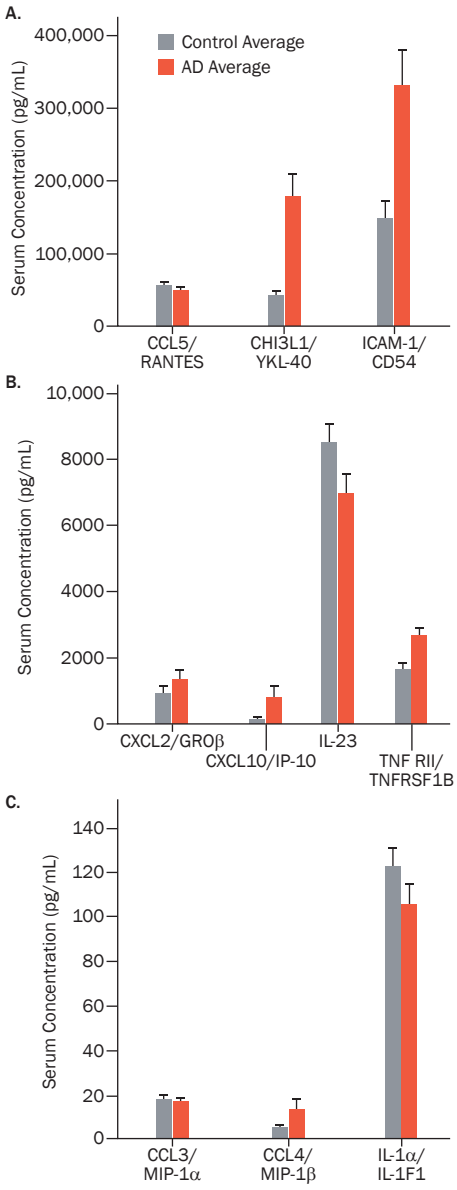
These assays are designed to maximize multiplexing capacity and flexibility while maintaining assay specificity.

- **Most Flexible** Choose from over 214 analytes. All analytes are available in either the polystyrene or magnetic microparticle format.
- **Largest Luminex Multiplex Selection Available** These screening assays allow you to maximize the use of your samples by simultaneously analyzing up to 100 analytes.
- **Unique Analytes Offered** Nearly 30% of our analytes are unique to us.

R&D Systems Luminex Performance Assays

These assays are designed to maximize assay accuracy and precision while preserving the benefits of multiplexing.

- **Most Accurate** Panel development and validation testing for these assays are similar to our gold-standard Quantikine® ELISA Assays.
- **Flexible** Select panels are available in either the polystyrene or magnetic microparticle format.
- **Customizable to Fit Your Needs** Users can choose their analytes of interest from established panels and select “premixed” or “end-user mixed” options.



Detection of Neuroinflammation and Alzheimer's Disease Biomarkers in Human Serum. The Human Luminex Screening Assay (Catalog # LXSAH) was used to measure 32 markers of neuroinflammation and Alzheimer's disease (AD) pathology in human serum samples. Samples were collected from individuals with AD (gray bars; N=20) and from apparently healthy individuals (orange bars; N=20); no medical histories were available. Histogram profiles for select analytes measured at high (**A**), moderate (**B**), and low (**C**) expression levels.

Luminex Screening Assays
Human Luminex Screening Assay Choose from 214 analytes, including 68 different cytokines and chemokines
Mouse Luminex Screening Assay Choose from 61 analytes, including 40 different cytokines and chemokines
Rat Luminex Screening Assay Choose from 17 analytes, including 14 different cytokines and chemokines
Selected Luminex Performance Assays
Human Adhesion Molecule 4-Plex*, *** Detects ICAM-1/CD54, E-Selectin/CD62E, P-Selectin/CD62P, VCAM-1/CD106
Human Cytokine Panel A Detects 22 different cytokines and chemokines
Human Cytokine Panel B* Detects 9 different cytokines and chemokines
Human High Sensitivity Cytokine Panel A Detects 12 different cytokines and chemokines
Human High Sensitivity Cytokine Panel B** Detects 18 different cytokines
Human MMP Panel Detects EMMPRIN/CD147 and 9 different MMP proteins
Multi-species TGF-β Panel*** Detects TGF-β1, TGF-β2, TGF-β3
Human TIMP 4-plex*** Detects TIMP-1, TIMP-2, TIMP-3, TIMP-4

*Analytes in this panel are only available in the polystyrene bead format.

**Analytes in this panel are only available in the magnetic bead format.

***Kit is only available in the premixed format.

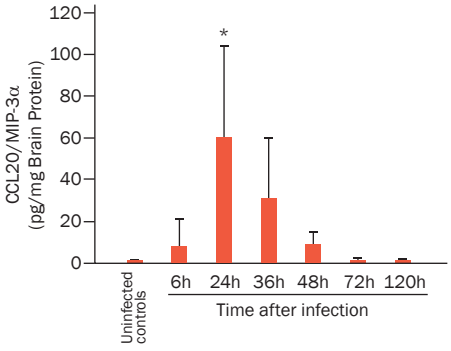
Measure Cytokine Levels

Measuring cytokine production during inflammatory responses can be achieved using R&D Systems Quantikine ELISA kits, the gold standard for measuring cytokine concentrations, and R&D Systems ELISpot Kits, which are used to detect a single cytokine secreting cell. Combine both techniques in one experiment to determine the mean production of your cytokine of interest by a single stimulated cell.

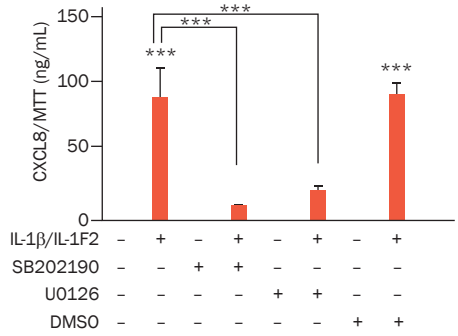
Quantikine ELISA Kits

Measure changes in cytokine concentrations using R&D Systems Quantikine ELISA Kits. These kits are complete, fully validated, ready-to-run sandwich ELISAs that are designed to measure the concentrations of natural or recombinant analytes. These kits can be used to measure proteins in cerebral spinal fluid, as well as other neural tissue including brain homogenates. In-house manufacturing and extensive validation testing ensure these kits provide the highest levels of specificity, accuracy, precision, and sensitivity, making them the industry gold standard.

- **Legendary Reproducibility** Our master calibrated assays ensure that our kits generate accurate data consistently over time. Results today can be compared to last month or even last year, and will be comparable to future results generated.
- **Confidence with Controls** Our assays have controls with assigned ranges which guarantee performance.
- **Unrivaled Reputation** Our ELISAs are the most referenced.
- **High Specificity** Every complete ELISA kit is extensively tested against related molecules and common interfering substances to ensure no cross-reactivity or interference.
- **Accurate Detection of Natural Proteins** Our antibody pairs recognize the supplied recombinant standard and natural proteins in biological samples in a parallel manner.



CCL20/MIP-3α Expression during Acute Pneumococcal Meningitis. CCL20/MIP-3α levels were measured in brain homogenates of C57BL6 mice infected with *Streptococcus pneumonia* type D39 at various time points after infection using the Mouse CCL20/MIP-3α Quantikine ELISA Kit (Catalog # MCC200). Brain CCL20/MIP-3α levels were also analyzed in uninfected controls. **P<0.01* compared with uninfected controls. *Graph from Klein, M. et al. (2014) PLoS One* **9***:e93057.*



IL-1β/IL-1F2-Induced CXCL8/IL-8 Expression in Astrocytes is Mediated by MAP Kinases. Human astrocyte cell cultures were treated with either an inhibitor specific for p38 (SB202190) or ERK (U0126), or DMSO (control) for 2 hours, followed by treatment with Recombinant Human IL-1β/IL-1F2 (Catalog # 201-LB). The levels of CXCL8/IL-8 in cell culture supernatants were measured using the Human CXCL8/IL-8 Quantikine ELISA Kit (Catalog # D8000C). CXCL8/IL-8 protein levels were normalized to the number of viable cells as measured using a MTT assay. ****P<0.001. Graph from Mamik, M.K. and A. Ghorpade (2012) PLoS One.* **7***:e45596.*

Selected ELISA Kits		
Analyte	**Species**	**Catalog #**
CCL2/MCP-1	Human	DCP00
	Mouse, Rat	MJE00
	Canine	CACP00
CCL3/MIP-1α	Human	DMA00
	Mouse	MMA00
CCL20/MIP-3α	Human	DM3A00
	Mouse	MCC200
CXCL1	Human	DGR00
	Mouse	MKC00B
	Rat	RCN100
CXCL8/IL-8	Human*	D8000C
	Canine	CA8000
	Porcine	P8000
IFN-γ	Human	DIF50
	Mouse	MIF00
	Rat	RIF00
	Canine	CAIF00
	Porcine	PIF00
IL-1β/IL-1F2	Human*	DLB50
	Mouse	MLB00C
	Rat	RLB00
	Porcine	PLB00B
IL-6	Human*	D6050
	Mouse	M6000B
	Rat	R6000B
	Canine	CA6000
	Porcine	P6000B
TGF-β1	Human	DB100B
	Mouse, Rat, Canine, Porcine	MB100B
TNF-α	Human*	DTA00C
	Mouse	MTA00B
	Rat	RTA00
	Canine	CATA00
	Porcine	PTA00
	Rhesus Macaque	RHMTA0
VEGF	Human	DVE00
	Mouse	MMV00
	Rat	RRV00
	Canine	CAVE00

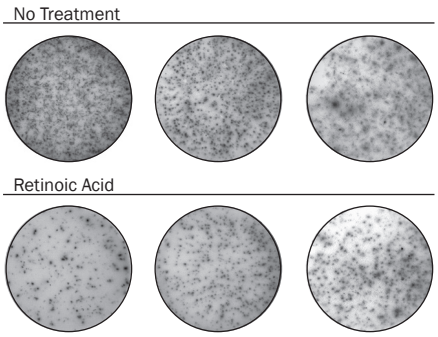
*Indicates a Quantikine High Sensitivity kit is available for this analyte and species.

ELISpot Kits

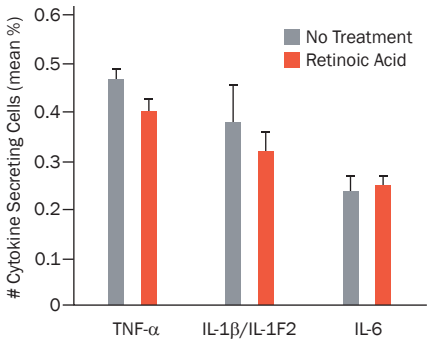
Determine the number of cells that secrete your cytokine of interest with R&D Systems ELISpot Kits. These microplate-based immunoassays are highly sensitive, allowing for the detection of a single cytokine secreting cell among 100,000 cells. Furthermore, use our dual-color ELISpot kits to detect parallel secretion of two cytokines by the same cell. These kits do not require any additional cell expansion or assay refinement.

- **Be Confident in the Data** Small, crisp spots allow for easier, more accurate quantitation and the largest dynamic range (number of detectable spots/well) on the market.
- **Detect Low Frequency Responses** Our kits can measure responses with frequencies well below 1 in 100,000 cells – up to 20% more sensitive than the competition.
- **Directly Compare Results – Even Years Later** We ensure lot-to-lot consistency by manufacturing multiple equivalent lots.
- **Choose from Multiple Formats** We offer ready-to-use kits for chromogenic and fluorescent detection formats. Do-it-yourself development modules for single analytes are also available.

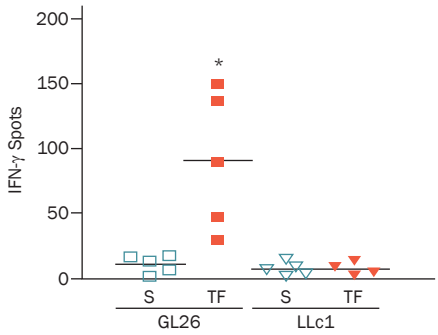
A.



B.



Cytokine Secretion by Microglia. Microglia were cultured from D1 Sprague Dawley rat pups. After 2 weeks in culture, the primary microglia cell cultures were incubated with Retinoic Acid for 17 hours (orange bars) or remained untreated (gray bars). **A.** The number of cells secreting TNF-α, IL-1β/IL-1F2, or IL-6 were analyzed using the Rat TNF-α (Catalog # SEL510), Rat IL-1β/IL-1F2 (Catalog # SEL501), or Mouse/Rat IL-6 (Catalog # SEL406) ELISpot Development Modules. **B.** Histogram profiles of the frequency (expressed as percent) of cytokine secreting cells, which was determined as the number of spots divided by the number of plated cells.



T Cells from Mice Bearing Intracranial Neoplasms Secrete IFN-γ. T lymphocytes were isolated from C57BL/6 mice bearing an intracranial neoplasm, which was generated by implanting GL26 mouse glioblastoma cells unilaterally into the right striatum. These intracranial brain tumors were treated with either an intratumoral injection of adenoviral vectors expressing Fms-like Tyrosine Kinase 3 Ligand and Thymidine Kinase (TF; closed symbols) or saline (S; open symbols) 17 days after tumor implantation. The isolated T lymphocytes were incubated with myeloid dendritic cells loaded with either the GL26 or LLc1 tumor antigen, and the number of T lymphocytes secreting IFN-γ in response to the tumor antigens was analyzed using the Mouse IFN-γ ELISpot Development Module (Catalog # SEL485). **P<0.05* TF versus S. *Graph from Curtin, J.F. et al. (2009) PLoS Medicine 6:e1000010.*

Selected ELISpot Kits

Analyte	Species	Single-Color ELISpot Kits / Dual-Color ELISpot Kits / ELISpot Development Modules	
		Catalog #s	
CXCL8/IL-8	Human		SEL208
	Canine		SEL1608
IFN-γ	Human	EL285	SEL285
	Mouse	EL485	SEL485
	Rat	EL585	SEL585
	Canine	EL781	SEL781
	Feline	EL764	SEL764
	Primate	EL961	SEL961
IFN-γ (from CD8α⁺ cells)	Human	EL3094	
IFN-γ (from CD4⁺ cells)	Mouse	EL2019	
IFN-γ/ Granzyme B	Human	ELD5818	
	Mouse	ELD5819	
IFN-γ/IL-2	Human	ELD4506	
	Mouse	ELD5006	
	Canine	ELD6314	
	Feline	ELD8069	
	Primate	ELD7595	
IFN-γ/IL-4	Human	ELD5008	
	Mouse	ELD5217	
IFN-γ/IL-5	Human	ELD7327	
	Mouse	ELD7420	
IFN-γ/IL-10	Human	ELD5505	
IFN-γ/IL-13	Human	ELD7328	
	Mouse	ELD7424	
IFN-γ/IL-17	Human	ELD5219	
	Mouse	ELD5007	
	Canine	ELD6555	
	Primate	ELD7596	
IL-1β/IL-1F2	Human		SEL201
	Rat		SEL501
IL-6	Human	EL206	SEL206
	Mouse, Rat	EL406	SEL406
	Canine	EL1609	SEL1609
	Feline		SEL2305
TGF-β1 (Latent)	Human	EL246	SEL246
TNF-α	Human	EL210	
	Mouse	EL410	
	Rat		SEL510
	Canine		SEL1507