Fusion BioLabs Fc Engineering Vector Datasheet

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

SKU#: AFV-18

Plasmid Information

- **Plasmid name:** pHC-IgG1-ADE(G236A/S239D/I332E)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide-MCS–C_H1–hinge(G236A)–C_H2(S239D/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 (C_H2) + I332E (C_H2)
- **Common name:** ADE Fc Variant
- **Engineering purpose:** Multi-domain engineered variant designed to simultaneously optimize FcγRIIIa binding for NK cell-mediated ADCC and maximize the FcγRIIa/FcγRIIb activation ratio for potent macrophage-mediated ADCP.

Functional Profile

Property	Effect
FcγRIIIa binding (V158 & F158)	Strongly Increased ↑↑↑ (~30–50× affinity boost)
FcγRIIa binding (H131 & R131)	Strongly Increased ↑↑ (A/I activation ratio optimized)
FcγRIIb binding (Inhibitory)	Lower relative engagement vs. FcγRIIa
ADCC	Potently Enhanced ↑↑↑
ADCP (Macrophage Phagocytosis)	Potently Enhanced ↑↑↑
CDC	WT Baseline / Preserved
FcRn binding & half-life	Normal / WT Baseline
Effector potency	High-Potency Dual ADCC/ADCP Enhancer

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 **ADE Fc variant** combines three synergistic amino acid substitutions across the lower hinge and C_H2 domain:

- **G236A** (lower hinge): Restructures backbone conformational flexibility at the hinge-C_H2 junction, specifically enhancing affinity for activating FcγRIIa (CD32a) while minimizing engagement with inhibitory FcγRIIb (CD32b).

- **S239D** (C_H2 Domain): Introduces a negative charge that establishes favorable electrostatic complementarity with positively charged surface residues on FcγRIIIa (CD16a).
- **I332E** (C_H2 Domain): Adds a second negatively charged carboxyl group at a critical receptor contact interface, further locking in high-affinity engagement.

Together, these mutations increase FcγRIIIa and FcγRIIa binding by one to two orders of magnitude, providing exceptional dual ADCC and ADCP activation while maintaining normal FcRn recycling and complement C1q binding.

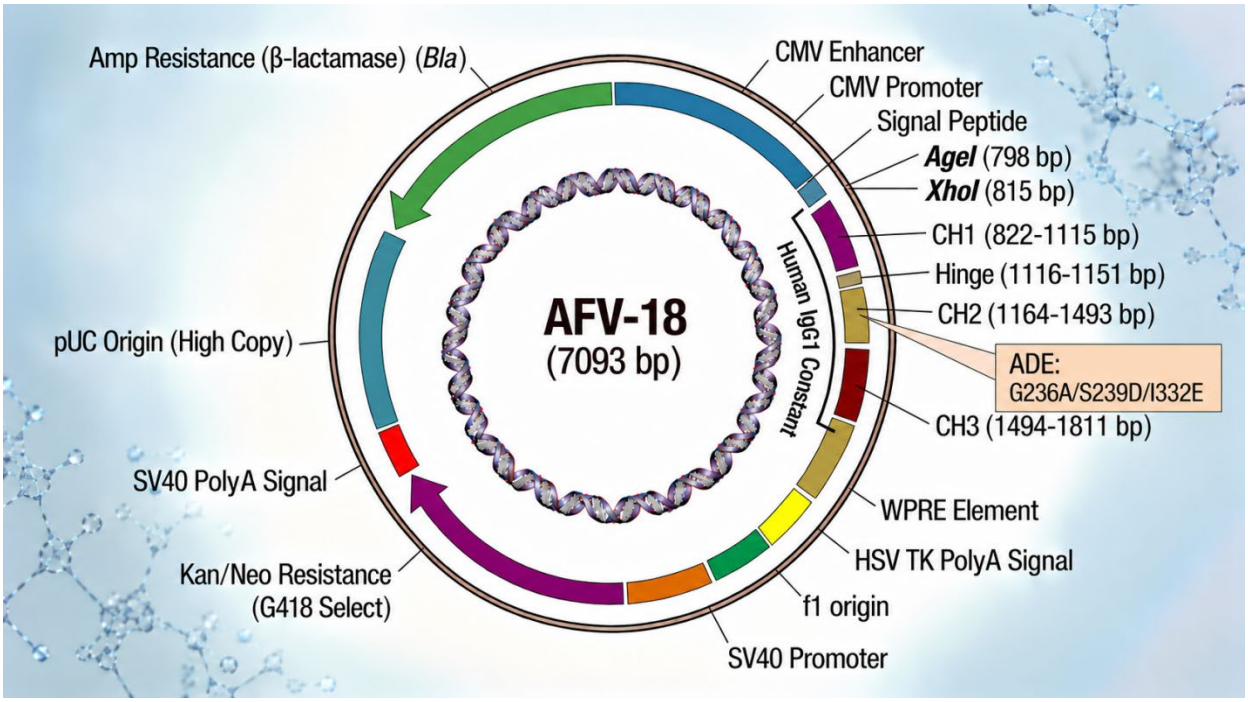
Phenotypic Effects

- Strong enhancement of NK cell recruitment and ADCC target cell lysis
- Optimized FcγRIIa/FcγRIIb binding ratio leading to superior macrophage phagocytosis (ADCP)
- High functional potency across all patient FcγRIIIa genotypes (V/V, V/F, and F/F)
- Preserves physiological complement activation (CDC baseline)
- Maintains standard FcRn endosomal recycling and in vivo systemic persistence

Applications

- Therapeutic oncology antibodies targeting cell surface antigens (e.g., CD20, HER2, EGFR)
- Solid tumor therapeutic formats requiring both NK cell killing and macrophage tumor clearance
- Comparative effector function profiling against DE, DLE, and GASDALIE modules
- Preclinical development of high-potency depleting antibodies with controlled complement activity

Vector Map



Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — native IgG1 core hinge
- **Lower Hinge(G236A)** — FcγRIIa-selective activation mutation
- **C_H2(S239D/I332E)** — ADCC/ADCP-enhancing electrostatic double mutation
- **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
- Handle using 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.

- The ADE Fc variant (G236A/S239D/I332E) represents a balanced, multi-mechanistic effector module in the Fusion BioLabs portfolio. By combining the phagocytosis-enhancing properties of G236A with the core electrostatic double mutant DE (S239D/I332E), ADE delivers potent dual ADCC/ADCP enhancement without requiring the structural hydrophobic packing mutation A330L present in GASDALIE

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)	Lower hinge FcγRIIa selective optimization	★★★☆☆+	Selective ADCP Enhancer. Specifically boosts macrophage phagocytosis by maximizing the FcγRIIa/FcγRIIb activation ratio.

G236A			
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

- *Richards JO, Karki S, Lazar GA, Chen H, Dang W, Dahiyat BI. Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther. 2008;7(8):2517-2527.*
- *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.*
- *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.*

Document Version

- **Datasheet version:** 3.1
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