Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG2 (K322A) — K322A Fc Variant

SKU#: AFV-24

Plasmid Information

- **Plasmid name:** `pHC-IgG2-K322A`
- **Backbone:** Human IgG2 heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2 (K322A)-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:** `K322A` (C_H2 domain)
- **Common name:** K322A Fc Variant
- **Engineering purpose:** Single amino acid substitution designed to selectively abolish C1q binding and complement-dependent cytotoxicity (CDC) while fully retaining native FcγR engagement ($ADCC^+$ / $ADCP^+$) and FcRn recycling.

Functional Profile

Property	Effect
FcγR Binding (I, IIa, IIIa)	Normal / WT Baseline
ADCC & ADCP	Normal / WT Baseline
CDC	Completely Ablated ↓↓↓ (CDC-Null)
FcRn binding	Normal / WT Baseline
Serum half-life	Normal / WT Baseline
Effector potency	Selective CDC-Null ($ADCC^+$ / $ADCP^+$ / CDC^{null})

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 K322A Fc variant replaces lysine at position 322 in the C_H2 domain with alanine (**Lys322 to Ala**). Lysine 322 serves as an essential charged contact point within the core globular C1q binding motif. Disruption of this side-chain interaction eliminates C1q recruitment and downstream complement cascade initiation without altering the structural integrity of the lower hinge or C_H2-C_H3 domain interfaces responsible for FcγR and FcRn interaction.

Phenotypic Effects

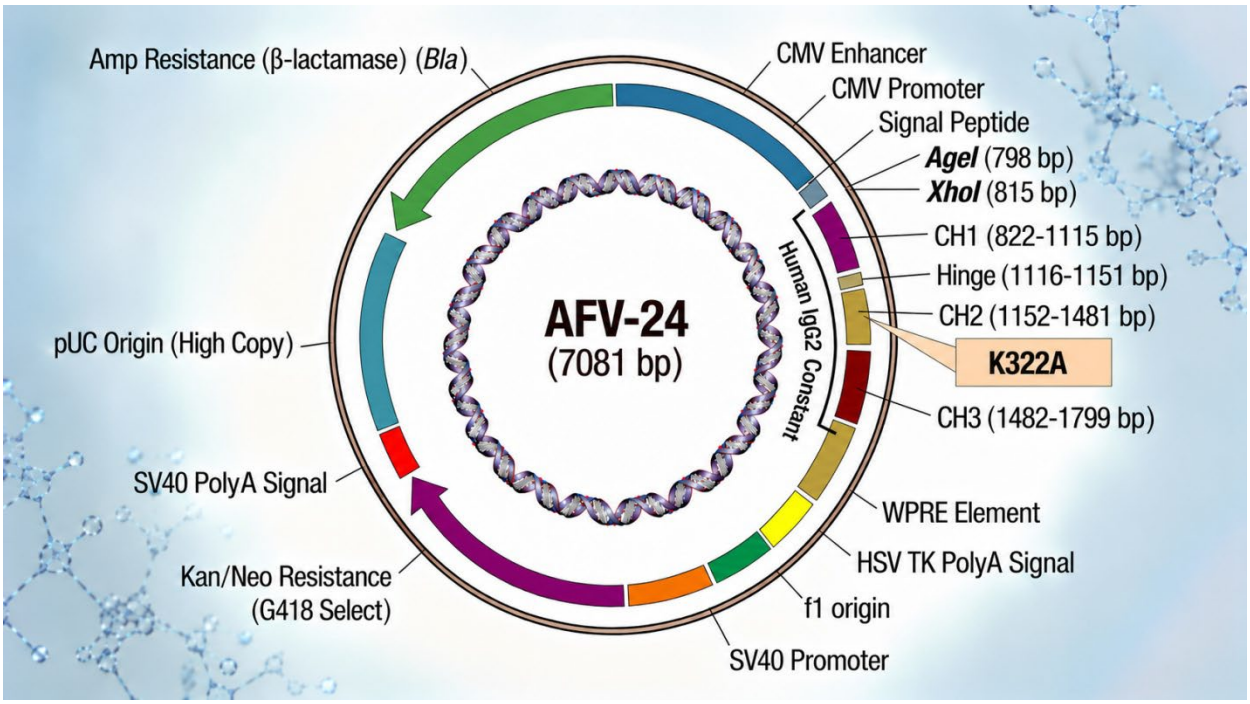
- Complete loss of C1q binding and complement-dependent cytotoxicity (CDC)

- Full retention of native FcγR-mediated effector functions (ADCC and ADCP)
- Eliminates complement-mediated systemic toxicity, cytokine release, or off-target cell lysis
- Preserves normal FcRn binding, endosomal recycling, and systemic pharmacokinetics

Applications

- Therapeutic oncology antibodies requiring ADCC/ADCP-driven cell depletion without complement activation
- Minimizing complement-related side effects in therapeutic antibody development
- Negative control for evaluating the specific contribution of CDC in target depletion assays
- Mechanistic dissecting of complement vs. cellular Fc-receptor effector pathways

Vector Map



Feature annotation:

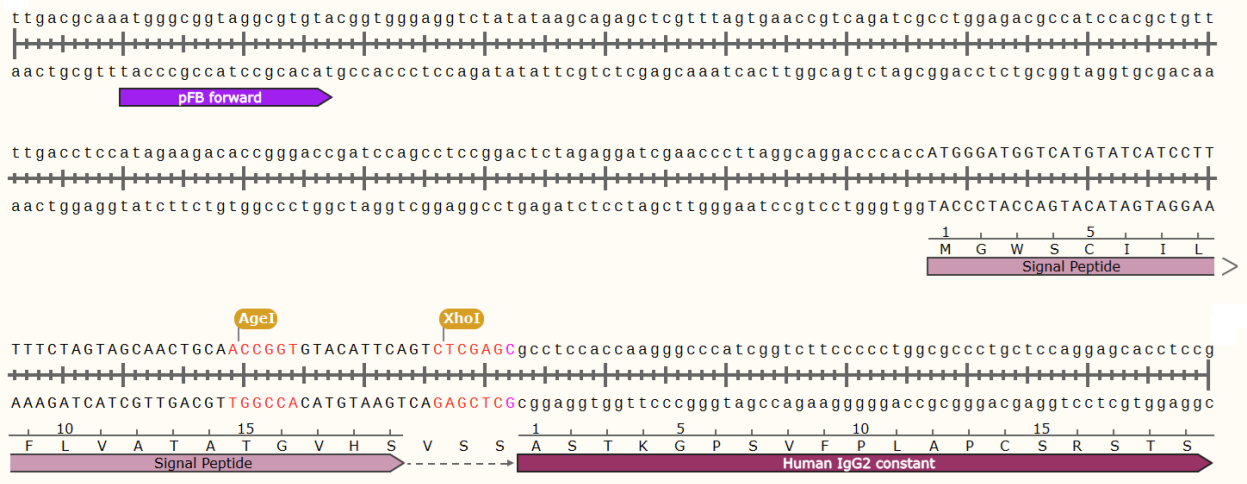
- C_H1 — constant heavy 1
- **Hinge** — native IgG2 hinge
- C_H2(K322A) — **selective CDC-ablating single mutation**
- C_H3 — native IgG2 C_H3

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 K322A mutation is the gold-standard engineering strategy for generating selective CDC-null human IgG2 therapeutics. First identified in landmark alanine-scanning studies, K322A provides surgical elimination of C1q binding while leaving cell-mediated effector mechanisms (ADCC/ADCP) completely intact.

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, Presta LG, Gazzano-Santoro H, Totpal K, Wong PY, Ultsch M, Meng YG, Mulkerrin MG. Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. J Immunol. 2000;164(8):4178-4184.*

- *Hezareh M, Hessell AJ, Jensen RC, van de Winkel JG, Parren PW. Effector function activities of a panel of mutants of a broadly neutralizing antibody against human immunodeficiency virus type 1. J Virol. 2001;75(24):12161-12168.*

Document Version

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