AdipoGen®
LIFE SCIENCES
insights

Proteins with Enhanced Activity & Stability

AdipoGen Life Sciences specializes in the development of recombinant proteins that show **enhanced activity and long half-life**, produced in **mammalian cells**. Using proprietary in-house technologies together with established and published technologies, we engineer, develop and produce an innovative panel of recombinant proteins for different research fields including **Cell Therapy Research**.

The development of recombinant proteins is performed using different production expression systems, including the mammalian cell lines CHO and HEK 293. The mammalian cell lines allow AdipoGen Life Sciences to produce exogenous recombinant proteins secreted into the cell medium with post-translational modifications and glycosylations closer to endogenous proteins compared to productions in *E. coli*. However, for some difficult-to-produce in mammalian cells proteins, we also use bacteria (*E. coli*) and/or insect cells as a host. AdipoGen Life Sciences' first goal is to provide **high purity**, **low endotoxin** and **biologically active** recombinant proteins, leveraging its in-house protein technologies.

Recombinant proteins are used throughout the life science academia and industry in a plethora of applications. Their use ranges from cell culture to bioprocessing and advanced cell and gene therapy. A defined composition of the cell culture medium to guarantee batch-to-batch reproducibility has become inevitable. The development of **animal-component-free** recombinant proteins eliminates the risk of contamination that has been associated with animal serum-derived media.

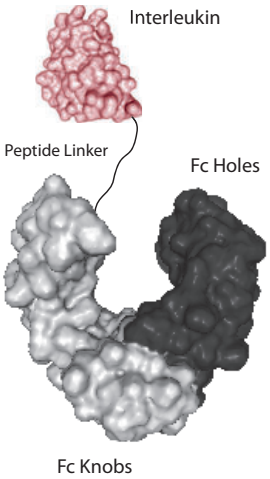


FIGURE: Schematic structure of a **Fc-KIH construct** with a monomeric cytokine.

Ask for

- ***BULK Quantities!***
- ***Animal-component-free Production!***

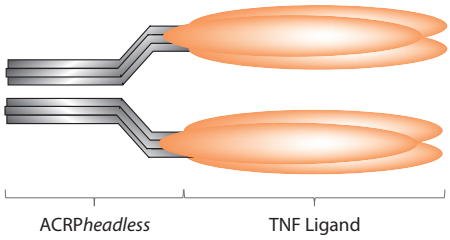
https://adipogen.com/enhanced-proteins

CONTENTS

TNF Ligands Multimeric Proteins

- Endogenous TNF superfamily ligands are either active as membrane-form (e.g. FasL, TRAIL, CD40L, OX40L) or are secreted and activated through oligomerization by the binding of proteoglycans at the surface of cells (e.g. APRIL).
- *Multimeric*Ligands™ are constructs in which two trimeric TNFSF ligands are linked via the oligomeric collagen domain of Adiponectin [ACRP30*headless*].
- *Multimeric*Ligands™ mimic the membrane-bound forms of the proteins and show high activity.
- For *in vitro* and *ex vivo* studies!

Hexamer Structure



PRODUCT NAME	PID	HOST	APPLICATIONS
APRIL (human) (multimeric) (rec.)	AG-40B-0017	HEK 293 cells	Stimulates B cell proliferation.
APRIL (human) (H98) (multimeric) (rec.)	AG-40B-0088	CHO cells	Stimulates B cell proliferation. Does not bind to proteoglycans.
CD40L (human) (multimeric) (rec.)	AG-40B-0010	CHO cells	Potent B cell and T cell expansion tool for Cell Therapy Application. Accelerates *ex vivo* TIL expansion for Immunotherapy.
CD40L (mouse) (multimeric) (rec.)	AG-40B-0020	CHO cells	Potent B cell expansion tool.
FasL (human) (multimeric) (rec.)	AG-40B-0130	HEK 293 cells	Potent apoptosis inducer in human T cells.
OX40L (mouse) (multimeric) (rec.)	AG-40B-0029	HEK 293 cells	Activates T cell proliferation.
TNF-α (human) (multimeric) (rec.)	AG-40B-0019	HEK 293 cells	Activates human and mouse TNF-R1 and TNF-R2.

*Multimeric*CD40L – Potent B Cell & T Cell Expansion Reagent

B cells contribute to immune regulation or act as potent antigen-presenting cells. CD40-activated B cells activate and expand naïve and memory $CD4^+$ and $CD8^+$, inducing specific T cell responses *in vitro* and *in vivo*. In contrast to dendritic cells, the generation of highly pure CD40-activated B cells is simple and time efficient. Normal or tumor-infiltrated **B cells can be activated efficiently using AdipoGen's *Multimeric*CD40L.**

LIT: Stimulation of tumor infiltrating B-cells improves ex-vivo TIL expansion for melanoma immunotherapy: R. Rosseti, et al.; J. ImmunoTherapy Cancer, Abstract **195** (2021) • Sensitive identification of neoantigens and cognate TCRs in human solid tumors: M. Arnaud, et al.; Nat. Biotechnol. **40,** 656 (2022)

CD40L (human) (multimeric) (rec.)
AG-40B-0010 Research Grade 10 µg | 3 x 10 µg

CD40L (human) (multimeric) (rec.) (Certified Serum Grade)
AG-40B-0010CSG Certified Serum Grade 100 µg

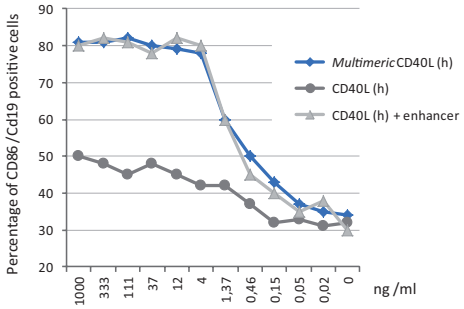


FIGURE: CD40L (human) (multimeric) (rec.) (Prod. No. AG-40B-0010) does not need an enhancer to induce B cell activation.

AdipoGen® LIFE SCIENCES

Improving *ex vivo* T Cell Expansion for Cellular Immunotherapy

With the continuous development of tumor biology and immunology, immune cell therapy has developed into an exciting new field of tumor treatment. Adoptive T cell transfer (ACT) is a type of T cell therapy in which T cells are isolated from a patient's blood or tumor tissue, expanded and activated *ex vivo*, and then re-infused back into the patient to target and eliminate cancer cells. T cells can be engineered to express **chimeric antigen receptors (CARs)** or **T cell receptors (TCRs)** that recognize tumor-specific antigens, or they can be expanded to enrich for **tumor-infiltrating lymphocytes (TILs)** that have natural tumor-specific reactivity.

The most widely used tumor-infiltrating lymphocyte (TIL) production method is to isolate infiltrating lymphocytes from tumor tissues and then culture and expand these cells *in vitro*. The patient's tumor cells can then interact with the enlarged TIL cells to screen effector TIL cells that can kill tumor cells. Dendritic cells or B cells loaded with tumor-specific antigen are used for further amplification, to cultivate and improve tumor-specific TIL. **B cells that are present together with TILs and activated with AdipoGen's *Multimeric*CD40L have been associated with improved clinical responses to cellular immunotherapy.** Finally, cells are transfused back into patients for treatment. Production of human T cells for cell therapy is a complex, multi-step process, including T cell isolation, activation and expansion. There are many opportunities for optimization to obtain maximum yield while retaining desired end phenotype and function. There are many important growth factors that can influence the effectivity of TIL (or other T cells) expansion as indicated below.

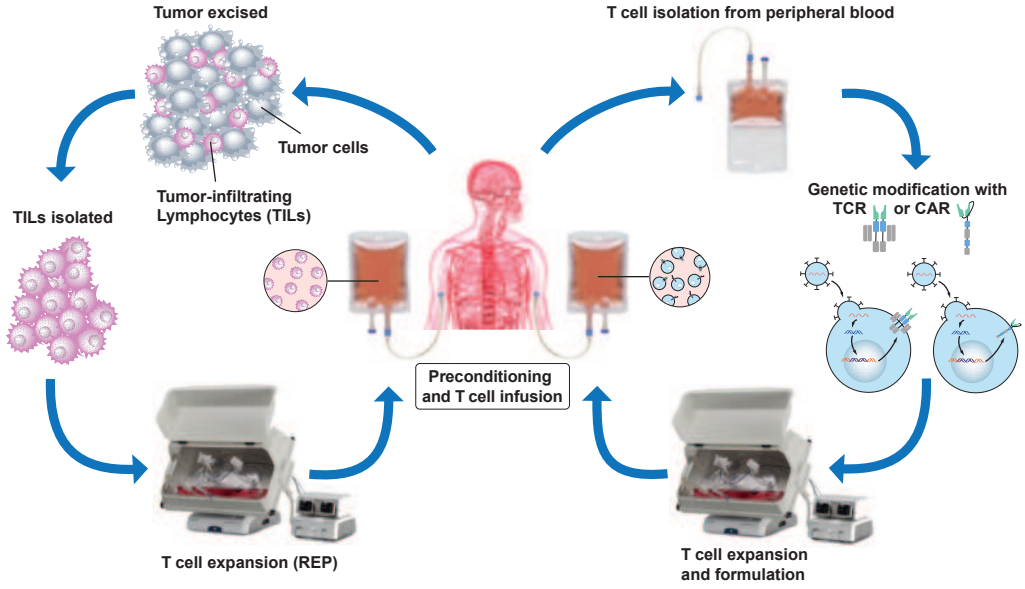


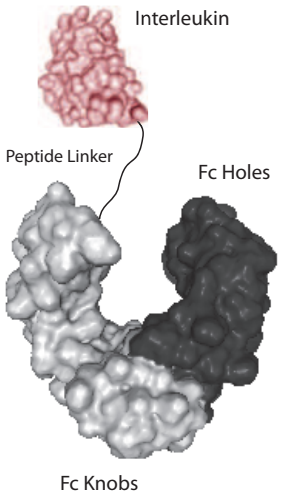
FIGURE: Adaptive T cell (ACT) workflow.

Standard TIL Expansion Proteins

- Next to CD40L, there are several cytokines and growth factors that can be used for activation and expansion of TIL (tumor-infiltrating lymphocyte) cells in *ex vivo* TIL cell therapy.
- **Interleukin-2** (IL-2) is critical for activation and proliferation of T cells, commonly used in **TIL cell therapy to promote T cell expansion**.
- **Interleukin-7** (IL-7) is used for ***ex vivo* T cell expansion**. T cells can be expanded and maintained *in vitro* for longer periods of time, while retaining their effector function and ability to target cancer cells.
- **Interleukin-15** (IL-15) promotes T cell activation and proliferation and has been shown to be effective in **expanding TILs with memory phenotype**.
- **Interleukin-21** (IL-21) promotes the differentiation and expansion of effector T cells and has been shown to **enhance the antitumor activity of TILs** in preclinical models.
- **CD137L** [4-1BB ligand] is a co-stimulatory molecule that binds to the CD137 receptor on T cells and promotes T cell activation and proliferation. CD137L has been shown to **enhance the antitumor activity of TILs** in preclinical models.

Monomeric Interleukins Fused to Fc (KIH-Technology)

- The Knobs-Into-Holes (KIH) strategy is a pioneering format, permitting Fc heterodimerization to create an "IgG with two different arms".
- The KIH technology can be used for antibodies as well as protein engineering.
- AdipoGen Life Sciences developed various interleukins (e.g. IL-2 and IL-38) into an Fc-fusion protein using the KIH technology.
- AdipoGen´s KIH proteins are Fc-fusion monomeric (see Figure) with substantially improved pharmacokinetics (PK) due to the Fc, while maintaining cytokine activity (since monomeric).
- These special monomeric Fc-constructs are produced **in mammalian cells** with **low endotoxin** content.
- For *in vitro* and *in vivo* studies!



LIT: Knobs-into-Holes (KIH) Technology: J.B. Ridgway, et al.; Protein Eng. **9,** 617 (1996)

PRODUCT NAME	PID	HOST	APPLICATIONS
IL-2 (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0224	HEK 293 cells	Activates T cell activation (CD4/CD8) and T reg (not wanted for Immunotherapy).
NEW IL-2 (human) (Switch-2) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0234	HEK 293 cells	Triggers $CD8^+$ T cell effector function more potently at acidic pH (tumor) than at neutral pH.
IL-2 (mouse) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0225	HEK 293 cells	Activates T cell activation (CD4/CD8) and T reg (not wanted for Immunotherapy).
IL-2 Superkine (monomeric):Fc-KIH (human) (rec.)	AG-40B-0222	HEK 293 cells	Induces T cell proliferation (CD4/CD8) (not T reg). Superior expansion of cytotoxic $CD8^+$ T cell and NK, leading to improved antitumor response *in vivo* compared to human IL-2 WT.
IL-2 Superkine H9T (monomeric):Fc-KIH (human) (rec.)	AG-40B-0223	HEK 293 cells	Helps to maintain activated $CD8^+$ T cells in a stem-cell-like state, with greater antitumor activity (*in vivo*, mice).
NEW IL-7 (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0238	HEK 293 cells	Binds to IL-7R expressed on the surface of T cells and stimulates their growth and survival.
NEW IL-23 (mouse):Fc-KIH (human) (rec.)	AG-40B-0235	CHO cells	IL-23 induces Th1 cell differentiation and activation of the antigen-presenting functions of dendritic cells.
NEW IL-27 (mouse):Fc-KIH (human) (rec.)	AG-40B-0236	CHO cells	Promotes NK and T cell proliferation and inhibits Th2 and Th17 cell activities.
NEW IL-33 (oxidation resistant) (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0233	HEK 293 cells	Activates ILC2 *in vivo*. The mutations protect IL-33 from oxidation and preserve its activity. IL-33 facilitates Treg expansion.
IL-38 (aa 20-152) (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0226	HEK 293 cells	Anti-inflammatory activity in A549 cells.
IL-38 (aa 3-152) (mouse) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0227	HEK 293 cells	Anti-inflammatory activity in A549 cells.
Fc-KIH (human) IgG1 Control (rec.)	AG-35B-0015	HEK 293 cells	Negative Control KIH construct.

Potent Monomeric IL-2 Superkine Proteins

The IL-2 Superkine has been shown to induce T cell (CD4/CD8) proliferation (not T reg), leading to superior expansion of cytotoxic CD8 T cells and NK cells, and consequently to improved antitumor response *in vivo* compared to human IL-2 WT. **IL-2 Superkine (monomeric):Fc-KIH** is much more potent compared to IL-2 Superkine:Fc (dimeric), and **equivalent or better than the Gold Standard IL-2 Aldesleukin**, as shown in the Figure below.

IL-2 Superkine (monomeric):Fc-KIH (human) (rec.)
AG-40B-0222 10 µg | 3 x 10 µg | 100 µg

NEW IL-2 Superkine H9T (monomeric):Fc-KIH (human) (rec.)
AG-40B-0223 10 µg | 3 x 10 µg | 100 µg

IL-2 (human) (monomeric):Fc-KIH (human) (rec.)
AG-40B-0224 10 µg | 3 x 10 µg | 100 µg

IL-2 (mouse) (monomeric):Fc-KIH (human) (rec.)
AG-40B-0225 10 µg | 3 x 10 µg | 100 µg

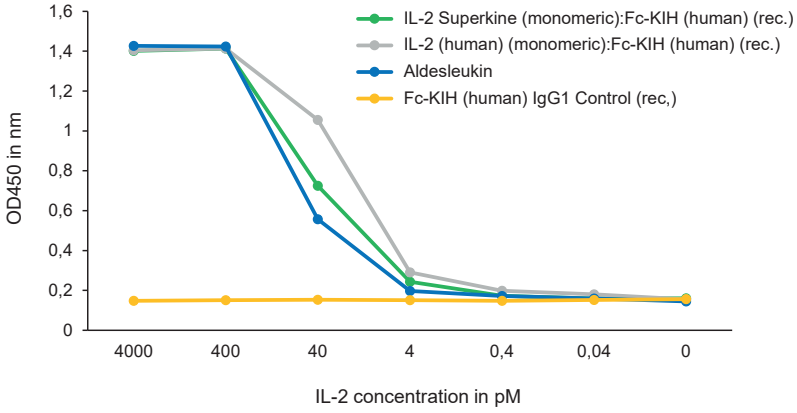
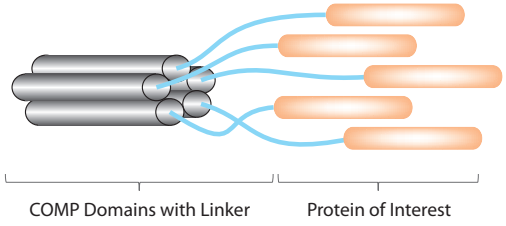


FIGURE: IL-2 Superkine (monomeric):Fc-KIH (human) (rec.) (AG-40B-0222) and IL-2 (human) (monomeric):Fc-KIH (human) (rec.) (AG-40B-0224) are equivalent or better than the Gold Standard IL-2 Aldesleukin to increase proliferation of CTLL-2 cytotoxic T cells.

COMP-Fusion Proteins – Improved Avidity & Biological Activity

- COMP-Fusion Proteins are based on the pentamerization domain (minimal coiled-coil domain) of the cartilage oligomeric matrix protein (COMP) which is fused through a specific linker to proteins of interest.
- The avidity (binding) and activity of selected proteins are improved by the addition of the COMP-oligomerization domain fused at the N- or C-terminus of the protein of interest.
- Using this technology AdipoGen Life Sciences generates cytokines with improved avidity and biological activity.
- For *in vitro* and *in vivo* studies!

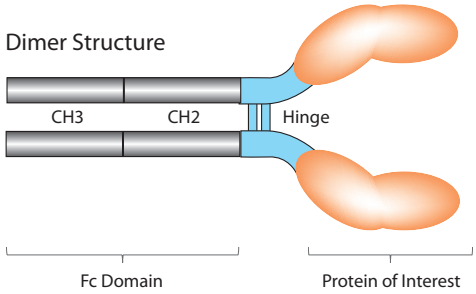
Pentamer Structure



PRODUCT NAME	PID	HOST	APPLICATIONS
Coming Soon COMP (rat):CD40L (human) (rec.)	AG-40B-0210	HEK 293 cells	Potent B cell and T cell expansion tool for Cell Therapy Application. Accelerates *ex vivo* TIL expansion for Immunotherapy.
COMP (rat):Angiopoietin-1 (human) (rec.)	AG-40B-0147	CHO cells	Potent angiogenic Ang-1 variant.
VISTA (human):COMP (mouse) (rec.) (His)	AG-40B-0183	HEK 293 cells	Immunosuppressive agonist *in vivo* inhibiting the proliferation of $CD4^+$ T cells.
VISTA (mouse):COMP (mouse) (rec.) (His)	AG-40B-0181	HEK 293 cells	Immunosuppressive agonist *in vivo* inhibiting the proliferation of $CD4^+$ T cells.

Fc-Fusion Proteins (wild-type or non-lytic)

- Fc-Fusion proteins (also known as Fc-Chimeric Fusion proteins, Ig-based Chimeric Fusion proteins and Fc-tag proteins) are composed of the Fc domain of IgG genetically linked to a protein of interest. Dimeric structures are formed during the production in mammalian cells.
- The fusion of a cytokine sequence to the Fc domain of an IgG (human or mouse) determines a prolonged circulating half-life time *in vivo*.
- The Fc domain folds independently and can improve the solubility and stability of the partner molecule both *in vitro* and *in vivo*.
- **Non-lytic:** Mutations to the complement (C1q) and FcgR I binding sites of the IgGs Fc fragment render the fusion proteins **incapable of antibody-directed cytotoxicity (ADCC) and complement-directed cytotoxicity (CDC).**
- For *in vitro* and especially *in vivo* studies!



PRODUCT NAME	PID	HOST	SPECIES
Fc (human):CD137L, Soluble (human) (rec.)	AG-40B-0173	HEK 293 cells	Human
CTLA-4 (human):Fc (human) (rec.) (non-lytic)	CHI-HF-220A4	CHO cells	Human
CTLA-4 (mouse):Fc (mouse) (rec.) (non-lytic)	CHI-MF-120A4	NS1 cells	Mouse
IL-2 Superkine (Fc)	AG-40B-0111	HEK 293 cells	Human, Mouse
IL-2 Superkine (Fc) (H9T)	AG-40B-0219	HEK 293 cells	Human, Mouse
IL-2 (human):Fc (human) (rec.) (non-lytic)	CHI-HF-22002	CHO cells	Human
IL-2 (mouse):Fc (mouse) (rec.) (non-lytic)	CHI-MF-12002	NS1 cells	Mouse
IL-4 (human):Fc (human) (rec.) (non-lytic)	CHI-HF-22004	CHO cells	Human
IL-4 (mouse):Fc (mouse) (rec.) (non-lytic)	CHI-MF-12004	CHO cells	Mouse
IL-10 (human):Fc (human) (rec.) (non-lytic)	CHI-HF-22010	CHO cells	Human
IL-10 (mouse):Fc (mouse) (rec.) (non-lytic)	CHI-MF-12010	CHO cells	Mouse
IL-15 (mutant) (human):Fc (human) (rec.)	CHI-HF-21015M	CHO cells	Human
IL-21 (mouse):Fc (mouse) (rec.) (non-lytic)	CHI-MF-12021	CHO cells	Mouse
IL-22 (human):Fc (human) (rec.) (non-lytic)	AG-40B-0230	HEK 293 cells	Human, Mouse
IL-22 (mouse):Fc (mouse) (rec.) (non-lytic)	AG-40B-0231	CHO cells	Mouse, Human
IL-35 (human):Fc (human) (rec.)	CHI-HF-21035	CHO cells	Human
IL-35 (mouse):Fc (human) (rec.)	CHI-MF-11135	CHO cells	Mouse
Jagged-1 (mouse):Fc (human) (rec.)	AG-40A-0157T	HEK 293 cells	Mouse
LAG-3 (human):Fc (human) (rec.)	AG-40B-0031	CHO cells	Human, Monkey, Mouse
LAG-3 (mouse):Fc (mouse) (rec.)	AG-40B-0039	CHO cells	Human, Mouse
PSGL-1 (human):Fc (human) (rec.)	AG-40B-0190	HEK 293 cells	Human
TAPBPL (human):Fc (human) (rec.)	AG-40B-0217	HEK 293 cells	Human
TAPBPL (mouse):Fc (human) (rec.)	AG-40B-0216	HEK 293 cells	Mouse
Tim-3 (mouse):Fc (human) (rec.)	AG-40B-0191	HEK 293 cells	Mouse
Tim-4 (human):Fc (human) (rec.)	CHI-HF-210T4	CHO cells	Human
Tim-4 (mouse):Fc (human) (rec.)	AG-40B-0180	HEK 293 cells	Human, Mouse
Tim-4 (mouse):Fc (human) (rec.) (Biotin)	AG-40B-0180B	CHO cells	Human, Mouse
NEW VSTM5 (human):Fc (human) (rec.) (non-lytic)	AG-40B-0237	HEK 293 cells	Human

Visit our Website for >350 Fc-Fusion Proteins

IL-22 – Controversial Cytokine in Tumor Development

IL-22 plays key functions in the intestine and therefore, plays a protective role in inflammatory bowel disease (IBD). IL-22 is also important for tumor development. In healthy conditions, it prevents tumor formation; however, once a tumor has been established, IL-22 promotes tumorigenesis.

IL-22 (human):Fc (human) (rec.) (non-lytic)
AG-40B-0230 50 µg

IL-22 (mouse):Fc (mouse) (rec.) (non-lytic)
AG-40B-0231 50 µg

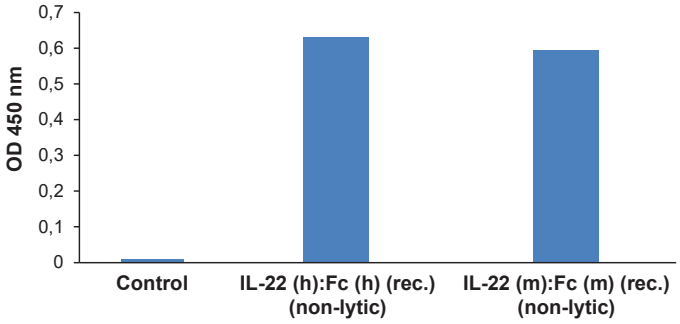


FIGURE: IL-22:Fc proteins (Prod. No. AG-40B-0230 & AG-40B-0231) induce IL-10 secretion in human COLO 205 cells. IL-10 is measured using an IL-10 (human) ELISA Kit. EC50 of this induction is ~ 90pM

Proteins with Improved Activity by Site-directed Mutations

AdipoGen Life Sciences provides several unique proteins with specific mutations described in the literature, which improve their function/activity.

PRODUCT NAME	PID	PROTEIN IMPROVEMENTS
DLL4 (human):Fc (human) (rec.) (highly active mutant)	AG-40B-0176	Several proprietary mutations result in a >20-fold increase in affinity relative to WT DLL4.
IL-2 Superkine (Fc)	AG-40B-0111	Mutations L80F / R81D / L85V / I86V / I92F to stabilize the structure and effectively eliminate the functional requirement of IL-2 for CD25 expression and elicit the proliferation of T cells.
IL-2 Superkine (Fc) (H9T)	AG-40B-0219	A new version of IL-2 Superkine with an additional mutation Q126T reduces the binding of IL-2Rγ and promotes the expansion of $CD8^+$ T cells without driving terminal differentiation.
IL-15 (mutant) (human):Fc (human) (rec.)	CHI-HF-21015M	Mutations at Q149D and Q156D make this protein specifically bind to the IL-15R, competitively inhibiting IL-15-triggered cell proliferation, promoting transplant tolerance and does not activate the STAT-signaling pathway.
IL-33 (oxidation resistant) (human) (rec.) (untagged)	AG-40B-0160	Amino acids 208 and 232 are mutated from cysteines to serines to protect IL-33 from oxidation.

Selected Other Specialty Proteins

AdipoGen Life Sciences' R&D department has developed proteins with unique active structures as well as proteins that need the absence of a tag to be active. Some examples are shown below.

PRODUCT NAME	PID	HOST	SPECIES
BAFF, Soluble (human) (60-mer) (rec.) (highly active)	AG-40B-0112	E. coli	Human, Mouse
Irisin (rec.) (untagged) (*E. coli*)	AG-40B-0103	E. coli	Human, Mouse
Progranulin (human) (rec.) (untagged)	AG-40A-0188Y	HEK 293 cells	Human

Recombinant Proteins for Preclinical Research

Immuno-oncology (e.g. cancer immunotherapy), including adoptive cell therapy also known as cellular immunotherapy, as well as immune-check point blockade strategy, are new protocols using the cells of our immune system and/or blocking antibodies to fight cancer. These therapies are constantly improving and providing new hope to cancer patients. Immuno-oncology-based therapies are currently being evaluated, both alone and in combination with other treatments, in a variety of cancer types in clinical trials.

To develop and improve successfully immuno-oncology, preclinical animal models are key for a better understanding of mechanisms, drug kinetics and toxicity, consequently leading to the selection of the best treatment, which should be translated into clinics. New preclinical models using animals such as mice or rats, *ex vivo* cell-based models or *in vivo* xenograft models have become available.

AdipoGen Life Sciences offers a broad range of unique high-quality research-grade proteins for preclinical studies in mice, humanized mice or rats. These recombinant proteins are produced mainly with specific mammalian expression systems (CHO cells or HEK 293 cells) and in animal-component-free media. The proteins are provided in lyophilized or liquid frozen form and are highly pure, contain low endotoxin levels and are biologically active.

Benefits of Using AdipoGen Life Sciences Animal-Component Free Proteins

- **Improved Cell Culture Consistency / Improved Cell Product Safety Profile / High Lot-to-Lot Consistency**
  ⇒ Defined composition of the cell culture medium
- **Facilitated Transition from Preclinical Research to Clinical Manufacturing**
  ⇒ Certified Master Cell Lines
- **Confirmed Bioactivity**
  ⇒ Binding Assays & Cell-based Assays
- **High Purity and Low Endotoxin Levels**
  ⇒ Over 95% purity and industry-leading guaranteed endotoxin level of <0.01 EU/µg by the LAL method.
- **Supply Reliability / Bulk Proteins at Discounted Prices**
  ⇒ Our in-house expertise & capacity in production allow a stable supply and scale up production for economical pricing.
- **Comprehensive Portfolio of Reagents / Custom Protein Capabilities**
  ⇒ We offer a broad range of protein constructs and we have the expertise and the systems necessary to develop custom proteins required to advance your research.

Protein Constructs for Preclinical Research