Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (HexaBody-Extended: E345K/E430G/E431K) — Hyper-CDC Fc Variant
SKU#: AFV-22

Plasmid Information

- **Plasmid name:** pHC-IgG1-HexaBody-Extended(E345K/E430G/E431K)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2(wild-type)-C_H3(E345K/E430G/E431K)
- **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 + E431K (C_H3 domain)
- **Common name:** HexaBody-Extended Fc Variant
- **Engineering purpose:** Premium triple mutation engineered to maximize intermolecular Fc-Fc interactions and drive ultra-efficient cell-surface hexamerization, achieving maximal CDC potency even against targets with ultra-low antigen density.

Functional Profile

Property	Effect
FcγRI/II/III binding	Normal / WT Baseline
ADCC & ADCP	Normal / WT Baseline
CDC	Hyper-Enhanced ↑↑↑↑↑ (Maximum C1q avidity)
FcRn binding	Normal / WT Baseline
Serum half-life	Normal / WT Baseline
Effector potency	Maximum CDC Potency (Top-tier complement activation)

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-Extended Fc variant combines three strategically engineered C_H3-domain substitutions—**Glu345 to Lys**, **Glu430 to Gly**, and **Glu431 to Lys**. The addition of E431K alongside E345K creates complementary salt bridges and electrostatic networks across adjacent Fc monomers, while E430G provides loop flexibility to optimize alignment. Upon antigen binding, this triple mutation dramatically lowers the threshold required for on-cell Fc-Fc self-assembly into hexameric platforms. The resulting ring structure presents six optimal binding sites for C1q, driving hyper-potent complement cascade activation and membrane attack complex (MAC) assembly.

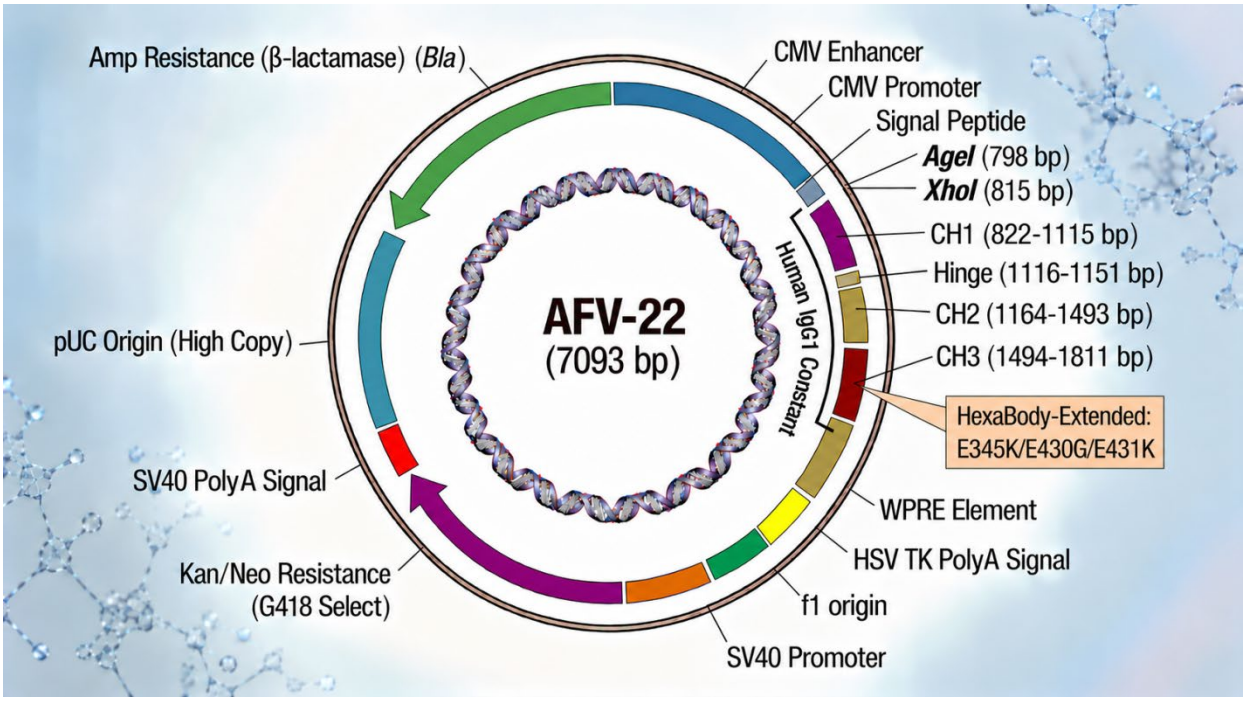
Phenotypic Effects

- Peak complement-dependent cytotoxicity (CDC) potency across the Fusion BioLabs catalog
- Triggers robust complement activation on targets with very low antigen expression or target density
- Preserves wild-type FcγR engagement and baseline cell-mediated cytotoxicity (ADCC/ADCP)
- Retains native FcRn endosomal recycling and systemic clearance kinetics
- Maintains standard monomeric IgG stability in solution prior to antigen engagement

Applications

- Therapeutic antibody programs targeting ultra-low density or refractory cell-surface antigens
- High-potency oncology therapeutics requiring maximum complement-mediated target depletion
- Anti-pathogen antibodies targeting sparse viral envelope or bacterial surface proteins
- Benchmark standard for evaluating the upper thermodynamic limits of Fc hexamerization

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/E431K) — **hyper-hexamerization triple 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.
Content	5.0 µg in Tris-EDTA 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 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 HexaBody-Extended (E345K/E430G/E431K) variant represents the ultimate complement-enhancing module in antibody engineering. By optimizing multiple intermolecular contact sites within the C_H3 interface, it surpasses canonical HexaBody (E345K/E430G) in driving hexamer formation on challenging targets where low antigen density normally limits C1q recruitment.

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

- *Diebolder CA, Beurskens FJ, de Jong RN, Koning RI, Strumane K, Lindorfer MA, Voorhorst M, Ugurlar D, Rosati S, Heck AJ, van de Winkel JG, Wilson IA, Koster AJ, Taylor RP, Saphire EO, Burton DR, Schuurman J, Gros P, Parren PW. Complement is activated by IgG hexamers assembled at the cell surface. Science. 2014;343(6176):1260-1263.*
- *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;14(1):e1002344.*

Document Version

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