Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (G236A) — ADCP-Selective Fc Variant

SKU#: AFV-19

Plasmid Information

- **Plasmid name:** `pHC-IgG1-G236A`
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2(G236A)-C_H3
- **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` (C_H2 lower hinge domain)
- **Common name:** G236A Single Mutation / GA Fc Variant
- **Engineering purpose:** Single amino acid substitution designed to selectively increase binding affinity for the activating receptor FcγRIIa over the inhibitory receptor FcγRIIb, driving macrophage-mediated antibody-dependent cellular phagocytosis (ADCP).

Functional Profile

Property	Effect
FcγRIIa binding (H131 & R131)	Enhanced ↑↑ (>6 x affinity boost)
FcγRIIa / FcγRIIb ratio	Substantially Increased ↑↑↑ (Selective activation)
ADCP (Phagocytosis)	Strongly Enhanced ↑↑↑ (Macrophage-Dominant)
FcγRIIIa binding / ADCC	Moderate / WT Baseline
CDC	WT Baseline to Mild Reduction
FcRn binding & half-life	Normal / WT Baseline
Effector potency	Selective ADCP-Dominant (High FcγRIIa/FcγRIIb selectivity)

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 `G236A` variant replaces glycine at position 236 in the lower hinge region of the C_H2 domain with alanine (**Gly236 to Ala**). Residue 236 directly engages the upper loop of FcγR contact interfaces. The substitution of alanine restricts local polypeptide backbone flexibility and introduces favorable hydrophobic interactions specifically tuned to the binding pocket of activating FcγRIIa. Critically, `G236A` enhances affinity for FcγRIIa without a proportional

increase in binding to the structurally homologous inhibitory receptor FcγRIIb, elevating the A/I (activating-to-inhibitory) ratio.

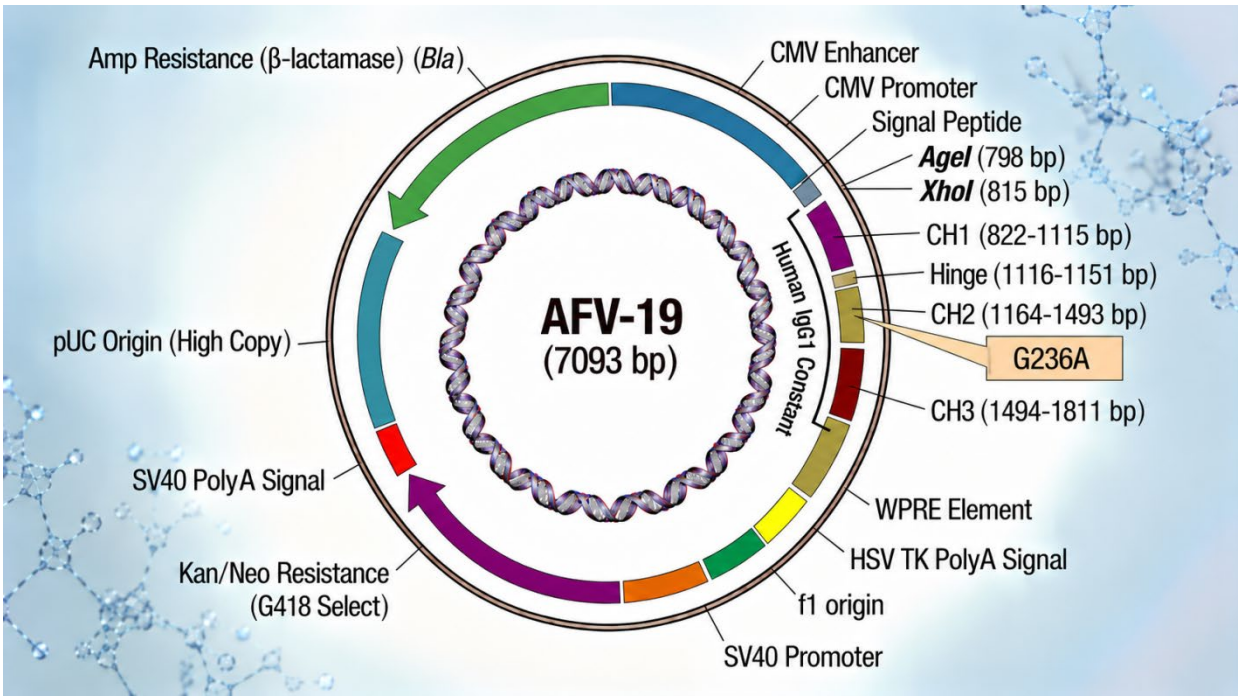
Phenotypic Effects

- Selective enhancement of macrophage and neutrophil recruitment for tumor cell phagocytosis (ADCP)
- Significantly improves the FcγRIIa/FcγRIIb binding ratio to overcome inhibitory signaling on myeloid cells
- Preserves standard FcRn-mediated recycling and systemic pharmacokinetics
- Distinguishes ADCP-driven clearance pathways from purely NK cell-mediated ADCC

Applications

- Therapeutic antibodies targeting solid tumors where myeloid/macrophage infiltration dominates over NK cells
- Macrophage-checkpoint target depletion requiring pure phagocytic enhancement without excessive NK cell hyperactivation
- Dissecting cellular effector mechanisms (ADCP vs. ADCC) in preclinical models

Vector Map



Feature annotation:

- C_H1 — constant heavy 1
- **Hinge** — native IgG1 hinge
- C_H2(G236A) — **selective ADCP-enhancing single mutation**
- C_H3 — 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)

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

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 when handling
- Suitable for transient or stable mammalian expression

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.

- While multi-mutation variants like GASDALIE include G236A as part of a high-affinity cluster, the isolated G236A single mutant serves as the core module for selective phagocytosis enhancement. Note that this substitution is distinct from the deletion variant ΔG236, which completely abolishes FcγR engagement (Effector-Null).

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

- *Yamane-Ohnuki N, Satoh M. Production of therapeutic antibodies with controlled fucosylation. MAbs. 2009;1(3):230-236.*
- *Richards JO, Karki S, Lazar GA, Chen H, Dang W, Desjarlais JR. Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther. 2008;7(8):2517-2527.*

Document Version

- **Datasheet version:** 3.1
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