Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (HexaBody: E345K/E430G) — Complement-Enhanced Hexamerization Fc Variant

SKU#: AFV-21

Plasmid Information

- **Plasmid name:** pHC-IgG1-HexaBody(E345K/E430G)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2(wild-type)-C_H3(E345K/E430G)
- **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:** E345K + E430G (C_H3 domain)
- **Common name:** Canonical HexaBody Fc Variant
- **Engineering purpose:** Next-generation complement-enhancing double mutation engineered to induce antigen-dependent Fc-Fc hexamerization on target cell membranes, driving ultra-potent C1q recruitment and complement-dependent cytotoxicity (CDC).

Functional Profile

Property	Effect
FcγRI/II/III binding	Normal / WT Baseline
ADCC & ADCP	Normal / WT Baseline
CDC	Ultra-Enhanced ↑↑↑↑ (Avidity-driven MAC assembly)
FcRn binding	Normal / WT Baseline
Serum half-life	Normal / WT Baseline
Effector potency	Ultra-Potent CDC Enhancement (Selective C1q recruitment boost)

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 HexaBody Fc variant introduces two structural substitutions—**Glu345 to Lys** and **Glu430 to Gly**—within the C_H3-C_H3 intermolecular contact interface. E345K introduces a positive charge that promotes favorable electrostatic interactions between adjacent Fc monomers. E430G increases local main-chain flexibility, lowering the thermodynamic barrier for self-assembly. Upon target antigen binding, these modifications drive the cooperative clustering of six antibody monomers into ordered hexameric rings at the cell surface. This arrangement creates a high-avidity docking platform that perfectly matches the

hexameric head structure of C1q, triggering massive complement activation without requiring fluid-phase aggregation.

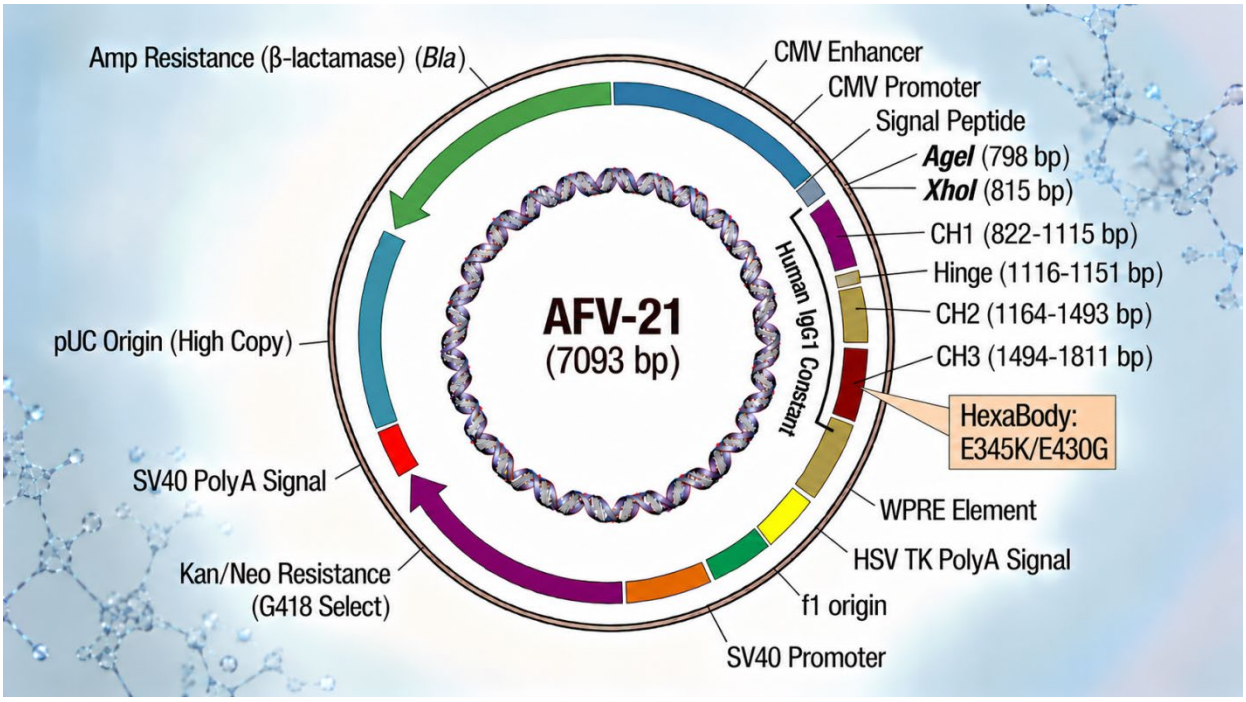
Phenotypic Effects

- Ultra-potent activation of complement-dependent cytotoxicity (CDC)
- Massive enhancement of C1q recruitment via high-avidity hexameric interface geometry
- Preserves baseline FcγR binding and cellular effector functions (ADCC/ADCP)
- Maintains normal FcRn-mediated recycling and systemic pharmacokinetics
- Excellent biophysical stability and monomeric profile in solution prior to antigen binding

Applications

- Therapeutic antibodies targeting low-to-moderate density cell-surface tumor antigens
- Enhancement of CDC-depleting mechanisms for oncology targets (e.g., CD20, CD19, CD38)
- Infectious disease therapeutics requiring rapid, complement-mediated pathogen or host-cell destruction
- Benchmark scaffold for studying cell-surface Fc-Fc clustering and complement cascade kinetics

Vector Map



Feature annotation:

- C_H1 — constant heavy 1
- **Hinge** — native IgG1 hinge
- C_H2(wild-type) — native IgG1 C_H2 domain (retains N297 glycosylation)
- C_H3(E345K/E430G) — **hexamerization-inducing double mutation**

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 9^{th}--8^{th} to 4^{th} 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 canonical HexaBody module (E345K/E430G) represents a paradigm shift in Fc engineering. Rather than altering direct contact residues for C1q within C_H2, HexaBody enhances C1q avidity by organizing monomeric IgGs into ordered cell-surface arrays upon antigen engagement. This preserves native solution-state monomeric stability while achieving CDC potencies orders of magnitude above wild-type IgG1.

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

- *de Jong RN, Beurskens FJ, Verploegen S, Strumane K, van Kampen MD, Voorhorst M, Horstman W, Engelberts PJ, Oostindie SC, Wang G, Heck AJ, Schuurman J, Parren PW. A Novel Platform for the Potentiation of Therapeutic Antibodies Based on Antigen-Dependent Formation of IgG Hexamers at the Cell Surface. PLoS Biol. 2016 Jan 6;14(1):e1002344.*

Document Version

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