Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (GASDALIE: G236A/S239D/A330L/I332E) — GASDALIE Fc Variant

SKU#: AFV-17

Plasmid Information

- **Plasmid name:** pHC-IgG1-GASDALIE(G236A/S239D/A330L/I332E)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS –C_H1–hinge-lower hinge(G236A)–C_H2(S239D/A330L/I332E)-C_H3(wild-type)
- **Resistance marker:** Ampicillin
- **Promoter:** CMV
- **Product Configuration:** Fc-engineered heavy-chain expression vector (single-vector format).
- **Compatible Light-Chain Vector:** A matching light-chain expression vector is required for complete IgG antibody expression and is available separately or as part of the optional Fc Engineering Set/Pair.

Fc Engineering Summary

- **Mutation set:** G236A(lower hinge) + S239D + A330L + I332E(C_H2 domain)
- **Common name:** GASDALIE Fc Variant
- **Engineering purpose:** Premier quadruple-mutation Fc module designed to achieve maximum binding affinity for FcγRIIIa and FcγRIIa, yielding ultra-high NK cell-mediated ADCC and macrophage-mediated ADCP potency against low-density target antigens.

Functional Profile

Property	Effect
FcγRIIIa binding (V158 & F158)	Maximum Hyper-Enhancement ↑↑↑↑ (>100× affinity boost)
FcγRIIa binding (H131 & R131)	Strongly Increased ↑↑↑ (A/I activation ratio optimized)
FcγRIIb binding (Inhibitory)	Lower relative engagement vs. FcγRIIa
ADCC	Ultra-High / Maximal ↑↑↑↑ (Industry Benchmark)
ADCP (Macrophage Phagocytosis)	Potently Enhanced ↑↑↑
CDC	Mildly Reduced / Secondary (due to local CH2 loop restructuring)
FcRn binding & half-life	Normal / WT Baseline
Effector potency	Maximal Engineered ADCC/ADCP Potency

Note: Functional properties are based on published Fc-engineering studies and may vary with antibody specificity, expression system, glycosylation, and assay conditions. Unless otherwise indicated, these results have not been independently validated for this specific Fusion BioLabs expression construct.

Mechanism of Action

The GASDALIE variant integrates four synergistic substitutions focused entirely within the lower hinge and C_H2 domain:

- G236A (Lower Hinge): Restricts local backbone flexibility at the lower hinge junction, enhancing binding selectivity for activating FcγRIIa over inhibitory FcγRIIb.
- S239D (C_H2 Domain): Introduces a negative charge that forms favorable electrostatic complementary interactions with FcγRIIIa surface residues.
- A330L (C_H2 Domain): Optimizes hydrophobic packing interactions and structural positioning at the receptor interface.
- I332E (C_H2 Domain): Inserts a second negatively charged residue to lock the receptor interface into a high-affinity conformation.

Together, these four mutations represent the highest-affinity engineered FcγRIIIa variant in the industry, enabling target cell lysis even at extremely low antigen density levels.

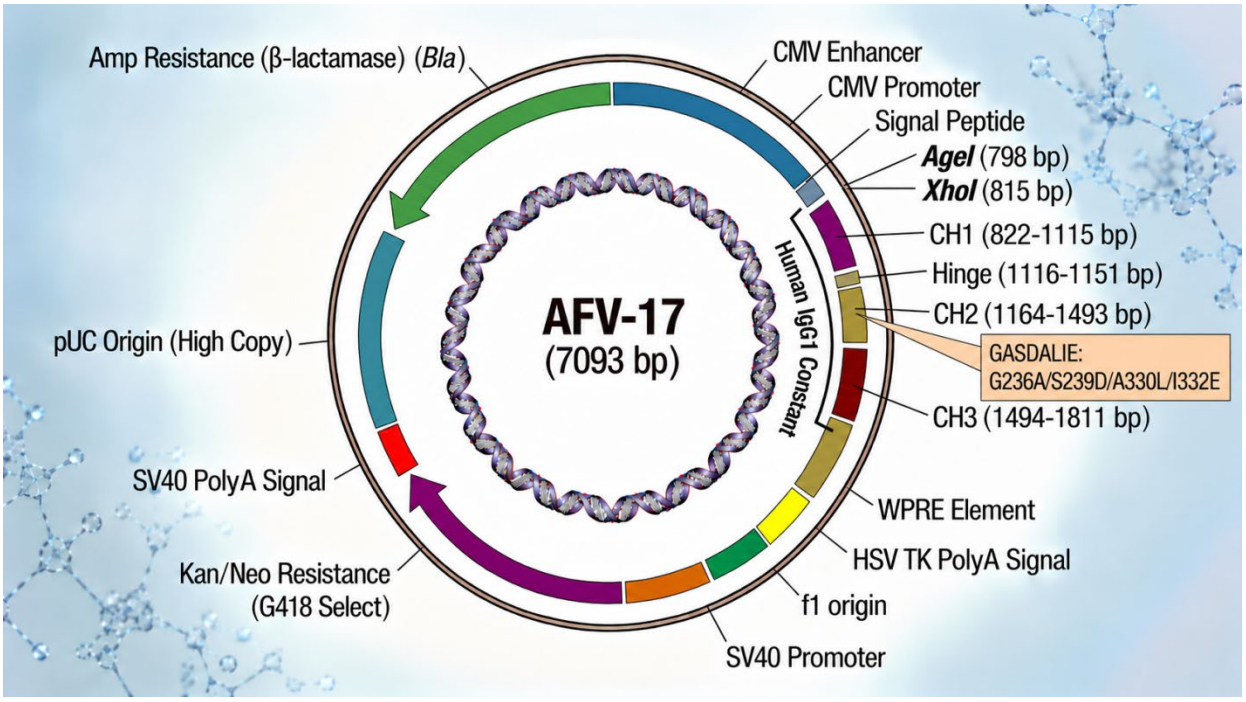
Phenotypic Effects

- Peak NK cell recruitment and maximal ADCC cytotoxicity (EC_{50} shifted up to 3 logs lower than wild-type)
- Overcomes low-affinity FcγRIIIa-F158 and FcγRIIa-R131 patient polymorphism limitations
- Potent activation of macrophage-mediated tumor cell phagocytosis (ADCP)
- Preserves standard FcRn endosomal recycling and systemic serum half-life
- Selective effector activation ideal for targets where CDC contribution is unnecessary or undesirable

Applications

- Therapeutic oncology antibodies targeting antigens expressed at low surface copy numbers
- Refractory cell depletion where standard IgG1 or DLE variants fail to elicit sufficient effector response
- Industry benchmark control for hyper-enhanced ADCC and structural FcγR engagement assays
- Preclinical antibody discovery against hard-to-deplete tumor targets

Vector Map



Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — native IgG1 core hinge
- **Lower Hinge(G236A)** — FcγRIIa-selective activation mutation
- **C_H2(S239D/A330L/I332E)** — ADCC/ADCP hyper-enhancing electrostatic & hydrophobic structural cluster
- **C_H3(WT)** — native IgG1 C_H3 domain

Vector Specifications

Antibiotic Resistance	Ampicillin (Amp^R)
Constitutive or Inducible System	Constitutive
Delivery Type	Transfection
Promoter	CMV
Product Type	Mammalian Expression Vector
Cloning Method	Restriction Enzyme (5'-AgeI; 3'-XhoI) or Homologous Assembly
Available Configurations	Single Heavy-Chain Vector or Fc Engineering Set/Pair
Content — Single Vector	5.0 µg Fc-engineered heavy-chain plasmid in TE buffer
Content — Set/Pair	Two separate plasmids: 5.0 µg Fc-engineered heavy-chain expression vector and 5.0 µg compatible light-chain expression vector, supplied individually in TE buffer.

Cloning Map



Primer Design for Restriction Enzyme Cloning (Traditional)

Forward primer: 5'- CTAGTAGCAACTGCAACCGGTGTACATTCAN_{VHF}(15-18)-3'

Reverse Primer: 5'- GGCCCTTGGTGGAGGCGCTCGAGACN_{VHR}(15-18)-3'

N_{VHF}(15-18): append 15-18 bases encoding 5-6 amino acids starting from the beginning of the VH region to the end of the forward primer.

N_{VHR}(15-18): append the Reverse Complement DNA Sequence of 15-18 base encoding 5-6 amino acids of starting from the end of the VH region to the reverse primer (i.e. from 9th--8th to 4th from the VH end, VSS has already been included in the reverse primer).

Primer Design for Homologous Recombination Cloning (Seamless)

Forward primer: 5'- CTAGTAGCAACTGCAACCGGTGTACATTCAN_{VHF}(15-18) -3'

Reverse Primer: 5'- GGCCCTTGGTGGAGGCN_{VHR}(15-18) -3'

N_{VHF}(15-18): append 15-18 bases encoding 5-6 amino acids starting from the beginning of the VH region to the end of the forward primer.

N_{VHR}(15-18): append the Reverse Complement DNA Sequence of 15-18 bases encoding 5-6 amino acids starting from the end of the VH region to the reverse primer.

Sequencing primer for confirming the correct VH insert (included in the Kit)

pFB forward: 5'- ATGGGCGGTAGGCGTGTA-3'

Storage & Handling

- Store plasmid at **–20°C**
- Avoid repeated freeze–thaw cycles
- Use sterile technique
- Suitable for transient or stable mammalian expression(CHO, HEK293, NS0)

Product Notes

- Complete Antibody Expression — Fc Engineering Set/Pair

For expression of a complete Fc-engineered IgG antibody, the Fc-engineered heavy-chain expression vector must be co-transfected with a compatible light-chain expression vector containing the corresponding antibody light-chain variable region.

Fusion BioLabs offers matching light-chain expression vectors through its Antibody Expression Vector product category. Customers may purchase the Fc-engineered heavy-chain vector individually or select the optional Fc Engineering Set/Pair, which includes the Fc-engineered heavy-chain expression vector and a compatible light-chain expression vector.

Note: The Fc Engineering Set/Pair provides compatible heavy-chain and light-chain expression vectors. Antibody-specific VH and VL sequences must be cloned into the corresponding vectors unless the product is explicitly supplied with pre-inserted variable-region sequences.

- GASDALIE (G236A/S239D/A330L/I332E) represents the apex variant in the Fusion BioLabs ADCC/ADCP-Enhanced Family. It is particularly recommended for research applications targeting ultra-low surface density receptors or when evaluating the maximum theoretical ceiling of NK- and macrophage-mediated cytotoxicity.

Comparison within Fusion BioLabs ADCC/ADCP-Enhanced Family

Variant	Mechanism	Strength	Primary Application & Notes
human IgG1 (WT) (Wild-Type)	Native baseline FcγR binding	★★☆☆☆	Baseline Control. Standard physiological FcγR binding and ADCC/ADCP baseline for comparative assays.
human IgG1 (G236A) G236A	Lower hinge FcγRIIa selective optimization	★★★☆☆+	Selective ADCP Enhancer. Specifically boosts macrophage phagocytosis by maximizing the FcγRIIa/FcγRIIb activation ratio.
human IgG1 (DE) S239D / I332E	C_H2 domain electrostatic interface enhancement	★★★☆☆	Moderate ADCC/ADCP Enhancer. Core double mutation boosting FcγRIIIa and FcγRIIa engagement.
human IgG1 (DLE) S239D / A330L / I332E	C_H2 domain structural interface enhancement	★★★★☆	Strong ADCC Enhancer. Classic triple mutation optimizing NK cell-mediated lysis.
human IgG1 (ADE) G236A / S239D / I332E	C_H2 domain multi-residue FcγRIIIa optimization	★★★★☆+	High-Potency ADCC/ADCP Enhancer. Increases FcγRIIIa binding while improving the FcγRIIa/FcγRIIb binding ratio to favor activation over inhibition.
human IgG1 (GASDALIE) G236A / S239D / A330L / I332E	C_H2 domain hyper-enhancement (FcγRIIIa & FcγRIIa)	★★★★★	Ultra-Potent ADCC/ADCP Enhancer. Maximum engineered affinity variant for low antigen density targets.

References

- *Ahmed AA, Keremane SR, Vielmetter J, Bjorkman PJ. Structural characterization of GASDALIE Fc bound to the activating Fc receptor FcγRIIIa. J Struct Biol. 2016;194(1):78-89.*
- *Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, Chan C, Chung HS, Eivazi A, Yoder SC, Vielmetter J, Carmichael DF, Hayes RJ, Dahiyat BI. Engineered antibody Fc variants with enhanced effector function. Proc Natl Acad Sci U S A. 2006;103(11):4005-4010.*

Document Version

- **Datasheet version:** 3.1
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