

3rd Edition

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

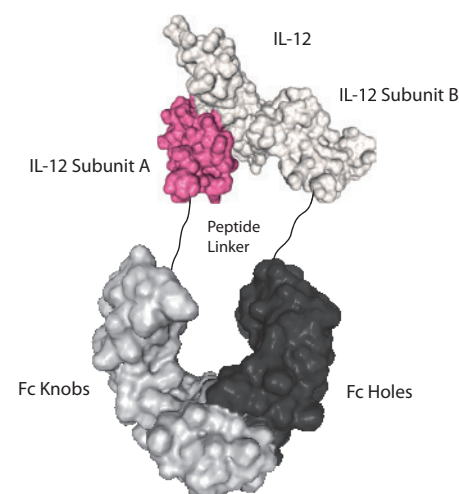


FIGURE: Example of a heterodimeric Fc-KIH structure.

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**

CONTENTS

• Naturally Occurring Cytokines Fused to Fc-KIH	2
• Silenced Fc-KIH Mutation	2
• IL-12 <i>In Vivo</i> Case Study!	3
• IL-27 <i>In Vivo</i> Case Study!	3
• IL-2 <i>In Vitro</i> Activity Case Study!	3
• InVivoKines [™] Product Overview	4

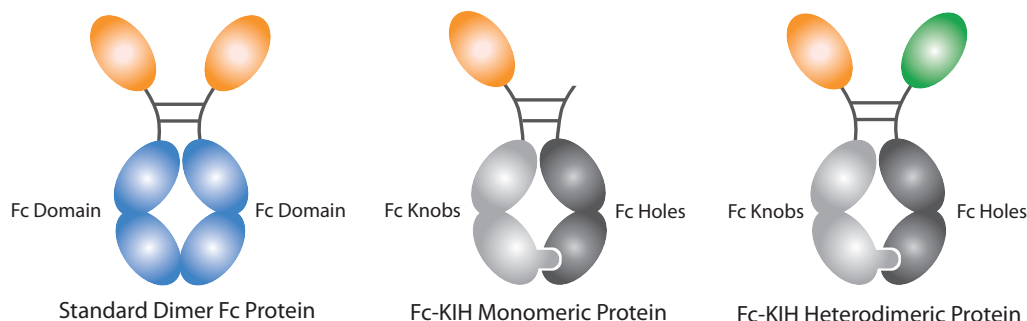
Naturally Occurring Cytokines Fused to Fc (KIH-Technology)

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 Fcγ receptors (FcγRs) and the complement C1q, which may result in ADCC, ADCP and CDC*.
- The Fc-KIH LALA-PG mutations inhibit binding to FcγRs 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 hIgG1-P329G LALA mutations, **with completely abolished FcγR 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 Fcγ receptors or to C1q, are inactive in functional cell-based assays and do not elicit inflammatory cytokine responses.

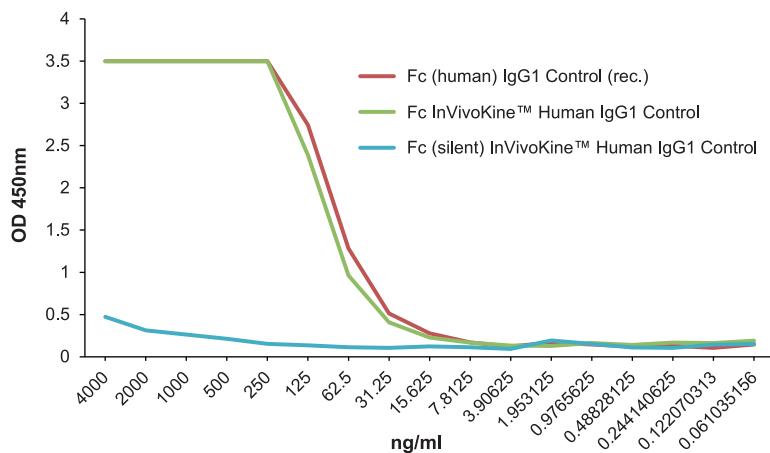


FIGURE: Fc (silent) InVivoKine™ Human IgG1 Control (Prod. No. AG-35B-0018) does not bind to the human Fcγ Receptor I.

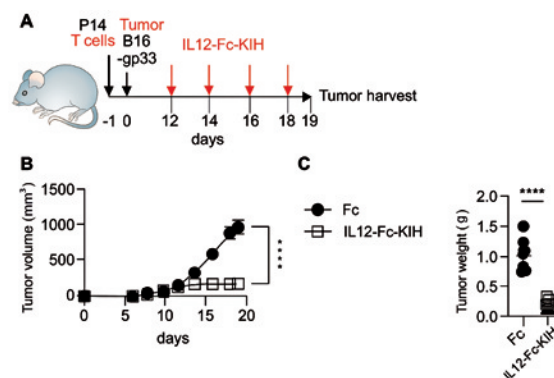
LITERATURE: (1) Knobs-into-Holes (KIH) Technology: J.B. Ridgway, et al.; Protein Eng. **9**, 617 (1996) • (2) Efficient Generation of Bispecific Murine Antibodies for Pre-Clinical Investigations in Syngeneic Rodent Models: A.F. Labrijn, et al.; Sci. Rep. **7**, 2476 (2017) • (3) Novel human IgG1 and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

IL-12 (mouse):Fc InVivoKine™ – *In Vivo* Case Study

IL-12 (mouse):Fc InVivoKine™ (Prod. No. AG-40B-0240) is a **heterodimeric** Fc-KIH construct that binds to mouse IL-12Rβ1/IL-12Rβ2 complex.

Treatment with IL-12 InVivoKine™ (2μg every 2 days) shows strong activity when injected in mice leading to a complete tumor growth stop (see Figures on the right). An optimized InVivoKine™ version with a silent Fc is also available.

FIGURE: IL-12 (mouse):Fc InVivoKine™ (Prod. No. AG-40B-0240) inhibits *in vivo* tumor growth in mice. Picture courtesy of Dr. Romain Veber, lab of Prof. Werner Held, University of Lausanne, Switzerland.



IL-27 (mouse):Fc (silent) InVivoKine™ – *In Vivo* Case Study

IL-27 (mouse):Fc (silent) InVivoKine™ (Prod. No. AG-40B-0249) is a **heterodimeric** Fc-KIH construct that binds to mouse & human IL-27R complex and activates Stat3 phosphorylation in mouse and human cells. Treatment with IL-27 InVivoKine™ (one injection 2,5μg) promoted robust expansion and differentiation of CD8⁺ T cells into IFNγ-producing cytotoxic effectors in an HBV *in vivo* setup, suggesting that IL-27 may have therapeutic potential in patients with chronic HBV infection.

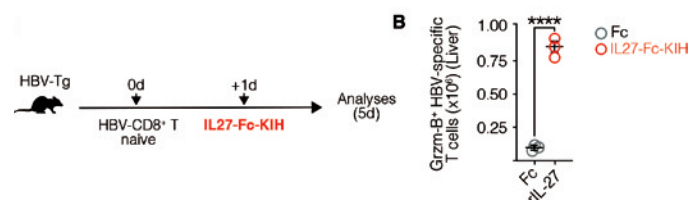


FIGURE: IL-27 (mouse):Fc (silent) InVivoKine™ (Prod. No. AG-40B-0249) reinvigorates the functionality of HBV-specific CD8⁺ T cells *in vivo*. Picture courtesy of Dr. Valentina Venzin, lab of Dr. Matteo Iannaccone, San Raffaele Scientific Institute, Milan, Italy.

Potent IL-2 InVivoKines™ – *In Vitro* Activity Case Study

IL-2 is a key lymphocyte growth factor that is required for the maintenance and expansion of effector T cells (CD4 and CD8), regulatory T cells (Tregs) and natural killer (NK) cells. **The monomeric IL-2 InVivoKines™ show better effect *in vivo* compared to standard IL-2-Fc and similar activity *in vitro* compared to the Gold Standard IL-2 Aldesleukin.** The IL-2 Superkine variant 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.

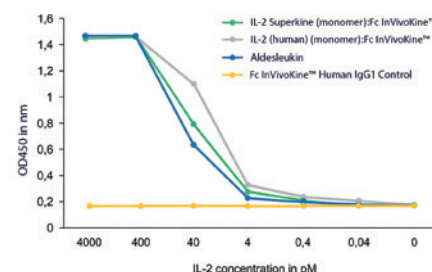


FIGURE: IL-2 InVivoKines™ (Prod. No. AG-40B-0222 and AG-40B-0224) are equivalent or better than the Gold Standard IL-2 Aldesleukin to increase proliferation of CTLL-2 cytotoxic T cells.

Product Name	PID	Protein Construct	Fc-KIH Silenced
IL-2 (human) (monomer):Fc InVivoKine™	AG-40B-0224	Monomer	No
IL-2 (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0263	Monomer	LALA-PG
IL-2 (mouse) (monomer):Fc InVivoKine™	AG-40B-0225	Monomer	No
IL-2 (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0264	Monomer	LALA-PG
IL-2 Superkine (monomer):Fc InVivoKine™	AG-40B-0222	Monomer	No
IL-2 Superkine (monomer):Fc (silent) InVivoKine™	AG-40B-0262	Monomer	LALA-PG
IL-2 Superkine H9T (monomer):Fc InVivoKine™	AG-40B-0223	Monomer	No
IL-2 (human) (Switch-2) (monomer):Fc InVivoKine™	AG-40B-0234	Monomer	No

See Last Page for ALL other available InVivoKines™ Overview

InVivoKines™ – Product Overview

Product Name	PID	Protein Construct	Fc-KIH Silenced
IL-1Ra (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0254	Monomer	LALA-PG
IL-2 InVivoKines™ (see Page 3)			
IL-4 (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0261	Monomer	LALA-PG
NEW IL-4 (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0269	Monomer	LALA-PG
IL-7 (human) (monomer):Fc InVivoKine™	AG-40B-0238	Monomer	No
IL-7 (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0247	Monomer	LALA-PG
IL-7 (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0256	Monomer	LALA-PG
NEW IL-10 (human):Fc (silent) InVivoKine™	AG-40B-0265	Homodimer	LALA-PG
IL-10 (mouse):Fc (silent) InVivoKine™	Coming Soon		
NEW IL-11 (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0266	Monomer	LALA-PG
NEW IL-11 (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0268	Monomer	LALA-PG
IL-12 (mouse):Fc InVivoKine™	AG-40B-0240	Heterodimer	No
IL-12 (mouse):Fc (silent) InVivoKine™	AG-40B-0255	Heterodimer	LALA-PG
IL-15:IL-15Ra (Sushi) (mouse):Fc (silent) InVivoKine™	AG-40B-0257	Heterodimer	LALA-PG
IL-18 (mouse):Fc (silent) InVivoKine™	Coming Soon		
NEW IL-18BP (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0276	Monomer	LALA-PG
NEW IL-18BP (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0277	Monomer	LALA-PG
IL-21 (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0258	Monomer	LALA-PG
IL-21 (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0250	Monomer	LALA-PG
IL-23 (mouse):Fc InVivoKine™	AG-40B-0235	Heterodimer	No
IL-23 (mouse):Fc (silent) InVivoKine™	AG-40B-0248	Heterodimer	LALA-PG
IL-27 (mouse):Fc InVivoKine™	AG-40B-0236	Heterodimer	No
IL-27 (mouse):Fc (silent) InVivoKine™	AG-40B-0249	Heterodimer	LALA-PG
IL-33 (oxidation resistant) (human) (monomer):Fc InVivoKine™	AG-40B-0233	Monomer	No
IL-33 (oxidation resistant) (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0259	Monomer	LALA-PG
IL-37 (human) (monomer):Fc InVivoKine™	AG-40B-0221	Monomer	No
IL-38 (aa 1-152) (human) (monomer):Fc InVivoKine™	AG-40B-0241	Monomer	No
IL-38 (aa 20-152) (human) (monomer):Fc InVivoKine™	AG-40B-0226	Monomer	No
IL-38 (aa 3-152) (mouse) (monomer):Fc InVivoKine™	AG-40B-0227	Monomer	No
GDF15 (human):Fc (silent) InVivoKine™	AG-40B-0253	Homodimer	LALA-PG
GDF15 (mouse):Fc (silent) InVivoKine™	AG-40B-0245	Homodimer	LALA-PG
NEW GLP-1R (human) (monomer):Fc (silent) InVivoKine™	AG-40B-0272	Monomer	LALA-PG
NEW IFN-β (mouse) (monomer):Fc (silent) InVivoKine™	AG-40B-0280	Monomer	LALA-PG
IFN-γ (mouse) (monomer):Fc (silent) InVivoKine™	Coming Soon		
Irisin (monomer): Fc (silent) InVivoKine™	AG-40B-0246	Monomer	LALA-PG
NEW PLXDC2 (mouse):Fc (silent) InVivoKine™	AG-40B-0270	Homodimer	LALA-PG
Fc InVivoKine™ Human IgG1 Control	AG-35B-0015	Control	No
Fc (silent) InVivoKine™ Human IgG1 Control	AG-35B-0018	Control	LALA-PG