Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (SEHF: S267E/H268F) — SEHF Fc Variant

SKU#: AFV-25

Plasmid Information

- **Plasmid name:** pHC-IgG1-SEHF(S267E/H268F)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2(S267E/H268F)-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:** S267E + H268F (C_H2 domain)
- **Common name:** SEHF Fc Variant
- **Engineering purpose:** High-potency double mutation designed to optimize the C1q binding loop, driving strong enhancement of C1q recruitment, C1q binding affinity, and complement-dependent cytotoxicity (CDC).

Functional Profile

Property	Effect
FcγRIIb binding	Strongly Increased ↑↑
FcγRIIIa binding / ADCC	Moderate / Baseline to Slight Shift
CDC	High-Potency Enhancement ↑↑↑ (>10 x C1q affinity)
FcRn binding	Normal / WT Baseline
Serum half-life	Normal / WT Baseline
Effector potency	High-Potency CDC-Dominant (with enhanced FcγRIIb engagement)

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 SEHF Fc variant introduces two targeted substitutions—**Ser267 to Glu** and **His268 to Phe**—within the C_H2 domain loop comprising residues 265–270. S267E introduces a strategic negative charge that enhances ionic interactions with C1q, while H268F provides favorable aromatic/hydrophobic packing. Together, this double mutation stabilizes the local backbone conformation to dramatically boost C1q binding affinity and accelerate membrane attack complex (MAC) assembly, providing a superior complement activation threshold compared to single-point or classical loop variants without requiring intermolecular hexamerization.

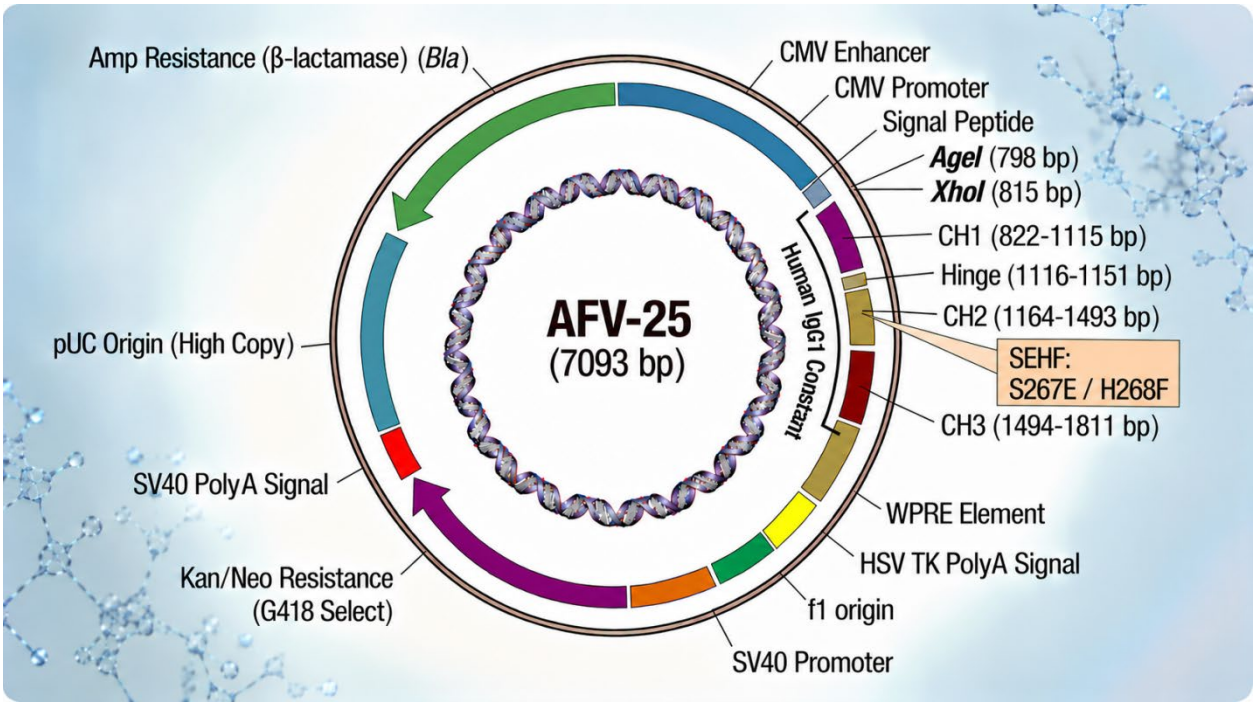
Phenotypic Effects

- High-potency enhancement of complement-dependent cytotoxicity (CDC)
- Substantially increased C1q binding affinity (>10-fold over wild-type IgG1)
- Superior lytic activity against targets expressing low-to-moderate antigen density
- Simultaneous enhancement of inhibitory FcγRIIb binding
- Preserves normal FcRn-mediated recycling and systemic pharmacokinetics

Applications

- Therapeutic antibodies targeting low-density cell-surface antigens requiring potent CDC
- Oncology antibody formats engineered for robust complement-mediated cell lysis
- Dual-functional candidates requiring combined CDC enhancement and FcγRIIb cross-linking
- Alternative complement-boosting strategy for constructs incompatible with hexamerizing mutations

Sequence Map



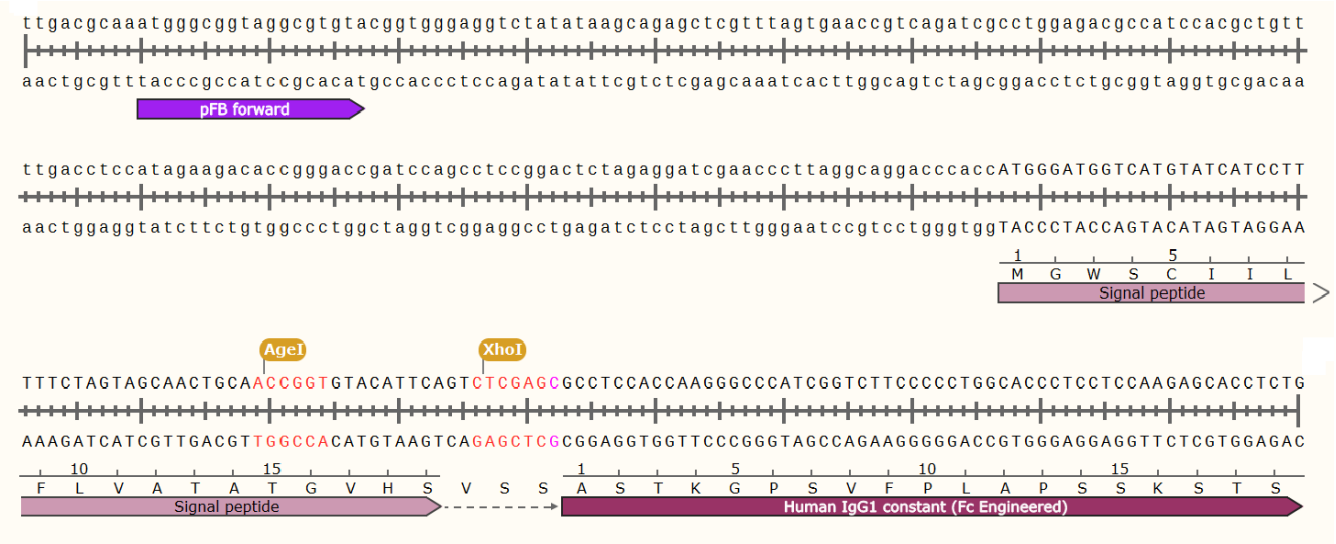
Feature annotation:

- C_H1 — constant heavy 1
- **Hinge** — native IgG1 hinge
- C_H2(S267E/H268F) — **high-potency C1q-enhancing double mutation**
- C_H3 — native IgG1 C_H3

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.

- The SEHF Fc variant represents a second-generation complement-enhancing module engineered via rational interface redesign. By directly optimizing the C_H2 loop interface for C1q, SEHF provides higher CDC activity than classic modifications like WS (K326W/E333S) while maintaining standard monovalent Fc structure and monomeric expression performance.

Comparison within Fusion BioLabs Complement-Modulated Family

Variant	Mechanism	Complement Effect	Primary Application & Notes
human IgG1 (WT)(Wild-Type)	Native C1q binding	★★☆☆☆	Baseline Control. Standard physiological C1q binding and CDC baseline for comparative assays.
human IgG1 (HexaBody-Extended) E345K / E430G / E431K	On-cell hexamerization	★★★★★+	Hyper-CDC Enhancer. Triple mutation maximizing Fc-Fc intermolecular interactions; yields ultra-potent C1q avidity against ultra-low density antigen targets.
human IgG1 (HexaBody) E345K / E430G	On-cell hexamerization	★★★★★	Canonical CDC-Boosting Fc. Enhances cell-surface hexamer assembly upon antigen binding to drastically boost C1q recruitment and membrane attack complex (MAC) formation.
human IgG1 (SEHF) S267E / H268F	C1q binding loop enhancement	★★★★☆	High-Potency CDC Enhancer. Optimizes local electrostatic and structural interactions with C1q to boost complement lysis without requiring hexamerization.
human IgG1 (WS) K326W / E333S	C1q binding loop enhancement	★★★☆☆	Strong CDC Enhancer. Synergistic double mutation in CH2 that significantly increases C1q binding affinity and complement killing.
human IgG1 (K322A) K322A	C1q contact site ablation	★☆☆☆☆	Selective Human IgG1 CDC-Null. Abolishes C1q binding and CDC while maintaining native FcγR engagement (ADCC+/ADCP+).
human IgG2 (K322A) K322A	C1q contact site ablation	★☆☆☆☆	Completely Silent Human IgG2 Control. Eliminates residual IgG2 C1q binding to yield a fully inert neutralizer (ADCCnull/ADCPnull/CDCnull).
mouse IgG2a (CompNull) L235E+E318A/K320A/K322A	Complement binding triad ablation	★☆☆☆☆	Murine CDC-Null Control. Completely abolishes complement activation in murine models while preserving mouse FcγR engagement for syngeneic in vivo studies.

References

- *Moore GL, Chen H, Karki S, Lazar GA. Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. MAbs. 2010;2(2):181-189.*
- *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
- **Last updated:** 1 November 2026