

InVivoKines[™]

Proteins with Enhanced Activity & Stability for *InVivo* Research

Cytokines are small proteins that facilitate communication among immune cells and orchestrate the response to infections and tumors as well as overall immune homeostasis, making them attractive for preclinical and clinical research for a variety of immune-related disorders. They have pivotal roles in immunity and engineered cytokine-based therapies represent a new evolution of immunotherapeutics. However, the widespread use of cytokines has been limited by their short blood half-lives, pleiotropism and unfavorable biodistribution. Increased knowledge of cytokine biology and innovative cytokine engineering and technologies allow to cope with such limitations, but often are not accessible for basic research purposes.

InVivoKines[™] are a new generation of recombinant fusion proteins for immunotherapeutic, preclinical and translational *in vivo* research, developed in-house by AdipoGen Life Sciences. InVivoKines[™] are Fc-based fusion proteins using the Knobs-into-Holes (KIH) technology.

InVivoKines[™] Quality Features:

- **Native Conformation** – Production in HEK 293 or CHO cells
- **Production under Animal-Free Conditions**
- **High Bioactivity tested by ELISA/Cell-based Assays**
- **Verified Purity & Homogeneity by SEC**
- **Low Endotoxin Levels**
- **Batch-to-Batch Consistency**

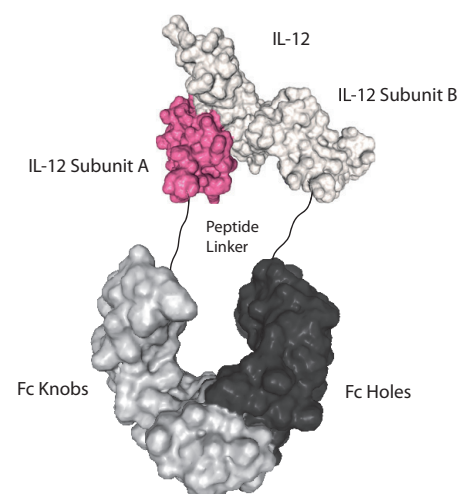


FIGURE: Example of a heterodimeric Fc-KIH structure.

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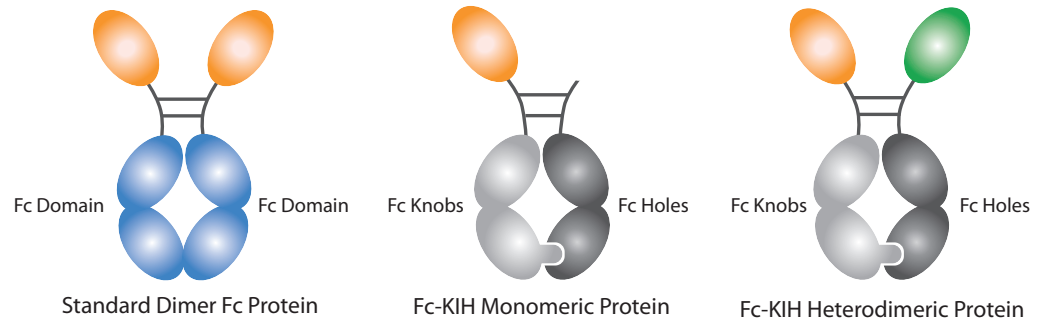
Naturally Occurring Cytokines Fused to Fc (KIH-Technology)

InVivoKines™ are Fc-based fusion proteins using the Knobs-into-Holes (KIH) technology.

- The Fc-KIH technology allows Fc heterodimerization to create a structure with two different arms, to design naturally occurring active monomeric, heterodimeric or homodimeric proteins.
- The Fc-KIH domain enhances the plasma half-life of the cytokine which substantially improves the pharmacokinetics (PK) while maintaining activity.
- The Fc-KIH domain engages with the Fc γ receptors (Fc γ R) and the complement C1q, which may result in ADCC, ADCP and CDC*.
- The Fc-KIH LALA-PG mutations inhibit binding to Fc γ R and C1q while FcRn binding and Fc stability remain unaffected.
- The Fc-KIH-constructs are produced in mammalian cells with low endotoxin content.
- The Fc-KIH proteins are developed for *in vivo* studies but are also suitable for *in vitro* experiments!

AdipoGen Life Sciences leveraged the KIH-technology to develop monomeric, heterodimeric or homodimeric Fc fusion proteins.

*Antibody-dependent cellular cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP), Complement-dependent cytotoxicity (CDC)



Silenced Fc-KIH Domain (LALA-PG Mutations)

AdipoGen Life Sciences engineers its silenced Fc-KIH domains using the IgG1-P329G LALA mutations, with completely abolished Fc γ R and C1q interactions, containing a limited number of mutations and with unaffected FcRn interactions and Fc stability. The LALA-PG mutations show no detectable binding to Fc γ receptors or to C1q, are inactive in functional cell-based assays and do not elicit inflammatory cytokine responses.

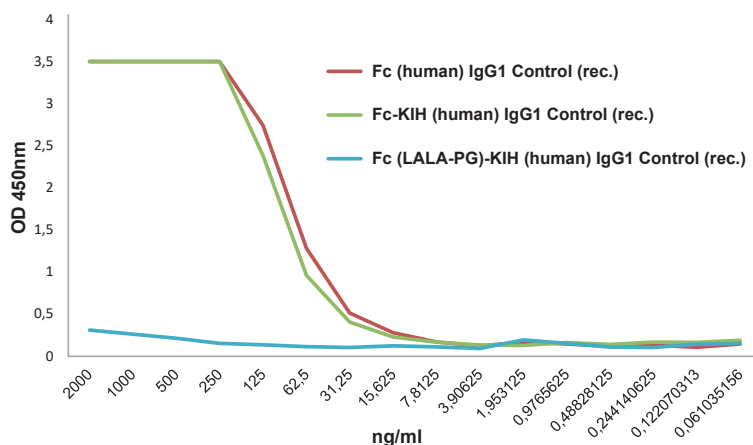


FIGURE: Fc (LALA-PG)-KIH (human) IgG1 Control (rec.) (Prod. No. AG-35B-0018) does not bind to the human Fc γ Receptor I.

LITERATURE: (1) Knobs-into-Holes (KIH) Technology: J.B. Ridgway, et al.; Protein Eng. **9**, 617 (1996) • (2) Efficient Generation of Bispecific Murine Antibodies for Pre-Clinical Investigations in Syngeneic Rodent Models: A.F. Labrijn, et al.; Sci. Rep. **7**, 2476 (2017) • (3) Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions: T. Schlothauer, et al.; Prot. Eng. Design Sel. **29**, 457 (2016)

Potent Monomeric IL-2 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**.

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

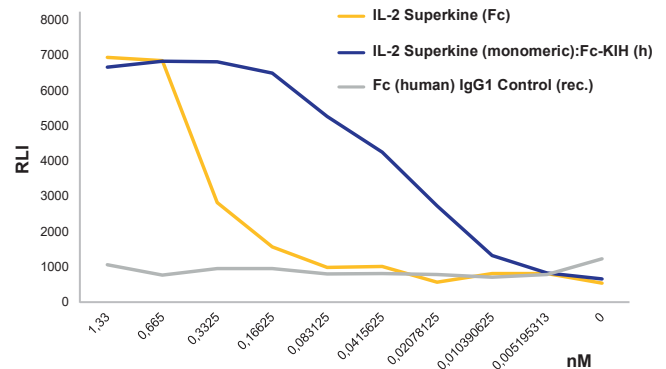


FIGURE: IL-2 Superkine (monomeric):Fc-KIH (human) (rec.) (Prod. No. AG-40B-0222) activates the IL-2 receptor better than the dimeric IL-2 Superkine (Prod. No. AG-40B-0111) using the IL-2 Bioassay (Promega JA2201).

NEW IL-27 (mouse):Fc-KIH (human) (rec.)

AG-40B-0236

IL-27 is composed of two subunits, IL-27p28 and EBI3. It promotes NK and T cell proliferation as well as the production of IFN- γ . IL-27 has potent antiviral activities against numerous viruses and antitumor activity via production of anti-angiogenic chemokines.

IL-27 (mouse):Fc-KIH (human) (rec.) is a heterodimeric Fc-KIH construct that binds to mouse & human IL-27R complex and activates Stat3 phosphorylation in mouse and human cells. Its activity is stronger compared to the classical dimeric IL-27 (mouse):Fc from competitors.

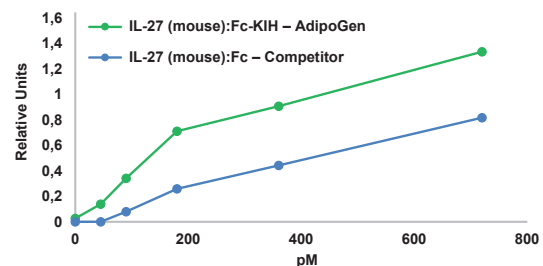


FIGURE: IL-27 (mouse):Fc-KIH (human) (rec.) (Prod. No. AG-40B-0236) (Heterodimer) is more active than an IL-27 (mouse):Fc from a competitor (Dimer of Heterodimer) in Stat3 phosphorylation in HepG2 cells (shown as a quantification figure of the western blot).

NEW Fc (LALA-PG)-KIH (human):GDF15 (mouse) (rec.)

AG-40B-0245

The effects of GDF15 are pleiotropic and include appetite regulation, actions on metabolism, pregnancy, cell survival, immune response and inflammation.

AdipoGen Life Sciences' homodimeric protein **Fc (LALA-PG)-KIH (human):GDF15 (mouse) (rec.)** is a unique protein with long-acting high activity for *in vivo* studies. It forms a biologically active homodimer, unlike other classical GDF15-Fc proteins that form inactive large aggregates.

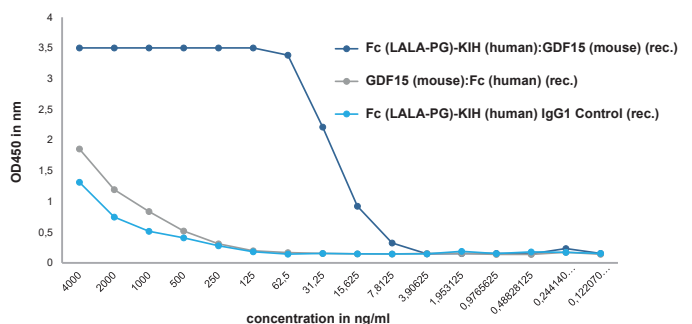


FIGURE: A binding assay shows that Fc (LALA-PG)-KIH (human):GDF15 (mouse) (rec.) (Prod. No. AG-40B-0245) binds with high affinity to its receptor GFRAL (mouse). The Fc (LALA-PG)-KIH (human):GDF15 (mouse) (rec.), which migrates as a dimer by SEC, binds to its receptor mouse GFRAL with an EC₅₀ ~15 ng/ml, while the GDF15 (mouse):Fc (human) (rec.), that migrates as an aggregate by SEC (>1000 kDa), binds similar to the control protein with low affinity.

InVivoKines™ – Product Overview

Product Name	PID	Protein Construct	Fc-KIH Silenced
IL-2 (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0224	Monomer	No
IL-2 (mouse) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0225	Monomer	No
IL-2 Superkine (monomeric):Fc-KIH (human) (rec.)	AG-40B-0222	Monomer	No
IL-2 Superkine H9T (monomeric):Fc-KIH (human) (rec.)	AG-40B-0223	Monomer	No
IL-2 (human) (Switch-2) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0234	Monomer	No
IL-7 (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0238	Monomer	No
NEW IL-7 (human) (monomeric):Fc (LALA-PG)-KIH (human) (rec.)	AG-40B-0247	Monomer	LALA-PG
IL-12 (mouse):Fc-KIH (human) (rec.)	AG-40B-0240	Heterodimer	No
NEW IL-21 (mouse) (monomeric):Fc (LALA-PG)-KIH (human) (rec.)	AG-40B-0250	Monomer	LALA-PG
IL-23 (mouse):Fc-KIH (human) (rec.)	AG-40B-0235	Heterodimer	No
NEW IL-23 (mouse):Fc (LALA-PG)-KIH (human) (rec.)	AG-40B-0248	Heterodimer	LALA-PG
IL-27 (mouse):Fc-KIH (human) (rec.)	AG-40B-0236	Heterodimer	No
NEW IL-27 (mouse):Fc (LALA-PG)-KIH (human) (rec.)	AG-40B-0249	Heterodimer	LALA-PG
IL-33 (oxidation resistant) (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0233	Monomer	No
IL-37 (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0221	Monomer	No
IL-38 (aa 1-152) (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0241	Monomer	No
IL-38 (aa 20-152) (human) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0226	Monomer	No
IL-38 (aa 3-152) (mouse) (monomeric):Fc-KIH (human) (rec.)	AG-40B-0227	Monomer	No
NEW Fc (LALA-PG)-KIH (human):GDF15 (mouse) (rec.)	AG-40B-0245	Homodimer	LALA-PG
NEW Fc (LALA-PG)-KIH (human):Irisin (monomeric) (rec.)	AG-40B-0246	Monomer	LALA-PG
Fc-KIH (human) IgG1 Control (rec.)	AG-35B-0015	Control	No
Fc (LALA-PG)-KIH (human) IgG1 Control (rec.)	AG-35B-0018	Control	LALA-PG

InVivoKines™ – Selected Structures

