Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (S239D/A330L/I332E) — DLE Fc Variant

SKU#: AFV-02

Plasmid Information

- **Plasmid name:** pHC-IgG1-DLE(S239D/A330L/I332E)
- **Backbone:** Human IgG1 heavy-chain expression vector
- **Insert:** Signal peptide-MCS–C_H1–hinge–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: S239D + A330L + I332E (C_H2 domain)
Common name: DLE Fc Variant
Engineering purpose: Classic triple mutation engineered to maximize structural hydrophobic packing and electrostatic complementarity at the FcγRIIIa interface, driving high-potency NK cell-mediated ADCC.

Functional Profile

Property	Effect
FcγRIIIa binding (V158 & F158)	Ultra-High ↑↑↑ (~100× affinity boost)
FcγRIIa / FcγRIIb binding	Increased ↑↑
ADCC	Potently Enhanced ↑↑↑↑
ADCP	Potently Enhanced ↑↑↑
CDC	WT Baseline / Neutral
FcRn binding & half-life	Normal / WT Baseline
Effector potency	Classic High-Potency ADCC Benchmark

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 DLE Fc variant combines three synergistic substitutions within the C_H2 domain: **Ser239 to Asp**, **Ala330 to Leu**, and **Ile332 to Glu**. While `S239D` and `I332E` introduce key electrostatic interactions with positively charged receptor residues, the addition of `A330L` provides hydrophobic contact optimization at the core Fc-FcγRIIIa binding interface. This triple

combination dramatically enhances receptor binding kinetics and structural stability with both V158 and F158 allotypes of FcγRIIIa, translating into high-potency NK cell activation and target cell lysis without impairing FcRn endosomal recycling or complement C1q binding.

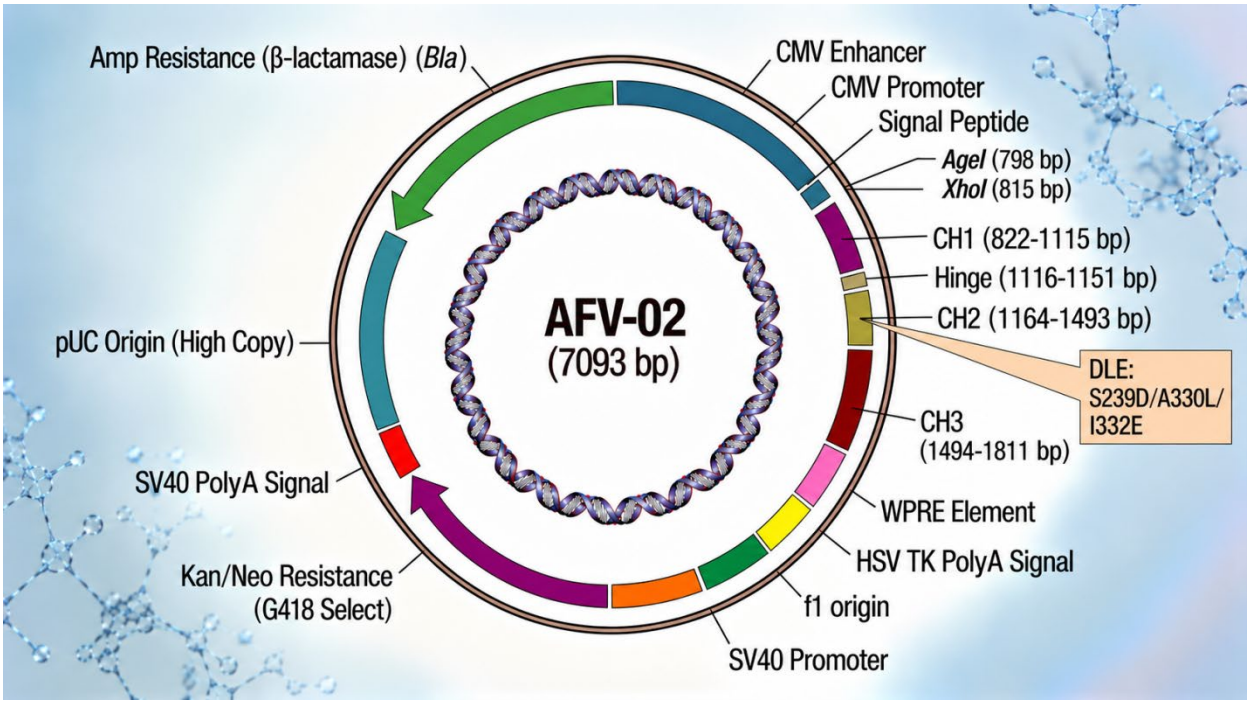
Phenotypic Effects

- Exceptional increase in NK cell recruitment and ADCC killing efficacy (~ 10-100 x potency boost over wild-type)
- Overcomes the low-affinity FcγRIIIa-F158 patient polymorphism barrier
- Robust activation of macrophage-mediated phagocytosis (ADCP)
- Maintains physiological complement activation (CDC) baseline
- Preserves standard FcRn-mediated recycling and systemic clearance kinetics

Applications

- High-potency oncology therapeutics requiring robust NK cell-mediated target elimination
- Cell-depleting antibody formats against low-density surface antigens
- Reference standard / benchmark control in Fc effector engineering studies
- Preclinical development of engineered therapeutic antibodies targeting solid and hematological tum

Vector Map



Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — native IgG1 hinge
- **C_H2(S239D/A330L/I332E)** — ADCC/ADCP high-potency structure triple

- **C_H3(WT)** — native IgG1 C_H3 domain

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'

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.

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.

- The DLE variant (S239D/A330L/I332E) is one of the most widely cited and benchmarked high-potency Fc variants in therapeutic antibody discovery. It delivers strong NK-cell recruitment and target cytotoxicity, serving as the immediate structural stepping stone between the double mutant DE (S239D/I332E) and the quadruple hyper-enhanced variant GASDALIE (G236A/S239D/A330L/I332E).

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

- *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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