Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (WS: K326W/E333S) — WS Fc Variant
SKU#: AFV-20

Plasmid Information

- **Plasmid name:** pHC-IgG1-WS(K326W/E333S)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2(K326W/E333S)-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:** K326W + E333S (C_H2 domain)
- **Common name:** WS Fc Variant
- **Engineering purpose:** Synergistic double mutation to significantly enhance C1q binding, boost complement-dependent cytotoxicity (CDC), and moderately increase ADCC.

Functional Profile

Property	Effect
FcγRIIIa binding	Moderately Increased ↑ (~ 1.5-2 x)
ADCC	Moderately Enhanced ↑
CDC	Strongly Increased ↑↑ (~ 5 x C1q affinity)
FcRn binding	Normal / WT Baseline
Serum half-life	Normal / WT Baseline
Effector potency	Dual Enhanced (CDC-dominant with moderate ADCC 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 WS Fc variant introduces two synergistic C_H2-domain mutations—**Lys326 to Trp** and **Glu333 to Ser**—that optimize the Fc–C1q interaction surface. K326W introduces a bulky hydrophobic tryptophan residue that improves packing at the C1q interface. E333S removes a negative charge, enhancing electrostatic complementarity with C1q. Together; these mutations increase C1q binding affinity by approximately 5-fold and drive robust complement activation and lysis, while also providing a modest boost in FcγRIIIa engagement.

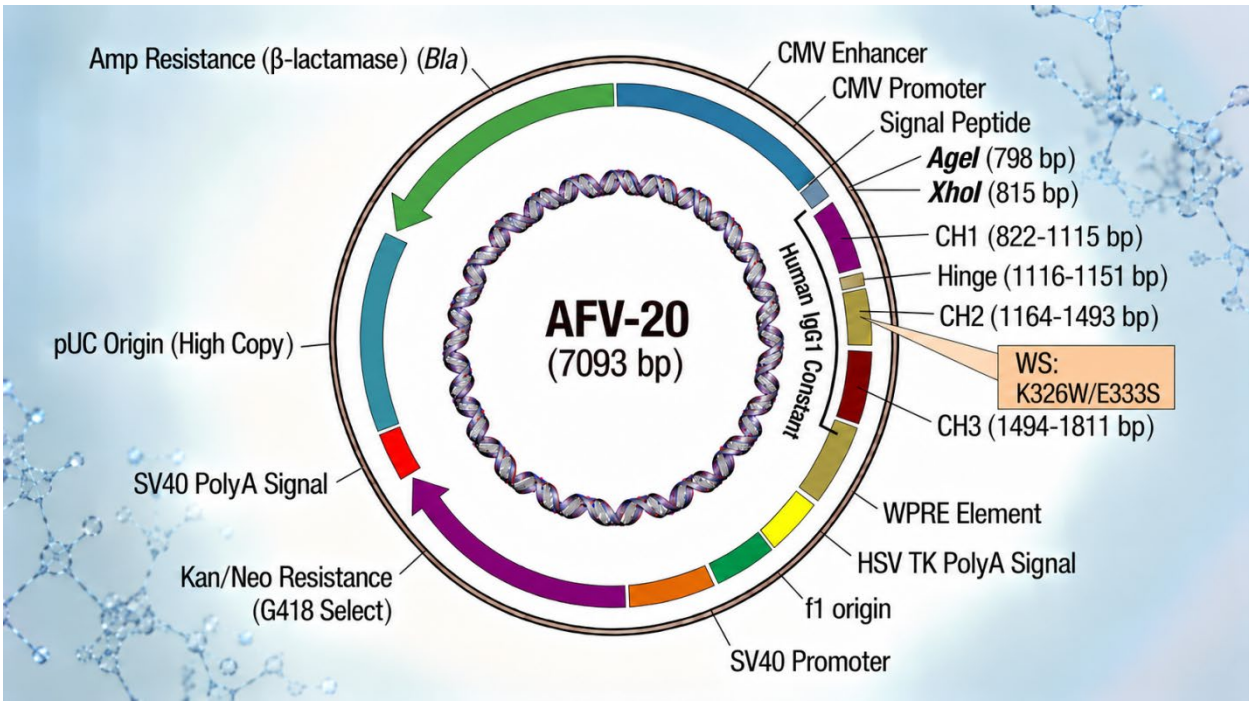
Phenotypic Effects

- Strongly enhanced complement-dependent cytotoxicity (CDC)
- Significantly increased C1q binding affinity (~ 5 x)
- Moderate enhancement of FcγRIIIa binding and ADCC activity
- Maintains normal FcRn-mediated endosomal recycling and serum half-life
- Ideal for cell-depleting antibodies requiring heightened dual-effector function

Applications

- Oncology antibodies where CDC is a primary mechanism of action
- Depleting therapeutics targeting low-density cell-surface antigens
- Preclinical optimization of dual CDC/ADCC-enhanced therapeutic candidates
- Mechanistic benchmark for C1q–Fc interface interactions

Vector Map



Feature annotation:

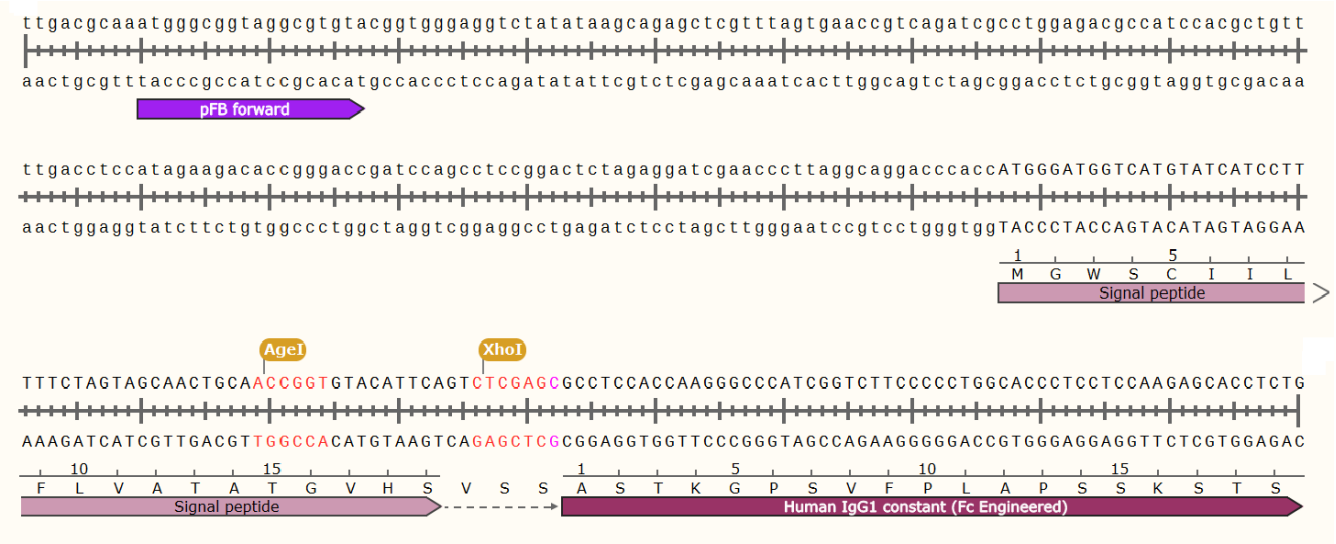
- C_H1 — constant heavy 1
- **Hinge** — native IgG1 hinge
- C_H2(K326W/E333S) — **complement-enhancing double mutation**
- C_H3 — native IgG1 C_H3

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 WS Fc variant is a classic complement-enhancing Fc module. It is one of the most widely cited CDC-enhancing double mutants in antibody engineering literature and serves as an ideal choice when heightened complement activation is required without sacrificing ADCC or FcRn stability.

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

- *Idusogie EE, Wong PY, Presta LG, Gazzano-Santoro H, Totpal K, Ultsch M, Mulkerrin MG. Engineered antibodies with increased activity to recruit complement. J Immunol. 2001;166(4):2571-2575.*

Document Version

- **Datasheet version:** 3.1
- **Last updated:** 1 November 2026