Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (S239D/I332E) — DE Fc Variant

SKU#: AFV-01

Plasmid Information

- **Plasmid name:** pHC-IgG1-DE(S239D/I332E)
- **Backbone:** Human IgG1 heavy-chain expression vector
- **Insert:** Signal peptide-MCS-C_H1–hinge–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: S239D + I332E (C_H2 domain)
Common name: DE Fc Variant
Engineering purpose: Core dual-mutation engineered to optimize electrostatic interactions at the FcγRIIIa and FcγRIIa interfaces, boosting NK cell-mediated ADCC and macrophage-mediated ADCP potency.

Functional Profile

Property	Effect
FcγRIIIa binding (V158 & F158)	Significantly Increased ↑↑ (~10–20× affinity boost)
FcγRIIa binding	Increased ↑ (Enhanced A/I activation ratio)
ADCC	Potently Enhanced ↑↑↑
ADCP	Enhanced ↑↑
CDC	WT Baseline / Neutral
FcRn binding & half-life	Normal / WT Baseline
Effector potency	Balanced High-Potency ADCC/ADCP

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 DE Fc variant introduces two synergistic amino acid substitutions in the C_H2 domain—Ser239 to Asp and Ile332 to Glu. Inserting negatively charged aspartate and glutamate residues at these positions optimizes electrostatic complementarity with positively charged residues on the extracellular domains of activating receptors, particularly FcγRIIIa (CD16a) and FcγRIIa (CD32a). This dual substitution substantially enhances receptor binding affinity without

perturbing the C_H2-C_H3 interface responsible for FcRn endosomal recycling or disrupting the complement C1q binding interface.

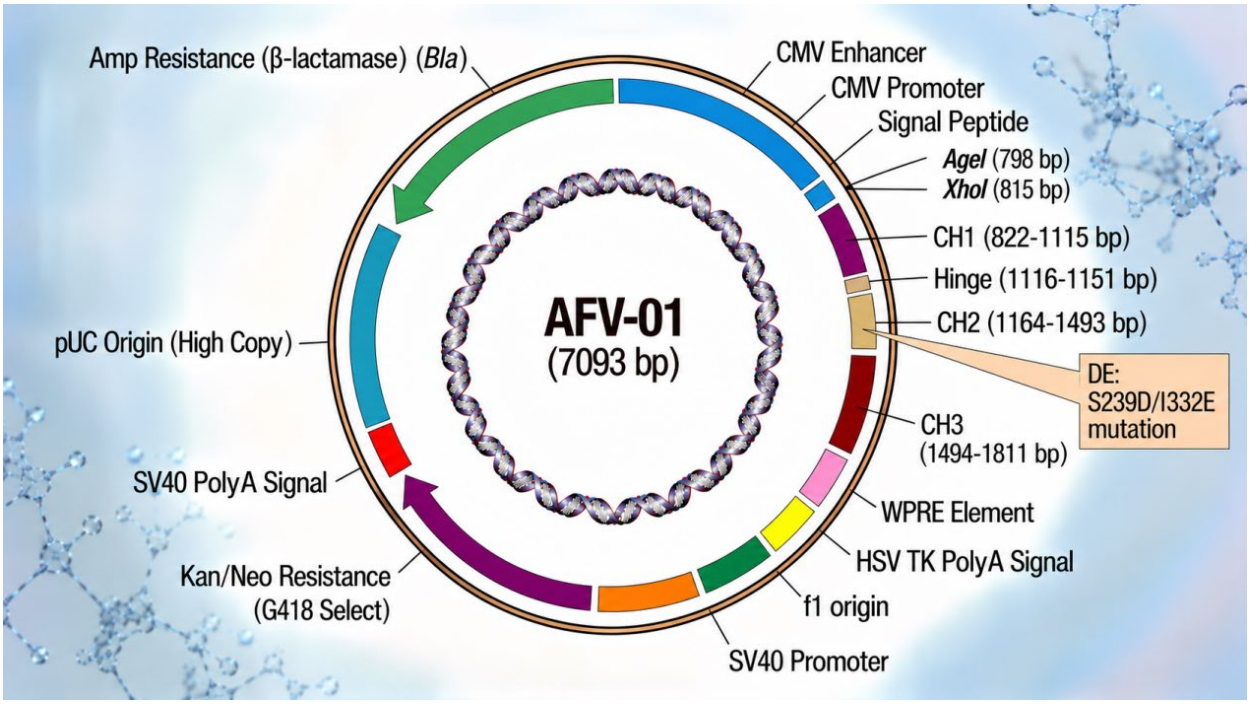
Phenotypic Effects

- Substantial increase in NK cell recruitment and ADCC killing activity
- Enhanced binding to both high-affinity (V158) and low-affinity (F158) FcγRIIIa patient allotypes
- Improved macrophage-mediated phagocytosis (ADCP)
- Retains physiological complement activation (CDC) baseline
- Preserves standard FcRn-mediated recycling and systemic clearance kinetics

Applications

- Therapeutic oncology antibodies requiring enhanced NK- and macrophage-mediated target cell depletion
- Cell-depleting antibody formats against targets with moderate-to-high antigen expression
- Foundational double-mutation module for comparative effector engineering studies
- Optimization of therapeutic formats where controlled, non-extreme ADCC enhancement is desired

Vector Map



Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — native IgG1 hinge
- **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 Fc-Engineered Heavy-Chain 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.

Storage & Handling

- Store plasmid at **–20°C**
- Avoid repeated freeze–thaw cycles
- Use sterile technique when handling
- Suitable for transient or stable mammalian expression

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'

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 DE variant (S239D/I332E) serves as the core foundational module in the Fusion BioLabs ADCC/ADCP-Enhanced Family. It provides a robust boost in effector function and serves as the structural building block for higher-order variants such as DLE (+A330L) and GASDALIE (+G236A/A330L).

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 /	C_H2 domain structural interface enhancement	★★★★☆	Strong ADCC Enhancer. Classic triple mutation optimizing NK cell-mediated lysis.

I332E			
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

- *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

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