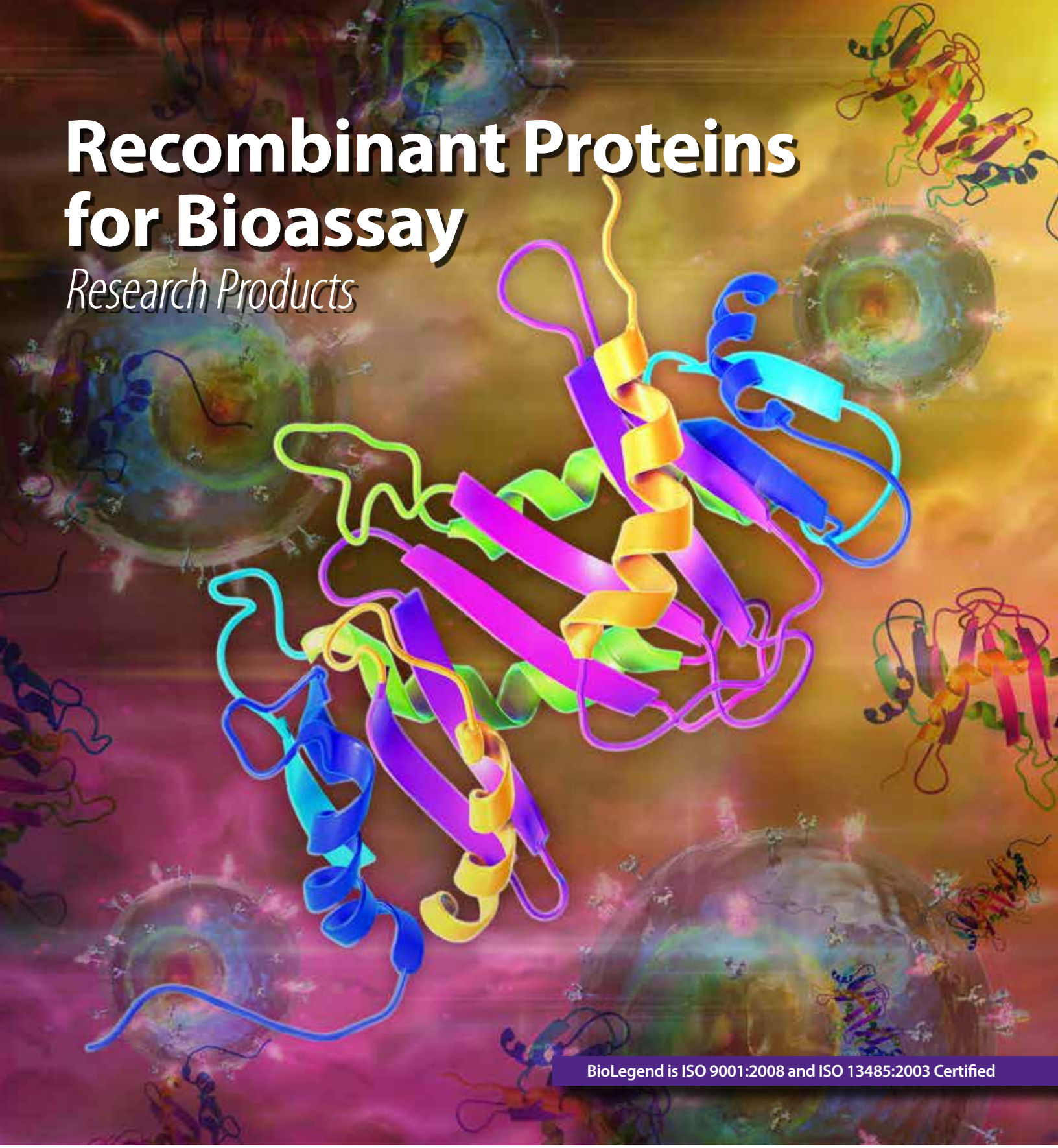
# Recombinant Proteins for Bioassay

*Research Products*



BioLegend is ISO 9001:2008 and ISO 13485:2003 Certified

BioLegend®

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**biolegend.com**

02-0007-02

World-Class Quality | Superior Customer Support | Outstanding Value

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# BioLegend Recombinant Proteins

BioLegend's growing portfolio of recombinant proteins now contains over 600 functional proteins, for human, mouse, and rat, that can be used for bioassays in several research areas including Immunology, Neurobiology, Stem Cell research, Cancer research, Glycobiology, and Cell Biology research.

## Why choose BioLegend?

Our recombinant proteins are:

- >95% pure
- Validated in-house through bioassays to ensure reproducibility and activity
- Biologically active and compare favorably against competitors' products
- Competitively priced
- Discounted for bulk orders
- Flexible packaging size

## Elevate your Research with BioLegend Recombinant Proteins

Our expanding catalog includes cytokines, chemokines, growth factors, enzymes, adhesion molecules, and more.

- Expressed with mammalian, *E.coli*, or insect protein expression system
- Both *in vivo* and *in vitro* functional assay applications
- Flexible, custom recombinant protein production
- Carrier-free formats for functional assays
- Animal-free formats to avoid animal pathogens and experimental variability

## Common uses of our Recombinant Proteins:

- Standard Cell Culture
- Cell Activation
- Cell Expansion
- Differentiation
- Enzymatic Cleavage
- Polarization
- Cytokine Production
- Growth and Proliferation
- Inhibition
- ELISA standards
- Stem Cell Differentiation
- Cytotoxicity
- Chemotaxis
- Cell Signaling Activation
- WB controls

**Learn more at: biolegend.com/recombinant_proteins**

# Stringent Quality Testing

To ensure that we deliver the highest quality products, BioLegend's recombinant proteins undergo rigorous testing before they reach your hands.

## Product Quality Testing

- Protein parameter confirmation: by SDS-PAGE, HPLC, and Mass Spec analyses
- Purity: by SDS-PAGE and HPLC analysis
- Protein content: by UV spectroscopy, and SDS-PAGE analyses
- Microbiological contamination: protein solutions are 0.2 µm-filtered prior to bottling by membrane filtration method.

## Product Functional Testing

Based on the expected function of the protein, our team tests the recombinants in several different bioassays for quality control purposes and to characterize the protein and measure its activity. Our bioassays include:

- Proliferation Assays
- Neurite Outgrowth Assays
- Chemotaxis Assays
- Enzymatic Assays
- Cytotoxicity Assays
- Cytokine Production Assays
- Adhesion Assays
- Differentiation Assays

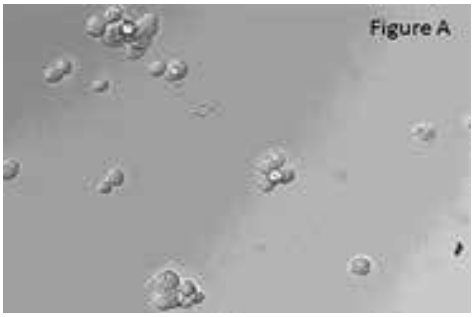



Figure A. 300 ng of human OMG, immobilized as a 3 µL droplet on a nitrocellulose-coated plate, completely inhibits neurite outgrowth of E13 chick DRG neurons induced by laminin substrate.

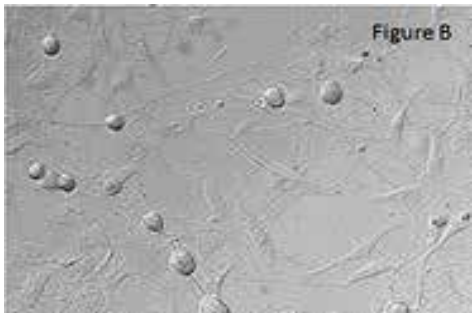
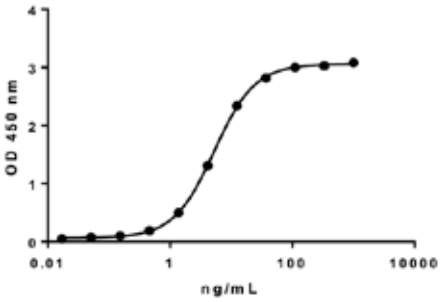



Figure B. 3 µL droplet of buffer control (PBS) on a nitrocellulose-coated plate shows no inhibition of neurite outgrowth of E13 chick DRG neurons induced by laminin substrate.



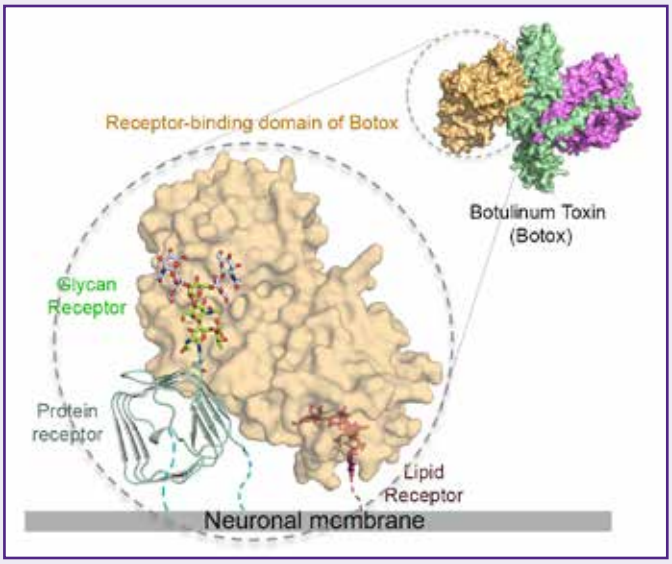

When mouse Syndecan-2 is immobilized at 5 µg/mL, human FGF-basic binds with $EC_{50}$ of 2-8 ng/mL in a functional ELISA.

# Recombinant Protein Services

## Custom Solutions

At BioLegend, our commitment is to provide solutions to our customers. This includes providing products and services not listed in our standard catalog. Save time and money by leveraging our expertise. We provide the following custom solutions:

- Custom Recombinant Protein Production
- Custom Bioassay Development

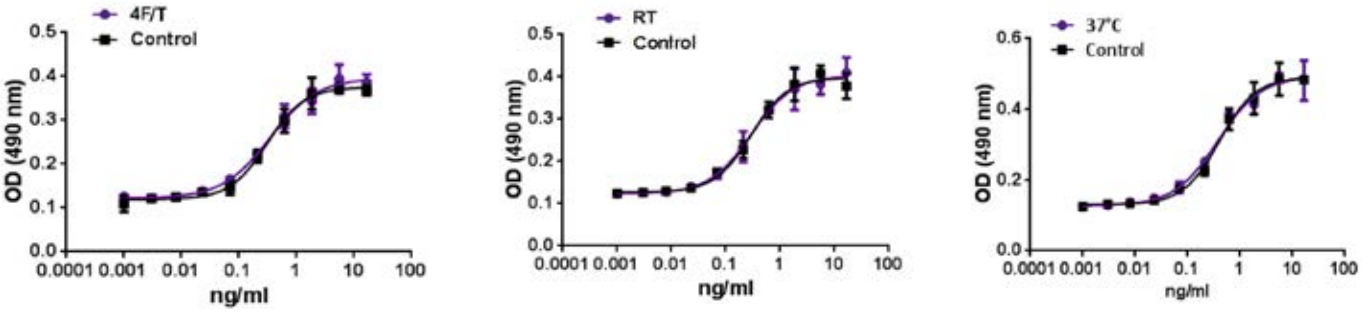



*Credit: Rongsheng Jin and Guorui Yao / UCI*
*Nat Struct Mol Biol. 2016 Jul; 23(7): 656–662.*

Botulinum toxin (Botox) is a large protein that is composed of three domains (upper right corner, crystal structure shown in yellow, green, and pink). The new study reveals a novel mechanism by which the toxin hijacks three receptors on the host neuronal surface as its "GPS" – the peptide moiety of protein SV2 (green-blue ribbon model), a conserved glycan modification of SV2 (green and light blue sticks), and a lipid (brown sticks) – to launch its attack. SUMO-gSV2C and gSV2C were expressed and secreted from HEK 293 cells from BioLegend.

## Stability Testing

Our recombinants are stability tested at different temperatures and time intervals to determine the optimal storage, handling, and shipping conditions. Our testing shows that most recombinant proteins are able to withstand room temperature (RT) and 37°C for a week without losing activity. In addition, most of our recombinant proteins are also able to withstand four cycles of freeze and thaw without losing activity.



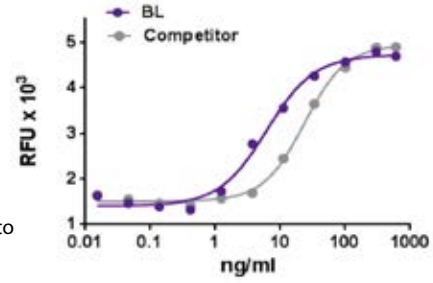

M-NFS60 cell proliferation induced by Recombinant Human M-CSF. Control refers to the protein stored at 4°C. 4F/T is 4 rounds of freeze/thaw of the protein.

## Endotoxin Testing

To assure it is safe for use in biological systems, our recombinant proteins are Endotoxin tested by the LAL (Limulus Amoebocyte Lysate) assay method. The endotoxin levels of our proteins are guaranteed to be less than 0.1 ng per µg protein or 1 EU per µg protein. However, for most proteins, the actual measured endotoxin values are consistently below this stated value.

## Competitor and Internal Control Testing

We internally compare new lots with previous lots for consistency. In addition, we also test our products against ones available from the competition. This ensures you can have full confidence when using our recombinant proteins.




Recombinant human IL-15 induces the proliferation of M07e human megakaryoblastic leukemia cell line in a dose dependent manner. BioLegend's protein was compared side-by-side to a competitor's equivalent product.

## Sample Testing

Need a sample to test your system and set-up before you purchase? Contact us. We are here to meet your needs.

## Bulk Product Requests

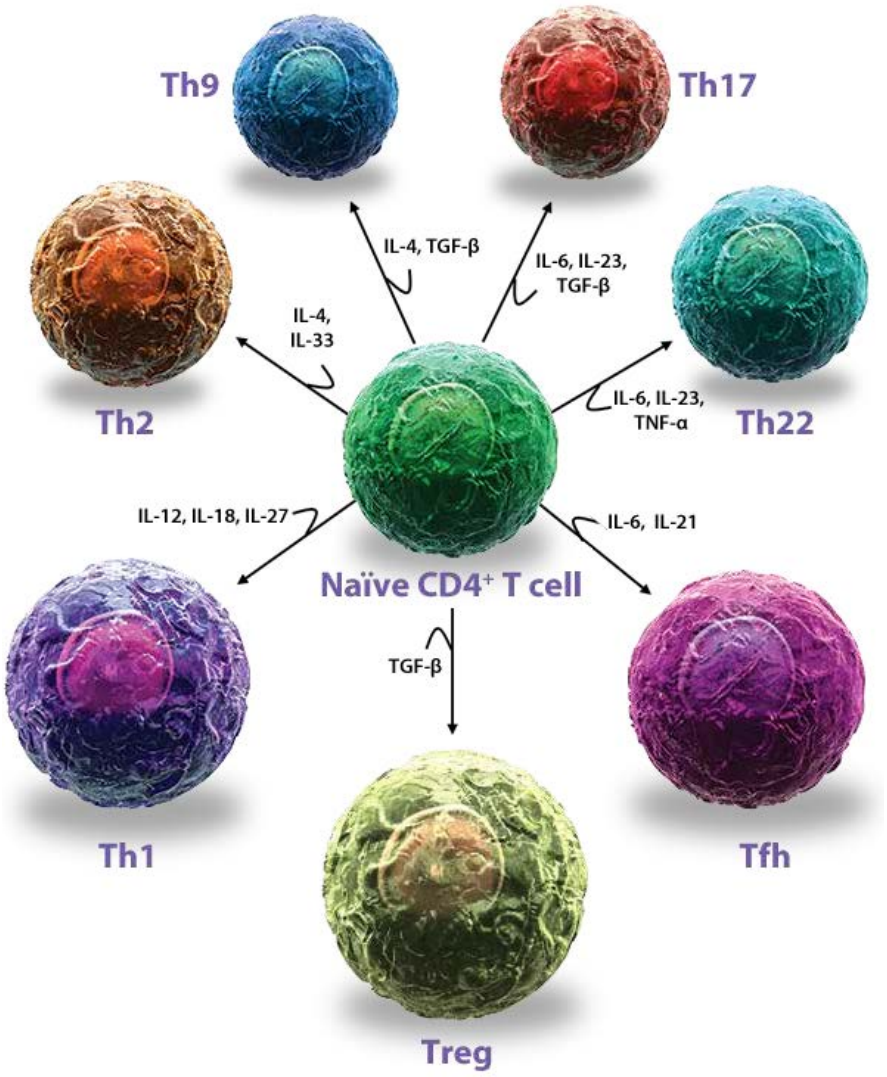
BioLegend is proud to offer special discounts for bulk orders on all of our protein products. Get large quantities of the same high quality recombinants you find in our single vials, at an exceptional price.

Email **sales@biolegend.com** (for US and Canada) or
**distributorsales@biolegend.com** (for international orders).

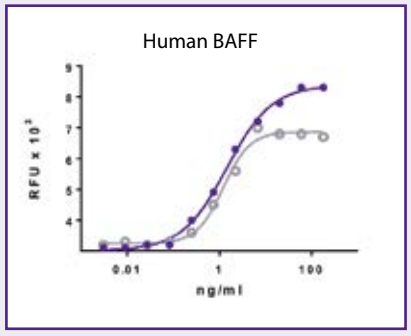
# Cytokines

Cytokines allow cells to communicate with one another, inducing a wide range of activities. These factors can incite or prevent inflammation, promote cell growth, or bias cells to differentiate to a particular phenotype. Cytokines mediate their function by binding to their respective receptors, initiating signaling cascades for gene transcription.

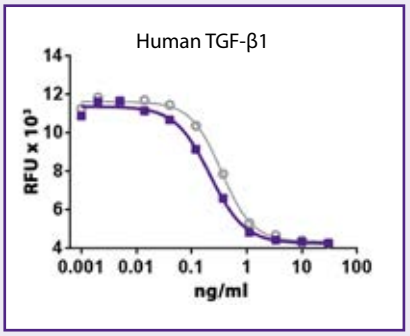
Naïve CD4+ T cells can give rise to a number of T helper classes, depending on the recombinant proteins used to induce their differentiation.



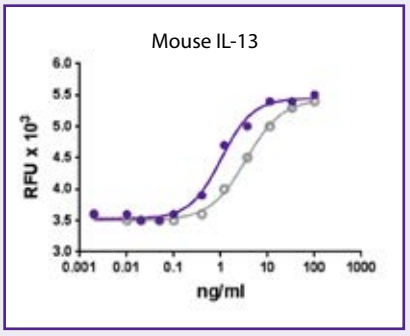

## Comparative Analysis ▪BioLegend ◦ Competitor A




Recombinant human BAFF induces the proliferation of IgM-stimulated mouse B cells in a dose dependent manner.




Human TGF-β1 inhibits the proliferation of mouse HT-2 cells induced by IL-4.




Recombinant mouse IL-13 induces the proliferation of TF-1 human erythroleukemic cells in a dose dependent manner.

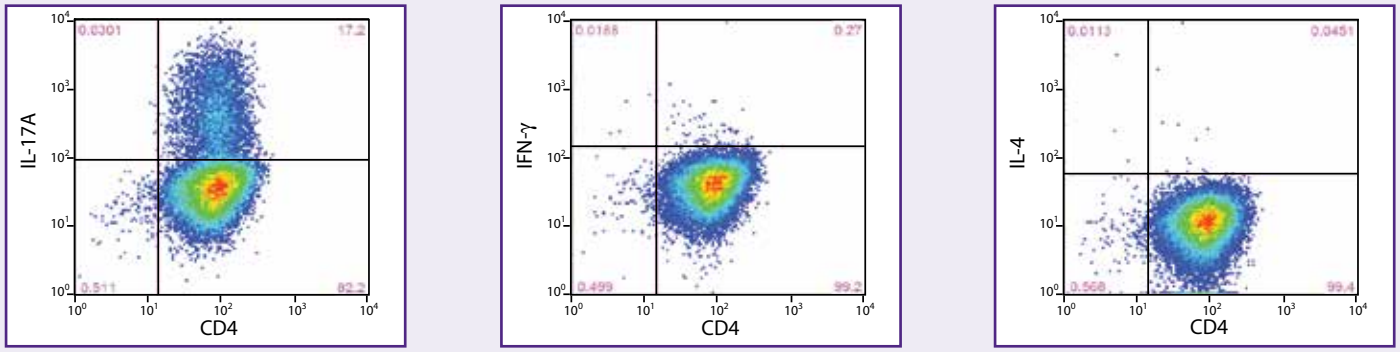
# Cytokines Product List (carrier-free format):

| **Human** | | |
|---|---|---|
| Cardiotrophin-1 | IL-10 (*E.coli* expressed) | IL-23 |
| HMGB1 | IL-10 (mammalian expressed) | IL-27 |
| IFN-α2 | IL-11 | IL-28A |
| IFN-γ | IL-12 (p70) | IL-32α |
| IL-1RA (IL-1RN) | IL-13 | IL-33 |
| IL-1α | IL-15 | IL-34 |
| IL-1β | IL-16 | IL-35-Fc fusion protein |
| IL-2 | IL-17A | IL-36a |
| IL-3 | IL-17A/F | IL-36Ra (IL-1F5) |
| IL-4 | IL-17B | IL-36β |
| IL-5 | IL-17E | LT-α (TNF-β) |
| IL-6 | IL-17F | MIF |
| IL-7 | IL-18 | TNF-α |
| IL-9 | IL-21 | |
| IL-9, His-tagged | IL-22 | |

| **Mouse** | | |
|---|---|---|
| Cardiotrophin-1 | IL-7 | IL-22 |
| IFN-α | IL-9 | IL-23 |
| IFN-α1 | IL-10 | IL-25 (IL-17E) |
| IFN-β1 | IL-11 | IL-27 |
| IFN-γ | IL-12 (p70) | IL-33 |
| IL-1α | IL-12 p40 Homodimer | IL-34 |
| IL-1β | IL-13 | IL-36a |
| IL-2 | IL-15 | IL-36Ra (IL-1F5) |
| IL-3 | IL-17A | IL-36β |
| IL-4 | IL-17A/F | IL-36γ |
| IL-5 | IL-17F | MIF |
| IL-6 | IL-21 | TNF-α |

| **Rat** |
|---|
| IFN-γ |
| IL-1β |
| IL-2 |
| IL-3 |
| IL-6 |
| IL-13 |
| TNF-α |

# Th17 Polarization with BioLegend Recombinant Proteins

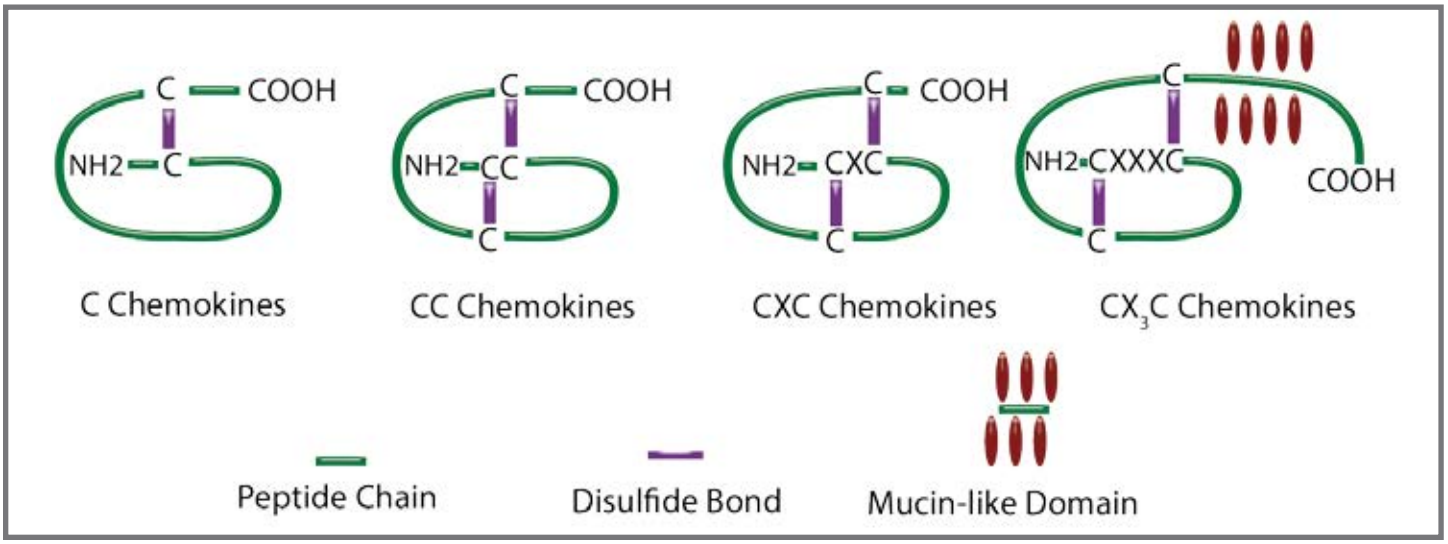



Mouse CD4$^+$ T cells were polarized with plate-bound anti-mouse CD3, soluble anti-mouse CD28, recombinant mouse IL-6, IL-23, and TGF-β, anti-mouse IL-4, and anti-mouse IFN-γ for 4 days. After re-stimulation with PMA/ionomycin in the presence of BFA or Monensin, the cells were harvested and surface stained with CD4-FITC, and intracellularly stained with IL-17A-PE, IFN-γ-APC, or IL-4-APC.

# Chemokines

Chemokines are relatively small cytokines focused on inducing cell movement, or chemotaxis. Chemokines contain several (usually four) cysteines in conserved positions within the protein. These cysteines (marked C in the figure below) provide tertiary structure for the chemokine through disulfide bonds.

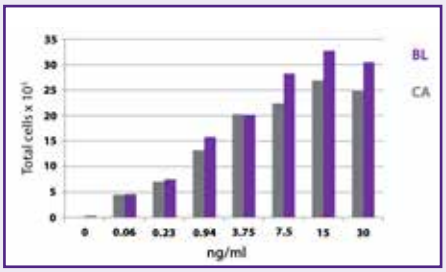
The spacing and intervening amino acid residues (denoted X) between the first two cysteines determine the type of chemokine. Chemokine receptors consist of seven transmembrane-spanning regions and most of them are promiscuous, binding to multiple ligands.




**Learn more at:** **biolegend.com/chemokine_receptors**

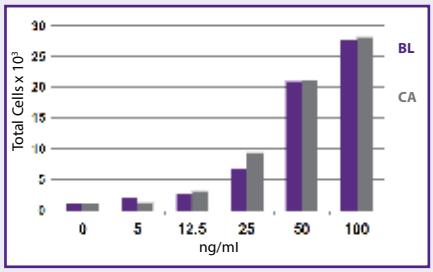
## Comparative Analysis ▪**BioLegend** • **Competitor A**

Mouse CCL2



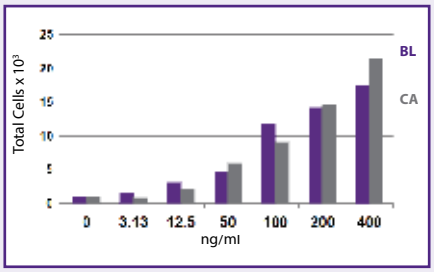

Mouse CCL2 attracts human monocytic THP-1 cells.

Human CCL3




BaF3-hCCR5 transfectants attracted by human CCL3.

Human CCL8




THP-1 cells attracted by human CCL8/MCP-2.

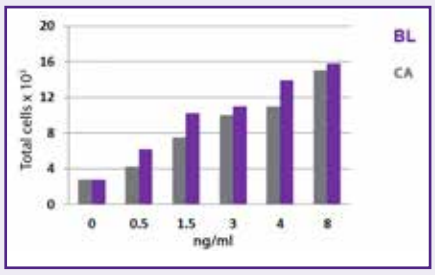
# Chemokines Product List (carrier-free format):

| Human | | |
|---|---|---|
| CCL1 (I-309) | CCL21 (6CKine) | CXCL7 (NAP-2) |
| CCL2 (MCP-1) | CCL22 (MDC) | CXCL9 (MIG) |
| CCL3 (MIP-1α) | CCL23 (MPIF-1) (Arg22-Asn120) | CXCL10 (IP-10) |
| CCL4 (MIP-1β) | CCL23 (MPIF-1) (Arg46-Asn120) | CXCL11 (ITAC) |
| CCL5 (RANTES) | CCL24 (Eotaxin-2) | CXCL12 (SDF-1α) |
| CCL6 (C10) | CCL25 (TECK) | CXCL12 (SDF-1β) |
| CCL7 (MCP-3) | CCL26 (Eotaxin-3) | CXCL13 |
| CCL8 (MCP-2) | CCL27 (CTACK) | CXCL14 (BRAK) |
| CCL20 (MIP-3α) | CCL28 (MEC) | CXCL16 |
| CCL11 (Eotaxin) | CX3CL1 (Fractalkine) | CXCL17 (VCC-1) |
| CCL13 (MCP-4) | CXCL1 (GRO-α) | IL-8 |
| CCL14 (HCC-1) | CXCL2 (Groβ) | TAFA2 (chemokine like) |
| CCL15 (MIP-1δ) | CXCL3 (GRO-γ) | XCL1 |
| CCL17 (TARC) | CXCL4 (PF-4) | XCL2 |
| CCL19 (MIP-3β) | CXCL5 (ENA-78) | |
| CCL20 (MIP-3α) | CXCL6 (GCP2) | |

| Mouse | | |
|---|---|---|
| CCL1 (I-309) | CCL19 (MIP-3β) | CXCL5 (LIX) |
| CCL2 (MCP-1) | CCL20 (MIP-3α) | CXCL7 (NAP-2) |
| CCL3 (MIP-1α) | CCL21 (6CKine) | CXCL9 (MIG) |
| CCL4 (MIP-1β) | CCL22 (MDC) | CXCL10 (IP-10) |
| CCL5 (RANTES) | CCL24 (Eotaxin-2) | CXCL11 (ITAC) |
| CCL6 (C10) | CCL25 (TECK) | CXCL12 (SDF-1α) |
| CCL7 (MCP-3) | CCL28 (MEC) | CXCL12 (SDF-1β) |
| CCL8 (MCP-2) | CX3CL1 (Fractalkine) | CXCL13 |
| CCL9 (MIP-1γ) | CXCL1 (KC) | CXCL14 (BRAK) |
| CCL11 (Eotaxin) | CXCL2 (MIP-2) | CXCL17 (VCC-1) |
| CCL12 (MCP-5) | CXCL3 | |
| CCL17 (TARC) | CXCL4 (PF-4) | |

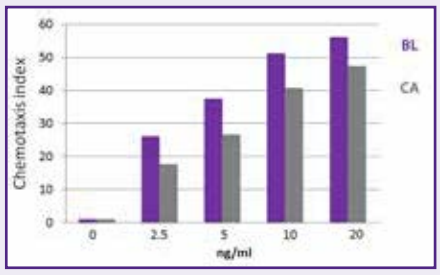
| Rat |
|---|
| CCL20 (MIP-3α) |
| CX3CL1 |

Human CXCL8/IL-8



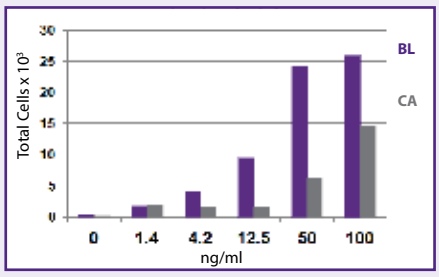

Chemoattraction of human neutrophils by IL-8.

Mouse CCL3




Mouse CCL3 induces chemotaxis of BaF3 mouse pro-B cells transfected with human CCR5.

Human CX3CL1



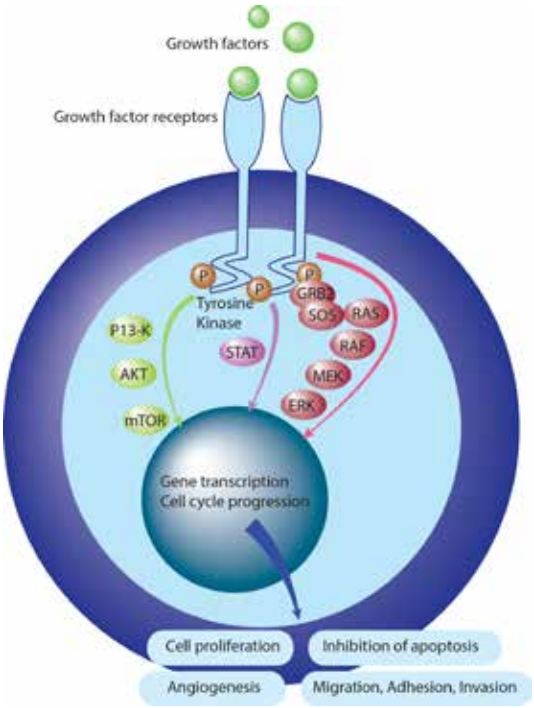

BaF3-mCX3CR1 transfectants attracted by human CX3CL1.

# Growth Factors

Growth factors are proteins produced by the body that control cell growth, differentiation, and survival. There are many different types of growth factors, and they work in different ways. Some tell cells what they should become (differentiation). Some make cells grow and divide into new cells.

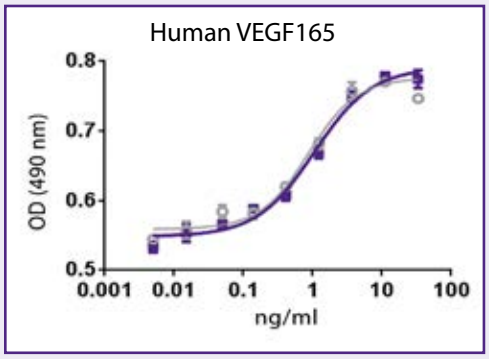
There are a number of different growth factors, and one way to classify them is by the ability of each family to affect specific cell types. Examples include:

- Epidermal growth factor (EGF) – controls cell growth.
- Vascular endothelial growth factor (VEGF) – controls blood vessel development.
- Platelet derived growth factor (PDGF) – controls blood vessel development and cell growth.
- Fibroblast growth factor (FGF) – controls cell growth.
- Transforming Growth Factor beta (TGF-beta) superfamily – Includes molecules like TGF-beta-1/2/3, Activins, Bone Morphogenetic Proteins (BMPs), Growth Differentiation Factors (GDFs).

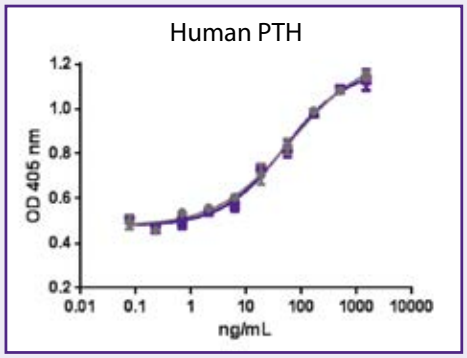



The activity of a growth factor is mediated via binding to its transmembrane receptor that often contains a cytoplasmic tyrosine kinase domain. This event initiates various downstream signaling cascades, such as ras-raf-MAP-fos or PI3K-Akt-mTOR pathways, eventually controlling growth and differentiation of the target cell. Typically, growth factors don't act in an autocrine fashion and act locally, due to their short half-lives and slow diffusion through the extracellular matrix.

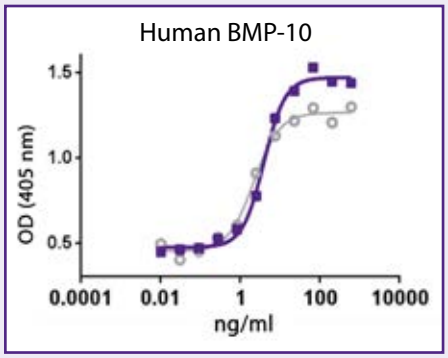
## Comparative Analysis ▪BioLegend ◦ Competitor A



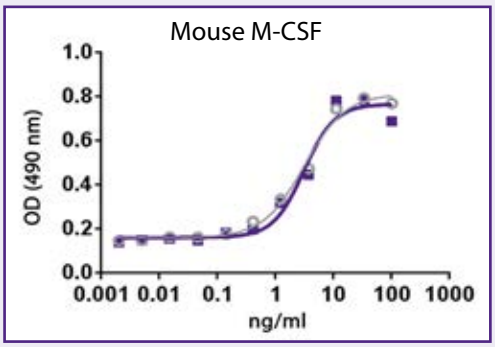

Human VEGF165 induces the proliferation of HUVEC cells.



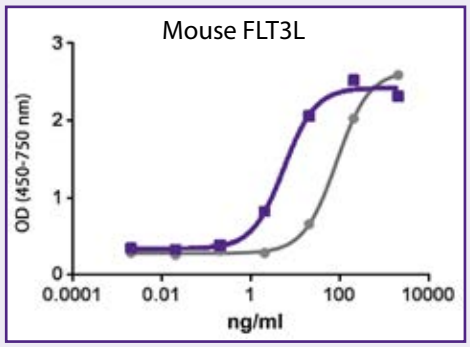

Recombinant Human PTH enhances BMP9 induced alkaline phophatase production in MC3T3-E1 cells in a dose dependent manner.



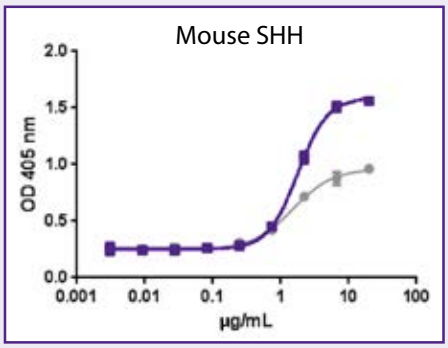

Recombinant human BMP-10 induces alkaline phosphatase production in the mouse chrondrogenic cell line ATDC5.




M-NFS60 cell proliferation induced by mouse M-CSF.




Mouse FLT3L induces IL-6 production in murine leukemia cell line M1 in the presence of mouse LIF recombinant protein.




Recombinant Mouse SHH induces C3H10T1/2 cell differentiation in the presence of BMP9 as measured by alkaline phosphatase production.

# Growth Factors Product List (carrier-free format):

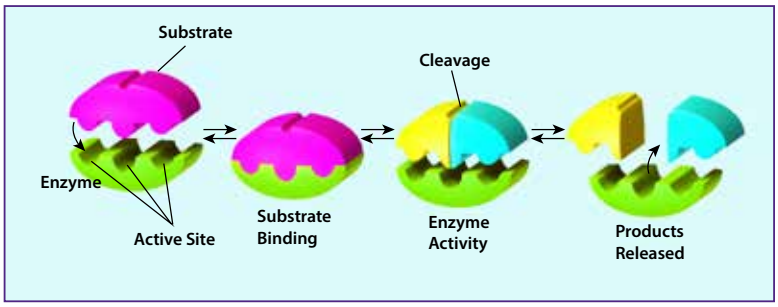
| Human | | |
|---|---|---|
| β-NGF | FGF-4 | Oncostatin M |
| Activin A | FGF-6 | OX40L |
| Angiopoietin-2 | FGF-9 | PDGF-BB |
| Artemin | FGF-10 | Persephin |
| Asprosin | FGF-17 | PLGF-1 |
| BAFF | FGF-18 | Prolactin |
| BDNF | FGF-21 | PTH |
| Betacellulin | FGF-basic/145aa | RANK (TNFRSF11A) |
| BMP-4 | FLT3L | S100A8/A9 Heterodimer |
| BMP-5 | G-CSF | SCF |
| BMP-6 | GDNF | Slit2-N |
| BMP-7 | GM-CSF | Sonic Hedgehog |
| BMP-9 | HB-EGF | TGF-α |
| BMP-10 | HGF | TGF-β1 |
| BMP-13 | IGF-I | TGF-β2 |
| BMP-14 (GDF-5) | IGF-II | TGF-β3 |
| CD27L | IHH | Thrombopoietin (TPO) |
| CD40L (TNFSF5) | LIF | TNFSF18 (GITRL) |
| CNTF | M-CSF | TRANCE (RANKL) |
| DHH | Midkine | TSLP |
| EG-VEGF | Neurturin | VEGF-121 |
| EGF | NNT-1 (BCSF-3) | VEGF-165 |
| Epigen | Noggin | VEGF-C |
| Epiregulin | NRG1 (Heregulin) EGF Domain | VEGF-D |
| Erythropoietin (EPO) | NRG1α | WISP-1 |
| FGF-1-acidic | NT-3 | WNT-7a |
| FGF-3 | NT-4 | VEGF-B167 |

| Mouse | | |
|---|---|---|
| β-NGF | FGF-1-acidic | NOV (CCN3) |
| Amphiregulin | FGF-10 | Oncostatin M |
| Asprosin | FGF-17 | PDGF-BB |
| BAFF | FGF-basic | Persephin |
| Betacellulin | FLT3L | Prolactin |
| BMP-4 | G-CSF (*E.coli* expressed) | SCF |
| BMP-9 | G-CSF (mammalian expressed) | Sonic Hedgehog (Cys25-Gly198) |
| BMP-14 (GDF-5) | GM-CSF | Sonic Hedgehog (Cys25/(Ile-Ile)-Gly198) |
| Cardiotrophin-1 (CT-1) | IGF-I | TGF-β1 |
| CD27L | IGF-II | Thrombopoietin (TPO) |
| CD30L | IHH | TRANCE (RANKL) |
| CNTF | KGF (FGF-7) | VEGF-120 |
| EGF | LIF | VEGF-164 |
| Epiregulin | M-CSF (carrier free) | |
| Erythropoietin (EPO) | Noggin | |

| Rat | |
|---|---|
| EGF | Oncostatin M |
| Erythropoietin (EPO) | Prolactin |
| GM-CSF | SCF |
| IGF-I | Thrombopoietin (TPO) |
| M-CSF | VEGF-164 |

# Enzymes and Regulators

Enzymes are molecules that act as biological catalysts regulating chemical reactions. Enzymes are very specific to the substrates with which they react. BioLegend offers several different enzymes, including proteases that can be used as useful research tools.



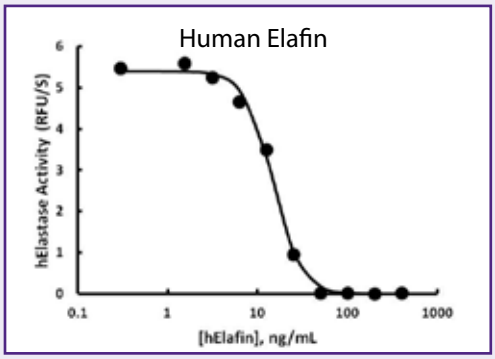

Proteases (also called Proteinases, Peptidases, or Proteolytic Enzymes) are enzymes that hydrolyze peptide bonds. Most proteases cleave α-peptide bonds between naturally occurring amino acids. Recent classification of proteases based on the mechanism of catalysis identifies six distinct classes:

- Serine proteases
- Threonine proteases
- Cysteine proteases
- Glutamic proteases
- Aspartic proteases
- Metalloproteases

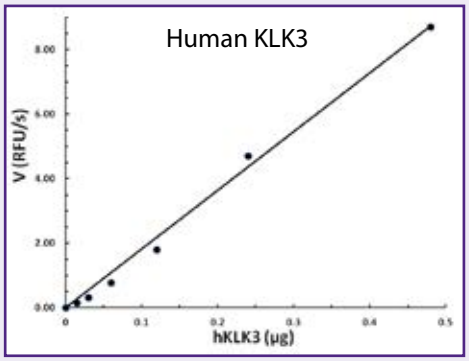
A seventh catalytic type of proteolytic enzymes, asparagine peptide lyase, was described recently in 2011. Its proteolytic mechanism is unusual since, rather than hydrolysis, it performs an elimination reaction.

Through highly specific reactions, enzymes and their regulators (such as inhibitors) modulate the fate, localization, and activity of many proteins, regulate protein-protein interactions, create new bioactive molecules, and contribute to signal transduction. As a result of these multiple actions, enzymes and their regulators influence critical cellular functions, including DNA replication and transcription, cell proliferation and differentiation, tissue morphogenesis and remodeling, neurogenesis, inflammation, immunity, autophagy, and apoptosis.

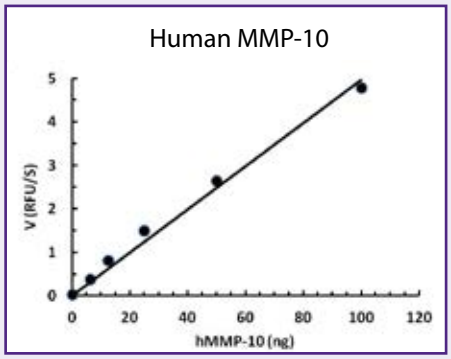
## Featured Data




The activity of recombinant Elafin was measured by its ability to inhibit human neutrophil elastase. The potency of inhibition was measured by monitoring the cleavage of a fluorogenic substrate (MeO-Suc-AAPV-AMC) in the presence of human neutrophil elastase.




The activity of recombinant human KLK3 is determined by its ability to cleave a chromogenic (MeO-Suc-Arg-Pro-Tyr-pNA) or fluorogenic peptide substrate (MeO-Suc-Arg-Pro-Tyr-AMC).




The activity of recombinant human MMP-10 was measured with a fluorogenic MMP substrate (Mca-RPKPVE-Nval-WRK(Dnp)-NH2).

## Enzymes and Regulators Product List (carrier-free format):

| **Human** | | |
|---|---|---|
| Arginase I | KLK7 | Serpin A12 (Vaspin) |
| Cathepsin A (CTSA) | MMP-1 | Serpin E1 (PAI-1) |
| Cathepsin B | MMP-2 | Serpin F1 |
| Cathepsin D | MMP-3 | SLPI |
| Cathepsin E | MMP-7 | ST8SIA1 |
| Cystatin C | MMP-8 | TIMP-1 |
| Elafin | MMP-9 | TIMP-2 |
| GALNT2 | MMP-9 (dimer) | t-Plasminogen Activator (t-PA) |
| Granzyme A | MMP-10 | u-Plasminogen Activator (Urokinase) |
| Granzyme B | PCSK9 | Visfatin |
| KLK3 | PLA2G7 | |

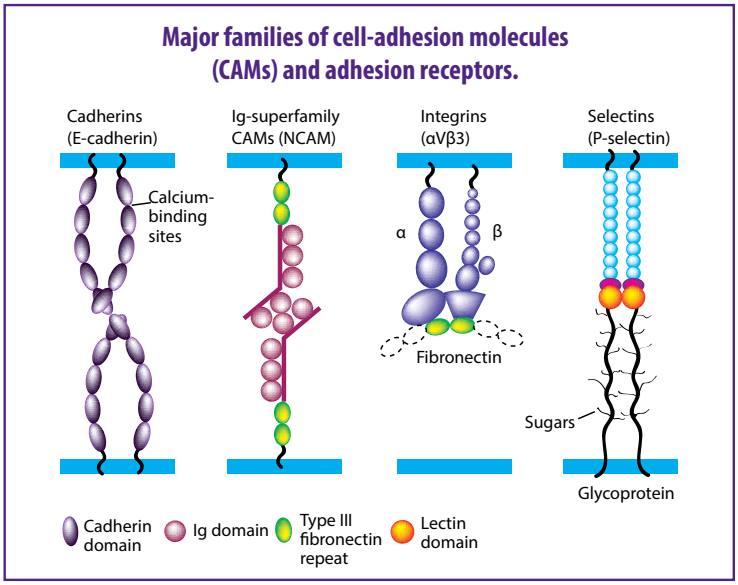
| **Mouse** | | |
|---|---|---|
| Cathepsin E (CTSE) | MMP-2 | Serpin A12 |
| Cathepsin B | MMP-3 | TIMP-1 |
| Granzyme B | MMP-9 (Gelatinase B) | |
| KLK7 | PCSK9 | |

## Comparative Analysis

| | **Activity Range** | | |
|---|---|---|---|
| **Enzyme** | **BioLegend** | **Competitor** | **Specific Activity Unit** |
| hGranzyme B | ≥ 1500 | ≥ 1000 | Pmol $\mu g^{-1}$ $min^{-1}$ |
| hKLK-3 | ≥ 150 | ≥ 70 | Pmol $\mu g^{-1}$ $min^{-1}$ |
| hMMP-2 | ≥ 1100 | ≥ 1000 | Pmol $\mu g^{-1}$ $min^{-1}$ |
| hPLA2G7 (LP-PLA2) | ≥ 14000 | ≥ 9000 | Pmol $\mu g^{-1}$ $min^{-1}$ |
| hPLAU | ≥ 300 | ≥ 190 | Pmol $\mu g^{-1}$ $min^{-1}$ |
| hELAFIN | ≤ 3.0 | ≤ 50 | nM ($IC_{50}$) |
| hSerpin E1 | ≤ 11 | ≤ 13 | nM ($IC_{50}$) |

# Adhesion Molecules

Cell adhesion molecules (CAMs) are proteins that account for cell-to-cell and/or cell-to-extracellular matrix (ECM) interactions. Most of the CAMs belong to four protein families: Ig (immunoglobulin) superfamily, the integrins, the cadherins, and the selectins. The biological effects that result from interactions mediated by CAMs can be either adhesive or repulsive (inhibitory) in nature. These interactions are associated with changes in intracellular signaling, cytoskeletal organization, or gene expression, hence, CAMs play vital roles in several cellular processes, including cell growth, differentiation, cell migration, and cancer metastasis.

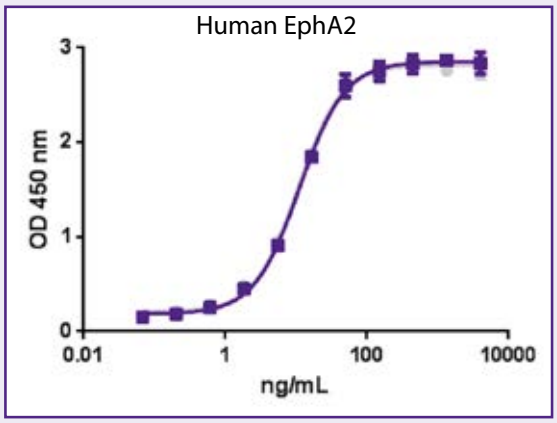



## Adhesion Molecules Product List (carrier-free format):

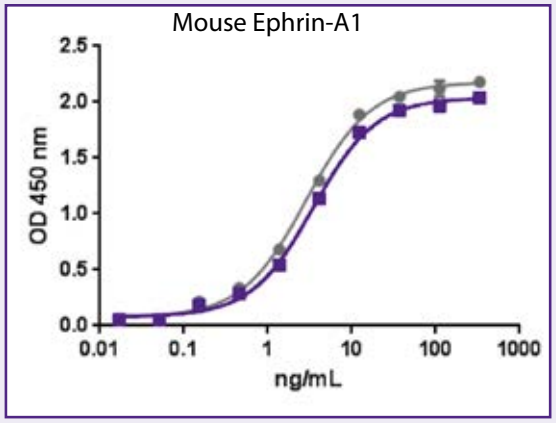
| **Human** | | |
|---|---|---|
| Aggrecan (G1-IGD-G2) | Galectin-3 | Siglec-5 |
| Clusterin | Galectin-4 | VAP-1 |
| EphA2 | Galectin-9 | VCAM-1-Fc Chimera |
| Ephrin-A1 | HVEM-Fc Chimera | Vitronectin |
| E-selectin | ICAM-1-Fc Chimera | |
| Galectin-1 | Siglec-3 | |

| **Mouse** | | |
|---|---|---|
| Cadherin-13 | Galectin-3 | Podoplanin-Fc Chimera |
| CLEC2-Fc Chimera | Galectin-4 | Siglec E-Fc Chimera |
| Ephrin-A1 | ICAM-1-Fc Chimera | |
| E-Selectin (CD62E)-Fc Chimeric | P-Selectin (CD62P)-Fc Chimeric | |

## Comparative Analysis ▪**BioLegend** ◦ **Competitor A**



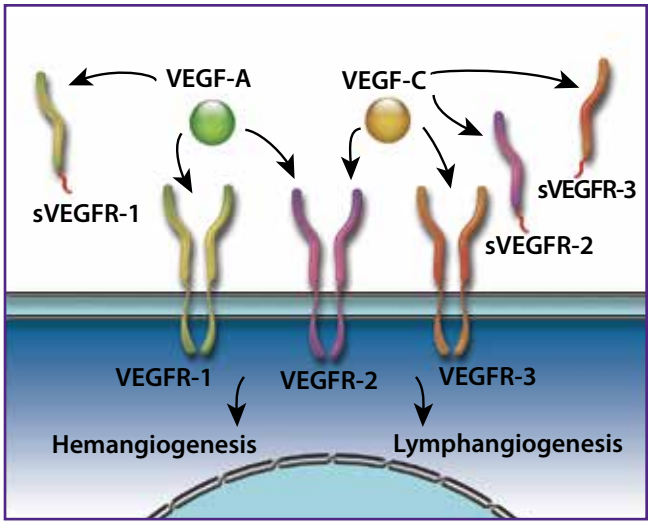

When human EphA2 is immobilized, human Ephrin-A1 binds with $EC_{50}$ of 3-12 ng/mL in a functional ELISA.




Mouse Ephrin-A1 binds to immobilized human EphA2 with an $EC_{50}$ of 3-12 ng/mL in a functional ELISA.

# Soluble Receptors

Receptors are proteins that recognize and respond to signals through their respective ligands. Each receptor is linked to a specific cellular biochemical pathway. The complex receptor system includes signaling receptors, non-signaling decoy receptors, receptor-associated proteins, and soluble receptors.

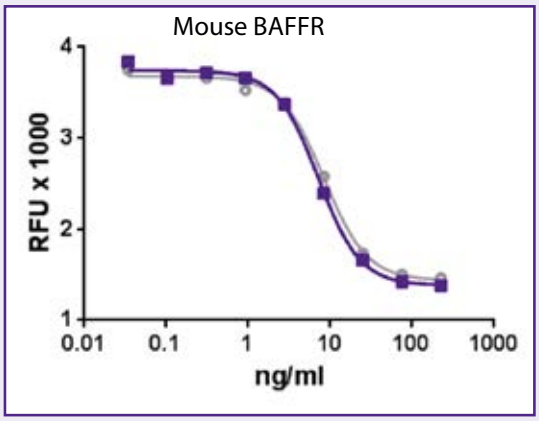



Naturally occurring soluble receptors can be generated by several mechanisms, which include proteolytic cleavage of cell-surface receptor ectodomains, alternative splicing of mRNA transcripts, transcription of distinct genes, cleavage of GPI-anchored receptors, and extracellular release of membrane-bound receptors within vesicles such as exosomes. Examples of soluble receptors include the cytokine receptors that bind cytokines and function as either agonists or antagonists of cytokine signaling. These receptors are important regulators of inflammation and immunity. BioLegend's receptor protein portfolio includes soluble receptors for many targets, including soluble cytokine receptors, soluble growth factor receptors, and soluble TNF superfamily receptors. These proteins are constructed to encode the extracellular domain only, and lack the transmembrane domain and cytoplasmic tail of the receptor.

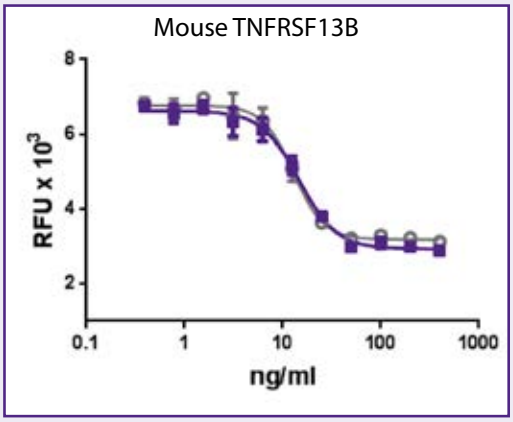
## Receptor Product List (carrier-free format):

| **Human** | | |
|---|---|---|
| B7-H1 (PD-L1, CD274)-Fc Chimera | GM-CSFRα | PLAUR (uPAR) |
| B7-H2-Fc Chimera | ICOS-Fc Chimera | sTNF-RI (TNFRSF1A) |
| CD14 | IL-1R | sTNF-RII (TNFRSF1B) |
| CD28-Fc Chimera | IL-1RL1 (ST2)-Fc Chimera | TIM-3-Fc Chimera |
| CTLA-4-Fc Chimera | IL-6Rα | TLR3 |
| EphA2 | IL-15Rα | TNFRSF11B |
| FAS (TNFRSF6)-Fc Chimera | LDLR | VEGFR1 |
| FGFR3 (IIIc)-Fc Chimera | NGFR (TNFRSF16)-Fc Chimera | VEGFR2-Fc Chimera |
| FGFR4-Fc Chimera | NTRK2 (TrkB) | VEGFR3-Fc Chimera |

| **Mouse** | | |
|---|---|---|
| B7-H1 (PD-L1, CD274)-Fc Chimera | ICOS-Fc Chimera | TNFRII (CD120b) |
| B7.1 (CD80)-Fc Chimera | IL-2Rα | TNFRSF11B |
| BAFFR (TNFRSF13C, CD268)-Fc Chimera | IL-15Rα | TNFRSF9 |
| CD28-Fc Chimera | LDLR | VEGFR2-Fc Chimera |
| CD30/TNFRSF8-Fc Chimera | sTNF-RI (TNFRSF1A) | |
| CTLA-4-Fc Chimera | TACI-Fc (TNFRSF13B) Chimera | |




Recombinant mouse BAFFR inhibits the proliferation of mouse B cells induced by BAFF in a dose dependent manner. BioLegend's protein was compared side-by-side to the leading competitor's equivalent product.




Mouse TNFRSF13B (TACI) inhibits mouse B cell proliferation induced by mouse BAFF (2.5 ng/mL). BioLegend's protein was compared side-by-side to the leading competitor's equivalent product.

# Other Proteins

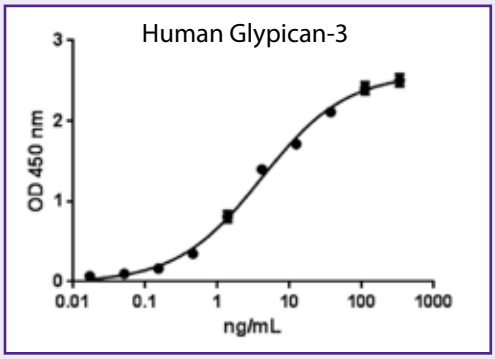
In addition to the aforementioned categories, we have other proteins that can be used in multi-functional assays and have been shown to play important cellular roles including cell migration, survival, regulation of immune responses, apoptosis, neuroinflammation, Alzheimer's disease, control of tumor cell phenotypes, and autoimmune diseases.

## Other Proteins Product List (carrier-free format):

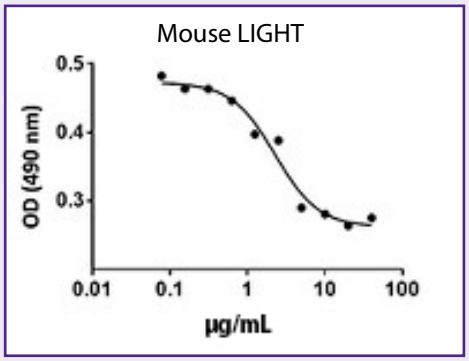
| **Human** | | |
|---|---|---|
| APCS (PTX2) | IGFALS | Osteopontin |
| Clusterin | IGFBP-1 | RBP4 |
| CRP | IGFBP-3 | Resistin |
| DLL1 | IGFBP-4 | SAA1 |
| Endostatin | IGFBP-6 | TFPI-2 |
| FASL (TNFSF6) | IGFBP-7 | TNFSF9 (4-1BBL) |
| GASP-1 | LIGHT (TNFSF14) | TNFSF15 |
| Glypican-1 | Mesothelin | TRAIL (TNFSF10) |
| Glypican-3 | NGAL (Lipocalin-2) | TWEAK (CD255) |
| Gremlin-1 | OMG | Twisted Gastrulation (TSG) |

| **Mouse** | | |
|---|---|---|
| DKK-1 | IGFBP-6 | Syndecan-2 |
| Endostatin | Isthmin1 | TACI-Fc Chimera |
| Glypican-1 | LIGHT (TNFSF14) | TNFRSF17-Fc Chimera |
| Glypican-3 | Mesothelin | TNFSF9 (4-1BBL) |
| IGFBP-1 | NGAL (Lipocalin-2) | TNFSF15 |
| IGFBP-2 | OMG | TNFSF18 (GITRL) |
| IGFBP-4 | Osteopontin | |
| IGFBP-5 | RBP4 | |

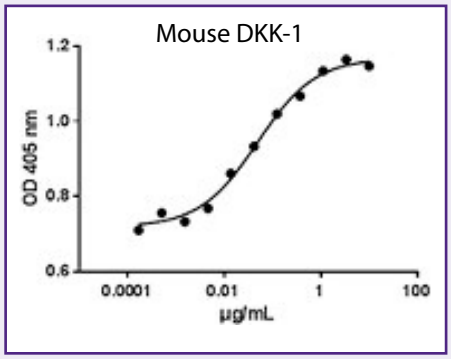
| **Rat** |
|---|
| Nogo-A/Nogo-66-Fc Chimera |




When human Glypican-3 is immobilized at 2 µg/mL, human FGF-basic binds with $EC_{50}$ of 3-12 ng/mL in a functional ELISA.




Recombinant mouse LIGHT's cytotoxic effect on HT-29 human colon adenocarcinoma cells.




Recombinant Mouse DKK-1 enhances BMP9 induced alkaline phosphatase production in MC3T3-E1 cells with $EC_{50}$ of 15 - 60 ng/mL.

# Animal-Free Recombinant Proteins

BioLegend's line of animal-free recombinant proteins greatly minimize the variables and potential contamination of mammalian pathogens during the production process. All of these proteins are produced in animal-free media, and the purification equipment itself is also animal component-free. The animal-free versions of these proteins function in a similar manner to their animal-derived counterparts. BioLegend provides the animal-free proteins in lyophilized format. Treat your cells to animal-free recombinant proteins and see how they prosper!


## Animal-Free Product List (carrier-free format):

| **Human** | | |
|---|---|---|
| β-NGF | Heregulin-β1 | IL-22 |
| Activin A | IFN-γ | IL-33 |
| BMP-4 | IFN-λ1 | IL-36γ |
| CCL2 (MCP-1) | IGF-1 | KGF (FGF-7) |
| CCL5 (RANTES) | IGF-II | LIF |
| CNTF | IL-1RA | M-CSF |
| EGF | IL-3 | NT-3 |
| FGF-1-acidic | IL-4 | NT-4 |
| FGF-4 | IL-6 | Oncostatin M |
| FGF-8 | IL-7 | PDGF-AA |
| FGF-9 | IL-8 | PlGF-1 |
| FGF-10 | IL-9 | sCD40L |
| FGF-basic (146 aa) | IL-10 | TGF-α |
| FGF-basic (154 aa) | IL-11 | Thrombopoietin (TPO) |
| Flt-3 Ligand | IL-15 | TNF-α |
| G-CSF | IL-16 | TRANCE (RANKL) |
| GDF-3 | IL-17A | TWEAK |
| GDNF | IL-17E | VEGF-165 |
| GM-CSF | IL-21 | |

| **Mouse** | | |
|---|---|---|
| FGF-basic | IL-2 | Noggin |
| G-CSF | IL-3 | Thrombopoietin (TPO) |
| GM-CSF | IL-4 | TNF-α |
| IFN-γ | IL-6 | VEGF-164 |

| **Rat** |
|---|
| GM-CSF |
| M-CSF |
| SCF |
| Thrombopoietin (TPO) |

Learn more at: **biolegend.com/recombinant_proteins**

# ELISA Standard Recombinant Proteins

Having the correct standards is critical for the accuracy and dependability of an ELISA assay. BioLegend provides lyophilized recombinant protein standards, optimized for use with our ELISA antibody pairs. Our ELISA standards are formulated with carrier proteins to ensure stability and to prevent the product from sticking to the walls of the vial.

## ELISA Standard Product List (with carrier protein):

| **Human** | | |
|---|---|---|
| APRIL (TNFSF13) | IL-2 | IL-32α |
| CCL5 (RANTES) | IL-4 | IL-33 |
| CCL8 (MCP-2) | IL-5 | IL-34 |
| CCL17 (TARC) | IL-6 | LAP (TGF-β1) |
| CCL28 (MEC) | IL-8 | Latent TGF-β |
| CTLA-4 | IL-9 | LIF |
| CXCL5 (ENA-78) | IL-10 | Mesothelin |
| CXCL10 (IP-10) | IL-12 (p70) | MIF |
| FGF-basic/145aa | IL-12/IL-23 (p40) | Nogo-B |
| GM-CSF | IL-13 | SCF |
| Granulysin | IL-15 | TGF-β1 |
| IFN-γ | IL-17A | TNF-α |
| IL-1α | IL-21 | TSLP |
| IL-1β | IL-22 | |

| **Mouse** | | |
|---|---|---|
| CCL28 (MEC) | IL-5 | IL-27 |
| GM-CSF | IL-6 | IL-33 |
| IFN-β | IL-10 | IL-34 |
| IFN-γ | IL-12 (p70) | Lactadherin |
| IL-1α | IL-12/IL-23 (p40) | Latent TGF-β |
| IL-1β | IL-17A | MCP-1 |
| IL-2 | IL-17A/F | TNF-α |
| IL-3 | IL-22 | TSLP |
| IL-4 | IL-23 | |

| **Rat** |
|---|
| IFN-γ |

# Protocols for Bioassay

The biological activity of our recombinant proteins is routinely measured using a bioassay and indicated with a specific activity range/ED50 on our datasheets. The specific activity of proteins is very much dependent on the cell type and the bioassay used for testing. Our bioassay team, consisting of expert scientists with extensive manufacturing and assay development experience, set-up the right experimental conditions based on the biological function of the protein and test every lot of our recombinant proteins in at least one out of over 20 different types of dose-response bioassays. Here are some example bioassay protocols and ED50 values for of our proteins (this is not an exhaustive list):

**1. Proliferation Assay:** Proliferation of cells induced by increasing concentrations of recombinant proteins is measured by metabolic fluorescence assay using our Deep Blue Cell Viability™ Kit (Cat. No. 424701).

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| Artemin | SHSY5Y cells | 3K/well | 300 ng/mL | 5 days | 4-16 ng/mL |
| BAFF | Mouse B cells | 200K/well | 180 ng/mL | 72 hr | 0.3-2.0 ng/mL |
| BDNF | C6 glioma cells | 2K/well | 30 µg/mL | 4 days | 1-3 µg/mL |
| Betacellulin | BalbC/3T3 cells | 2K/well | 3 ng/mL | 48 hr | 0.04-0.24 ng/mL |
| CD40L | Human B Cells | 100K/well | 2000 ng/mL | 72 hr | 20-100 ng/mL |
| CNTF | TF-1 cells | 25K/well | 12 µg/mL | 72 hr | 30-180 ng/mL |
| EGF | A431 cells | 1.2K/well | 100 ng/mL | 5 days | 1-2 ng/mL |
| EG-VEGF | MIA Paca 2 cells | 1K/well | 50 µg/mL | 72 hr | 1-4 µg/mL |
| EPO | TF-1 cells | 25K/well | 10 ng/mL | 48 hr | 0.1-0.6 ng/mL |
| FGF basic | NIH/3T3 cells | 1.5K/well | 60 ng/mL | 48 hr | 1-4 ng/mL |
| GM-CSF | TF-1 cells | 10K/well | 2 ng/mL | 72 hr | 0.10- 0.30 ng/mL |
| HB-EGF | BalbC/3T3 cells | 2K/well | 30 ng/mL | 48 hr | 0.2-1.2 ng/mL |
| hVEGF165 | HUVEC | 3.6K/well | 300 ng/mL | 48 hr | 1-6 ng/mL |
| IL-1α | D10.G4.1 cells | 25K/well | 900 pg/mL | 48 hr | 5-15 pg/mL |
| IL-1β | D10.G4.1 cells | 20K/well | 1 ng/mL | 48 hr | 5-15 pg/mL |
| IL-1RA | D10.G4.1 cells | 25K/well | 5000 ng/mL | 48 hr | 7-35 ng/mL |
| IL-2 | CTLL2 cells | 25K/well | 20 ng/mL | 48 hr | 0.05-0.3 ng/mL |
| IL-3 | TF-1 cells | 25K/well | 10 ng/mL | 48 hr | ≤ 0.1 ng/mL |
| IL-4 | TF-1 cells | 25K/well | 40 ng/mL | 72 hr | 0.2-0.6 ng/mL |
| IL-6 | 7TD1 cells | 8K/well | 3 ng/mL | 48 hr | 4-20 pg/mL |
| IL-7 | PHA activated PBL | 100K/well | 100 ng/mL | 72 hr | 0.1-0.5 ng/mL |
| IL-9 | M07e cells | 25K/well | 16.67 ng/mL | 72 hr | 0.1-0.5 ng/mL |
| IL-11 | 7TD1 cells | 8K/well | 1000 ng/mL | 48 hr | 4-12 ng/mL |
| IL-13 | TF-1 cells | 20K/well | 300 ng/mL | 48 hr | 1.5-3 ng/mL |
| IL-15 | M07e cells | 25K/well | 600 ng/mL | 72 hr | 2-10 ng/mL |
| IL-33 | D10.G4.1 cells | 25K/well | 12 ng/mL | 48 hr | 0.05-0.25 ng/mL |
| LIF | TF-1 cells | 20K/well | 50 ng/mL | 48 hr | 0.03-0.12 ng/mL |
| M-CSF | M-NFS-60 cells | 20K/well | 100 ng/mL | 48 hr | 0.5-2 ng/mL |
| Oncostatin M | TF-1 cells | 15K/well | 60 ng/mL | 72 hr | 0.5-2.5 ng/mL |
| PDGF-BB | NIH/3T3 cells | 2.5K/well | 450 ng/mL | 48 hr | 10-20 ng/mL |
| S100A8/A9 | Astrocytes | 1K/well | 10 µg/mL | 4 days | 0.15-0.6 µg/mL |
| SCF | TF-1 cells | 20K/well | 600 ng/mL | 72 hr | 3 -12 ng/mL |
| VEGF-121 | HUVEC | 5K/well | 90 ng/mL | 72 hr | 0.5-2.5 ng/mL |

**1. Proliferation Assay:** *(Continued)*

| Mouse Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| BAFF | Mouse B cells | 200K/well | 100 ng/mL | 72 hr | 0.5-3 ng/mL |
| EPO | TF-1 cells | 25K/well | 60 ng/mL | 48 hr | 0.5-2.5 ng/mL |
| FGF basic | NIH/3T3 cells | 1.5K/well | 60 ng/mL | 48 hr | 0.3-2 ng/mL |
| G-CSF | M-NFS-60 cells | 25K/well | 30 ng/mL | 48 hr | 0.5-3 ng/mL |
| IL-1α | D10.G4.1 cells | 25K/well | 900 pg/mL | 48 hr | 1- 5 pg/mL |
| IL-1β | D10.G4.1 cells | 25K/well | 1000 pg/mL | 48 hr | 1-5 pg/mL |
| IL-2 | HT-2 cells | 10K/well | 20 ng/mL | 48 hr | 01-0.4 ng/mL |
| IL-3 | M-NFS-60 cells | 20K/well | 30 ng/mL | 48 hr | 20-100 pg/mL |
| IL-4 | CTLL-2 cells | 25K/well | 400 ng/mL | 48 hr | 0.3-1.8 ng/mL |
| IL-5 | BCL-1 cells | 7.5K/well | 10 ng/mL | 72 hr | 0.03-0.15 ng/mL |
| IL-6 | 7TD1 cells | 6.5K/well | 1000 pg/mL | 72 hr | < 0.01 ng/mL |
| IL-7 | PHA activated PBL | 100K/well | 100 ng/mL | 72 hr | 1.0-5 ng/mL |
| IL-9 | M07e cells | 25K/well | 50 ng/mL | 72 hr | 0.04-0.16 ng/mL |
| IL-10 | MC/9 cells | 20K/mL | 10 ng/mL | 92 hr | 0.1-0.5 ng/mL |
| IL-21 | CTLL-2 cells | 25K/well | 3000 ng/mL | 48 hr | 15-60 ng/mL |
| IL-33 | D10.G4.1 cells | 25K/well | 10 ng/mL | 48 hr | 0.1-0.6 ng/mL |
| IL-34 | M-NFS-60 cells | 20K/well | 4000 ng/mL | 48 hr | 20-30 ng/mL |
| M-CSF | M-NFS-60 cells | 20K/well | 300 ng/mL | 48 hr | 2-6 ng/mL |
| SCF | TF-1 cells | 20K/well | 400 ng/mL | 72 hr | 5-20 ng/mL |
| VEGF-120 | HUVEC | 5K/well | 90 ng/mL | 72 hr | 1-4 ng/mL |
| VEGF-164 | HUVEC | 5K/well | 300 ng/mL | 72 hr | 1-4 ng/mL |

| Rat Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| EPO | TF-1 cells | 20K/well | 60 ng/mL | 48 hr | 0.5-2.5 ng/mL |
| IL-1β | D10.G4.1 cells | 10K/well | 10 ng/mL | 48 hr | ≤ 0.1 ng/mL |
| IL-2 | CTLL-2 cells | 25K/well | 30 ng/mL | 48 hr | 0.102-0.295 ng/mL |
| IL-3 | M-NFS-60 cells | 25K/well | 400 ng/mL | 48 hr | 1-5 ng/mL |
| IL-13 | TF-1 cells | 20K/well | 300 ng/mL | 72 hr | 0.8-5.0 ng/mL |
| M-CSF | M-NFS-60 cells | 20K/well | 400 ng/mL | 48 hr | 2.5-12.5 ng/mL |
| SCF | TF-1 cells | 20K/well | 4000 ng/mL | 72 hr | 10-40 ng/mL |
| TPO | M07e cells | 20K/well | 14.16 ng/mL | 72 hr | 0.08-0.4 ng/mL |
| VEGF | HUVEC | 3.6K/well | 800 ng/mL | 48 hr | 0.6-3.6 ng/mL |

**2. Inhibition of Proliferation Assay:** This assay detects the inhibition of cell proliferation with increasing concentration of the recombinant protein using Deep Blue Cell Viability™ Kit (Cat. No. 424701).

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| TGF-β1 | HT-2 cells | 25K/well | 30 ng/mL | 48 hr | 0.05-0.2 ng/mL |
| TGF-β2 | HT-2 cells | 25K/well | 270 ng/mL | 48 hr | 1-4 ng/mL |
| TGF-β3 | HT-2 cells | 25K/well | 6 ng/mL | 48 hr | 0.10-0.4 ng/mL |

| Mouse Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| EGF | A431 cells | 1.2K/well | 100 ng/mL | 5 days | 1-2 ng/mL |
| LIF | Myeloid leukemia M1 cells | 10K/well | 10 ng/mL | 72 hr | < 0.05 ng/mL |

**3. Cytotoxicity Assay:** Cytotoxic effects of increasing concentration of the recombinant protein is measured by fluorescence assay using our Deep Blue Cell Viability™ Kit (Cat. No. 424701).

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| IFN-γ | HT-29 cells | 3K/well | 500 ng/mL | 72 hr | 0.3-2.0 ng/mL |
| TNF-α | L929 cells | 6K/well | 10 ng/mL | 24 hr | 0.020-0.10 ng/mL |
| TNF-β | L929 cells | 6K/well | 10 ng/mL | 24 hr | ≤0.05 ng/mL |
| TRAIL | L929 cells | 6K/well | 300 ng/mL | 24 hr | 4-16 ng/mL |

| Mouse Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| TNF-α | L929 cells | 6K/well | 3 ng/mL | 24 hr | 0.004-0.020 ng/mL |

| Rat Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| EGF | A431 cells | 1.2K/well | 100 ng/mL | 5 days | 0.4-2 ng/mL |
| TNF-α | L929 cells | 6K/well | 3 ng/mL | 24 hr | 5- 15 pg/mL |

**4. Cytokine Induction Assay:** Detection of induced cytokines in the cell by increasing concentrations of the recombinant protein is detected by cytokine-specific ELISA assay.

| Human Target Cytokine | Readout | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|---|
| HMGB1 | Induction of mTNF-α | RAW264.7 cells | 6K/well | 40 µg/mL | 4 days | 3.0-15 µg/mL |
| IL-12 | Induction of IFN-γ | Activated PBMC | 100K/well | 10 ng/mL | 48 hr | 0.05-0.25 ng/mL |
| IL-17A | Induction of IL-6 | Human skin fibroblasts | 2.5K/well | 100 ng/mL | 48 hr | 2-4 ng/mL |
| IL-17A/F | Induction of IL-6 | Human neonate fibroblasts | 2.5K/well | 3200 ng/mL | 48 hr | 15 -25 ng/mL |
| IL-17F | Induction of CXCL1 | Human skin fibroblasts | 2.5K/well | 10000 ng/mL | 48 hr | 400-800 ng/mL |
| IL-21 | Induction of IFN-γ | NK-92 cells | 100K/well | 40 ng/mL | 18 hr | 0.3-0.5 ng/mL |
| IL-22 | Induction of IL-10 | Colo-205 cells | 20K/well | 6 ng/mL | 48 hr | 0.062-0.177 ng/mL |
| IL-23 | Induction of mouse IL-17A | Mouse splenocytes | 200K/well | 180 ng/mL | 72 hr | 0.4-2.0 ng/mL |
| IL-27 | Inhibition of IL-2 production | Activated PBMC | 200K/well | 1000 ng/mL | 48 hr | 30-150 ng/mL |
| IL-34 | Induction of MCP-1 | PBMC | 200K/well | 400 ng/mL | 24 hrs | 20 -80 ng/mL |

| Mouse Target Cytokine | Readout | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|---|
| FLT3L | Induction of IL-6 | Myeloid leukemia M1 cells | 100K/well | 1000 ng/mL | 48 hr | 5.0-25.0 ng/mL |
| IL-12 | Induction of mIFN-γ | Mouse splenocytes | 100K/well | 10 ng/mL | 48 hr | 0.10-0.20 ng/mL |
| IL-17A | Induction of IL-6 | Fetal mouse skin fibroblasts | 2.5K/well | 100 ng/mL | 48 hr | 0.25-1 ng/mL |
| IL-17A/F | Induction of IL-6 | Fetal mouse skin fibroblasts | 2.5K/well | 3750 ng/mL | 48 hr | 125-175 ng/mL |
| IL-17F | Induction of CXCL1 | Fetal mouse skin fibroblasts | 2.5K/well | 5000 ng/mL | 48 hr | 300-600 ng/mL |
| IL-22 | Induction of hIL-10 | Colo-205 cells | 20K/well | 6 ng/mL | 48 hr | 0.062-0.177 ng/mL |
| IL-23 | Induction of mIL-17A | Mouse splenocytes | 200K/well | 100 ng/mL | 72 hr | ≤1.5 ng/mL |

**5. Inhibition of Cytokine Production Assay:** Inhibition of cytokine production by the cells is measured by ELISA in the presence of increasing concentrations of the recombinant protein.

| Human Target Cytokine | Readout | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|---|
| IL-10 | Inhibition of IFN-γ | PHA activated PBMC | 200K/well | 10 ng/mL | 72 hr | 0.1-0.3 ng/mL |
| IL-27 | Inhibition of IL-2 production | Activated PBMC | 200K/well | 1000 ng/mL | 48 hr | 30-150 ng/mL |

**6. RBC Agglutination Assay:** Agglutination of RBCs is a simple visual assay using different titrations of the recombinant proteins.

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| Galectin 1 | Red blood cells | 2% | 25 µg/mL | 1 hr | < 5.0 µg/mL |
| Galectin 3 | Red blood cells | 2% | 25 µg/mL | 1 hr | 1.0-4.0 µg/mL |
| Galectin 4 | Red blood cells | 2% | 50 µg/mL | 1 hr | 6.25 µg/mL |
| Galectin 9 | Red blood cells | 2% | 25 µg/mL | 1 hr | 0.5-2.0 µg/mL |

**7. Binding Assay:** Binding of receptor-ligands is measured by functional ELISA, and binding of proteins-cells is determined by fluorescence assay.

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| EphA2 | Ephrin-A1 binding | NA | 2 µg/mL | 24 hr | 3-12 ng/mL |
| Ephrin-A1 | EphA2 binding | NA | 300 ng/mL | 24 hr | 3-12 ng/mL |
| GDNF | GFRa1 binding | NA | 0.5 µg/mL | 24 hr | 10-50 ng/mL |
| Glypican-1 | FGF-basic binding | NA | 2 µg/mL | 6 hr | 2-8 ng/mL |
| Glypican-3 | FGF-basic binding | NA | 2 µg/mL | 6 hr | 3-12 ng/mL |
| Neurturin | GFRa2 binding | NA | 0.5 µg/mL | 24 hr | 20-80 ng/mL |
| Vitronectin | HUVEC | 30K/well | 3 µg/mL | 45 min | 0.03-0.12 µg/mL |

| Mouse Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| Cadherin-13 | HUVEC | 30K/well | 30 µg/mL | 1 hr | 1-5 µg/mL |
| NOV | BalbC/3T3 cells | 40K/well | 30 µg/mL | 1 hr | 0.2-0.8 µg/mL |
| Ephrin-A1 | EphA2 binding | NA | 300 ng/mL | 24 hr | 3-12 ng/mL |
| Glypican-1 | FGF-basic binding | NA | 2 µg/mL | 6 hr | 3-12 ng/mL |

**8. Differentiation Assay:** Differentiation of cells by varying concentrations of the protein is measured by alkaline phosphatase induction assay.

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| PTH | MC3T3-E1 cells | 5K/well | 1 µg/ml | 72 hr | 10-60 ng/mL |
| SHH | C3H10T1/2 cells | 5K/well | 10 µg/mL | 72 hr | 0.15-0.6 µg/mL |

| Mouse Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| SHH (C25II) | C3H10T1/2 cells | 5K/well | 10 µg/mL | 3 days | 0.2-1 µg/mL |
| SHH (native) | C3H10T1/2 cells | 5K/well | 30 µg/mL | 3 days | 1-5 µg/mL |

**9. Neurite Assay:** Neurite outgrowth or inhibition is determined by checking the morphology of neurons in the presence of varying concentrations of the protein.

| Human Target Cytokine | Readout | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|---|
| Midkine | Neurite outgrowth | E18 rat embryonic cortical neuron | 20K/well | 10 µg/mL | 48 hr | 0.4 µg/mL |
| OMGp | Neurite inhibition | E13 chick DRG neurons | 6K/well | 600 ng/3 µL droplet | 48 hr | 150 ng/3 µL droplet |
| Slit2-N | Neurite outgrowth | E18 rat embryonic cortical neuron | 20K/well | 10 µg/mL | 48 hr | 2.5 µg/mL |
| TAFA-2 | Neurite outgrowth | E18 rat embryonic cortical neuron | 20K/well | 30 µg/mL | 48 hr | 2 µg/mL |

**9. Neurite Assay:** *(Continued)*

| Mouse Target Cytokine | Readout | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|---|
| OMGp | Neurite inhibition | E13 chick DRG neurons | 6K/well | 600 ng/3 µL droplet | 48 hr | 150 ng/3 µL droplet |

**10. Enzymatic Assays:** Activity of the enzyme or inhibitor is measured by its ability to cleave a fluorogenic or chromogenic substrate in the presence of increasing concentrations of the protein.

| Inhibitor | Readout (Ex/Em nm) | Target Enzyme | Target Enzyme concentration | Top concentration | Incubation Time (minutes) | IC50 |
|---|---|---|---|---|---|---|
| hCystatin C | 380/460 | Papain | 50 ng/mL | 5.0 µg/mL | 10 | 45 ng/mL |
| hElafin | 380/460 | Human Neutrophil Elastase | 100 ng/mL | 400 ng/mL | 30 | 17 ng/mL |
| hSerpin A12 | 320/405 | Human KLK7 | 1000 ng/mL | 16.0 µg/mL | 60 | 1.0 µg/mL |
| hSerpin E1 | 380/460 | Human uPA | 100 ng/mL | 2.5 µg/mL | 30 | 230 ng/mL |
| hSLPI | 380/460 | Human Neutrophil Elastase | 100 ng/mL | 400 ng/mL | 30 | 30 ng/mL |
| hTFPI | 320/405 | Bovine Trypsin | 10 ng/mL | 128 ng/mL | 30 | 7.0 ng/mL |

| Enzyme | Readout (Ex/Em nm) | Target Substrate (µM) | Top concentration | Top Specific Activity (pmol/µg/min) |
|---|---|---|---|---|
| hBACE1 | 320/405 | Mca-SEVNLDAEFRK(Dnp)RR-NH2 | 20.0 µg/mL | 16 |
| hKLK-3 | 380/460 | MeO-Suc-RPY-AMC | 4.8 µg/mL | 300 |
| hMMP-2 | 320/405 | Mca-PLGL-Dpa-AR-NH2 | 400 ng/mL | 2200 |
| hMMP-10 | 320/405 | Mca-RPKPVE-Nval-WRK(Dnp)-NH2 | 1.0 µg/mL | 400 |
| huPA | 380/460 | Bz-GGR-AMC | 400 ng/mL | 5200 |

**11. Chemotaxis Assays:** The ability of the recombinant protein to induce chemotaxis is measured by counting the cells by FACS, or by Deep Blue Cell Viability™ Kit (Cat. No. 424701) to detect cell migration.

| Human Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| CCL1 (I-309) | BW.5147.3 | 50K | 800 ng/mL | 2 hr | 0.5–2.5 ng/mL |
| CCL3 (MIP-1α) | BaF3-hCCR5 | 50K | 20 ng/mL | 2 hr | 1-10 ng/mL |
| CCL5 (RANTES) | T cells | 500K | 800 ng/mL | 3 hr | 5-15 ng/mL |
| CCL11 (Eotaxin) | BaF3-hCCR3 | 50K | 400 ng/mL | 2 hr | 0.25-1.5 ng/mL |
| CCL17 (TARC) | BaF3-hCCR4 | 50K | 100 ng/mL | 2 hr | 0.2 -1.2 ng/mL |
| CCL20 (MIP-3α) | BaF3-hCCR6 | 50K | 50 ng/mL | 2 hr | 0.4-2 ng/mL |
| CXCL8 (IL-8) | human neutrophils | 150K | 50 ng/mL | 1 hr | 1-5 ng/mL |
| CXCL9 (MIG) | BAF3-hCXCR3 | 150K | 1 µg/mL | 2 hr | 0.4-0.8 µg/mL |
| CXCL10 (IP-10) | Human T cells | 150K | 3 µg/mL | 2 hr | 30-180 ng/mL |
| CXCL11 (ITAC) | BAF3-hCXCR3 | 50K | 6 µg/mL | 2 hr | 1-5 ng/mL |
| CXCL12 (SDF-1α) | Human T cells | 500K | 500 ng/mL | 3 hr | 80-120 ng/mL |

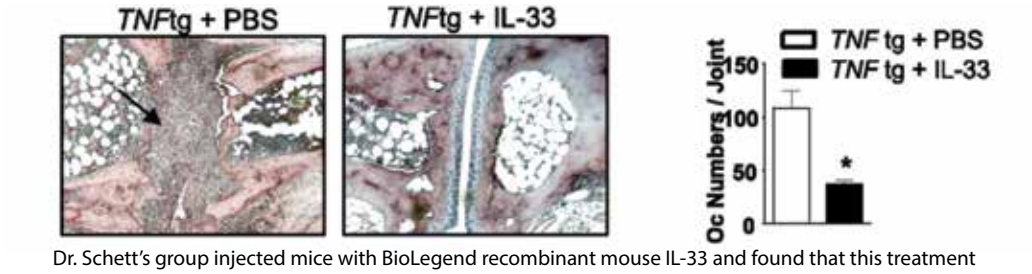
| Mouse Target Cytokine | Target Cell Line/Binding Partner | Cell Number | Top Concentration | Incubation Time | ED50 |
|---|---|---|---|---|---|
| CCL3 (MIP-1α) | BaF3-hCCR5 | 495K | 150 ng/mL | 3 hr | 1-5 ng/mL |
| CCL6 (C10) | BaF3-hCCR1 | 450K | 500 µg/mL | 2 hr | 0.2 - 1 µg/mL |
| CCL9 (MIP-1γ) | BaF3-hCCR1 | 50K | 1000 ng/mL | 2 hr | 2 - 10 ng/mL |
| CCL12 (MCP-5) | THP-1 | 200K | 100 ng/mL | 3 hr | 1 -5 ng/mL |
| CCL19 (MIP-3β) | BaF3-hCCR7 | 50K | 10 ng/mL | 2 hr | 0.25-1.25 ng/mL |
| CCL20 (MIP-3α) | BaF3-hCCR6 | 50K | 400 ng/mL | 1.5 hr | 0.25-1.5 ng/mL |
| CXCL9 (MIG) | BAF3-hCXCR3 | 50K | 10 ng/mL | 2 hr | 8.5 ng/mL |
| CXCL12 (SDF-1α) | BAF3-hCXCR4 | 50K | 100 ng/mL | 2 hr | 0.15–0.6 ng/mL |

**For detailed information about these or any other protocols, please contact: tech@biolegend.com**

# Researcher Spotlight

## Dr. George Schett

Photo: Dr. H.E. Langer



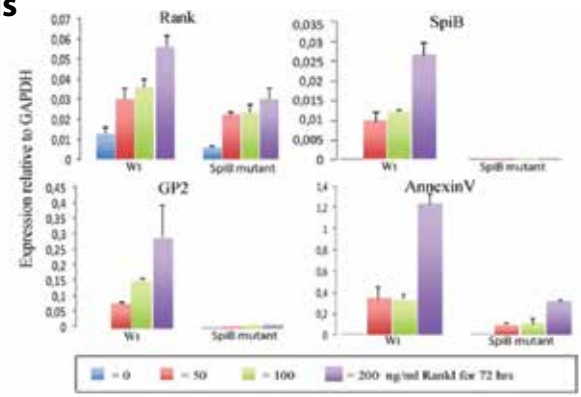

Dr. Schett's group injected mice with BioLegend recombinant mouse IL-33 and found that this treatment prevented differentiation of cells into inflammatory osteoclasts (Oc) and bone loss.
Zaiss, M.M. *et al.* 2011. *J. Immunol.* 186:6097.

"I am currently the Professor of Internal Medicine and Chairman of the Department of Internal Medicine 3 at the University Erlangen-Nuremberg. My research focuses on basic, translational, and clinical forms of research of several autoimmune diseases. BioLegend's recombinant mouse IL-33 allowed us to analyze its effects on prevention of bone destruction."

-Dr. George Schett, University Erlangen-Nuremberg

## Dr. Edward Nieuwenhuis

The Lab of Dr. Edward Nieuwenhuis (third from the right)



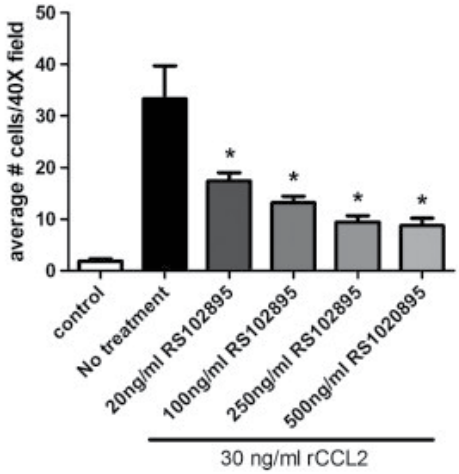

Dr. Nieuwenhuis's group utilized BioLegend recombinant mouse RANKL to stimulate wild-type or SpiB mutant minigut organoid cultures to detect gene expression of RANK, SpiB, GP2, and Annexin V.
de Lau, W., *et al.* 2012. *Mol. Cell. Biol.* 32:3639.

"Our lab of pediatric gastroenterology focuses on the immune system of the gut and different enteropathies. By using BioLegend's recombinant mouse RANKL, we successfully established a culture system for large amounts of M-cells, a rare cell type in the small intestine which plays an important role in immune homeostasis of the gut. This culture system gives us the unique opportunity to further study the differentiation and function of M-cells in health and disease."

-Dr. Edward Nieuwenhuis, University Medical Centre Utrecht

## Dr. Steven Dow

Photo: College of Veterinary Medicine & Biomedical Sciences



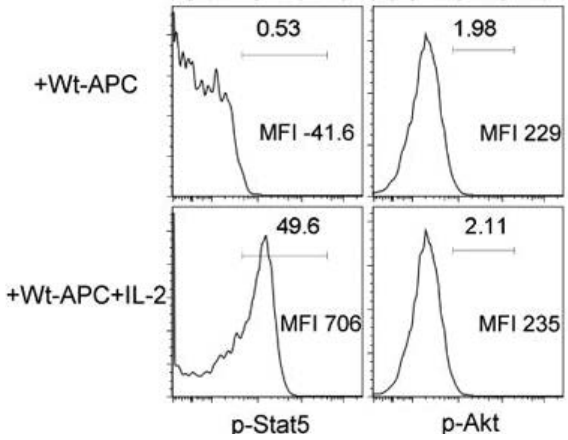

Dr. Dow's group measured the ability of an antagonistic drug (RS102895) to prevent chemotaxis induced by BioLegend recombinant mouse CCL2.
Mitchell, L.M. *et al.* 2013. *Intl Immupharmacol.* 15:357.

"Our lab uses BioLegend mouse rCCL2 in monocyte migration assays. These studies are done with Boyden chambers and are used to identify novel compounds or repurposed drugs that can be applied *in-vivo* for vaccine enhancement and cancer therapeutics."

-Dr. Steven Dow, Colorado State University

## Dr. Xian C. Li

Dr. Xian C. Li (Left).




Dr. Li's group stimulated mouse Tregs with BioLegend recombinant mouse IL-2 and measured Stat5 and Akt phosphorylation through flow cytometry. Xiao, X. *et al*. 2012. *J. Immunol.* 188: 892.

"We are interested in IL-2 and IL-15 and how such cytokines regulate different facets of T cells, particularly Tregs and memory T cells. The proteins from BioLegend are instrumental in our endeavor in this area."

-Dr. Xian C. Li, Harvard Medical School

## Dr. Tatiana Scorza

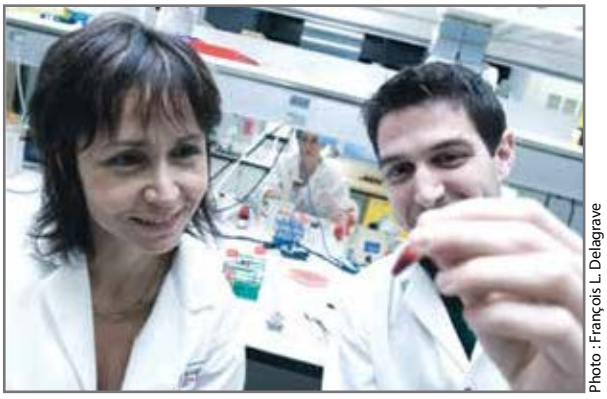
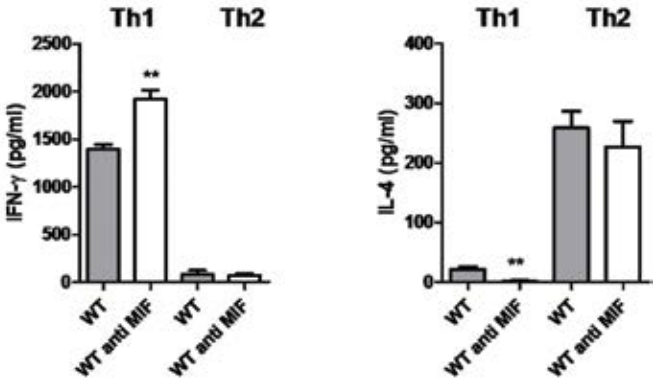


Photo : François L. Delagrave

Dr. Tatiana Scorza (Left)




Dr. Scorza's group tested the effect of neutralization of migration inhibitory factor on production of hallmark Th1 and Th2 cytokines by cells polarized with BioLegend recombinant proteins and antibodies. Malu, D.T. *et al.* 2011. *J. Immunol.* 186:6271.

"Our lab is focused on studying the interaction between malaria parasites and the immune system, particularly the induction of immunosuppressive factors in macrophages following contact with Plasmodium or with damaged red blood cells. BioLegend has provided numerous recombinant proteins which have allowed us to explore the mechanism of the host response to malarial infection."

-Dr. Tatiana Scorza, Université du Québec à Montréal

# Frequently Asked Questions

## How does the activity of your recombinant proteins compare to competitors?

We quality control each and every lot of recombinant protein. Not only do we check its bioactivity, but we also compare it against other commercially available recombinant proteins. We make sure each recombinant protein's activity is at least as good as or better than the competitor's. In order to provide you with the best possible product, we ensure that our testing process is rigorous and thorough. If you're curious and eager to make the switch to BioLegend recombinants, contact **sales@biolegend.com** today!

## What is the specific activity or ED50 of my recombinant protein?

The specific activity range of the protein is indicated on the product datasheets. Because the exact activity values on a per unit basis can largely fluctuate depending on a number of factors, including the nature of the assay, cell density, age of cells/passage number, culture media used, and end user technique, the specific activity is best defined as a range and we guarantee the specific activity of all our lots will be within the range indicated on the datasheet.

## Does specific activity of a recombinant protein vary between lots?

Specific activity will vary for each lot and for the type of experiment that is done to validate it, but all passed lots will have activity within the established ED50 range for the product and we guarantee that our products will have lot-to-lot consistency. Please do your own experiment specific validations to find out the optimal ED50 for your specific system.

## How do you convert activity as an ED50 in ng/mL to a specific activity in Units/mg?

Use the formula Specific activity (Units/mg) = $10^6$/ED50 (ng/mL)

## What is the difference between carrier-free and non carrier-free recombinant proteins?

All our carrier-free and animal-free formats of recombinant proteins do not have any additional carrier proteins such as BSA in the formulation. Typically our ELISA standard recombinants have carrier proteins added to the formulation for added stability and to avoid the product from sticking to the wall of the vial. When the presence of carrier is not desirable (e.g., *in-vivo* applications), carrier-free proteins can be used directly. When carrier proteins do not affect the outcome in a study, the customer can decide what type of carrier protein they would like to use and whether it is necessary to add it to their stock.

## How are BioLegend's recombinant proteins shipped?

All our recombinants are shipped on blue ice. These products have been validated to maintain activity after shipping using blue ice.

## What is the difference between carrier-free and animal-free categories of recombinant proteins?

Our animal-free products go through the entire production process without touching any animal-containing components. This includes using animal-free media and purification equipment. Studies which are particularly sensitive to contamination by mammalian pathogens may require the use of animal-free products. Our carrier-free products do not contain any carrier protein, but they are produced using animal-containing components. Both versions are expected to have similar activity and function, though specific activity is lot-dependent.

## What should I reconstitute the protein with? What do you recommend for its long-term storage?

Most of our carrier-free recombinants are shipped in liquid form, so no need for reconstitution. Our animal-free recombinant proteins are shipped in lyophilized form and protein reconstitution information is indicated on the respective datasheets. If you need to make dilutions, refer to the formulation on the product data sheet. Stock solutions should be prepared at 50-100 µg/mL in buffer containing carrier protein such as 1% BSA or HSA or 10% FBS (for chemokines, use either BSA or HSA). For long-term storage, aliquot into polypropylene vials and store in a manual defrost freezer. Avoid repeated freeze/thaw cycles.

## Do you test the bioactivity of your recombinant proteins with *in-vivo* assays?

We typically validate the activity of the proteins with *in-vitro* assays as described on the data sheet and not with *in-vivo* testing. However, all our carrier-free and animal-free formats can be used for *in-vivo* applications as is evident from many customers who have successfully used it for this purpose.

## Are BioLegend's recombinant proteins suitable for *in-vitro* and *in-vivo* bioassays?

BioLegend's recombinant protein solutions are 0.2 µm-filtered prior to bottling by membrane filtration method. In addition, our recombinants are endotoxin tested and are guaranteed to have levels less than 0.1 ng per µg protein. All our recombinants can be safely used for both *in-vitro* and *in-vivo* bioassays.

## What is the difference between laboratory (observed) units and international units?

There is no direct relationship between International Units and the units that are calculated using the inverse of the specific activity because we do not use the International Standard provided by WHO (National Institute for Biological Standards and control). The best way to compare the activity of two sources of recombinants is by doing the bioassay side by side using the same system.

# References Using BioLegend Recombinant Proteins

## Human

**CXCL10**

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2. Ramirez, L. A., *et al.* 2014 *J. Leukoc. Biol* 96:1055.

**GM-CSF**

1. Chalubinsk, M., *et al.* 2014. *Food Chem Toxicol.* 69:289.
2. Byrd, D., *et al.* 2014. *J. Virol.* 88:6819.

**FGF-basic/145aa**

1. Wang, J., *et al*. 2013. *Biochem Biophys Res Commun.* 41:143.

**IFN-γ**

1. Meissner, T.B., *et al*. 2012. *J. Immunol*. 188:4951.
2. Meissner, T.B., *et al*. 2010. *PNAS*. 107:13794.

**IL-2**

1. Wingender, G., *et al*. 2011. *J. Exp. Med.* 208:1151.
2. Wang, N., *et al*. 2010. *J. Immunol.* 185:5683.

**IL-4**

1. Chalubinsk, M., *et al.* 2014. *Food Chem Toxicol.* 69:289.

**IL-8**

1. Popova T, *et al*. 2016. *PLoS One*. 11: 0163163.
2. DuMont A, *et al*. 2015. *Infect. Immun.* 83: 3825 - 3837.

**M-CSF**

1. Nijaguna M, *et al*. 2015. *J. Biol. Chem.* 290: 23401-23415.
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