Fusion BioLabs Fc Engineering Vector Datasheet

Mouse IgG2a (CompNull: L235E + E318A/K320A/K322A) — Complement-Null Mouse Fc Variant

SKU#: AFV-10

Plasmid Information

- **Plasmid name:** pMC-IgG2a-CompNull(L235E+E318A/K320A/K322A)
- **Backbone:** Mouse IgG2a heavy chain expression vector
- **Insert:** Signal peptide–MCS-C_H1-hinge-C_H2(L235E/E318A/K320A/K322A)-C_H3(wild-type)
- **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:** L235E + E318A + K320A + K322A (C_H2 domain)
- **Common name:** Mouse IgG2a CompNull Variant
- **Engineering purpose:** Four coordinated substitutions engineered to completely abolish C1q binding and complement activation in murine models while preserving native mouse FcγR engagement ($ADCC^+$ / $ADCP^+$).

Functional Profile

Property	Effect
Mouse FcγRI/IIb/III/IV binding	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 Murine 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 mouse IgG2a CompNull Fc variant targets two core elements of the classical complement activation interface within the C_H2 domain. **Leu235 to Glu** alters the hydrophobic lower-hinge region required for optimal C1q docking. Concurrently, **Glu318 to Ala**, **Lys320 to Ala**, and **Lys322 to Ala** ablate the canonical C1q-binding motif (the "E-K-K triad"). Together, these four modifications eliminate C1q recruitment and downstream membrane attack complex (MAC) assembly while keeping the mouse FcγR and FcRn interaction surfaces intact.

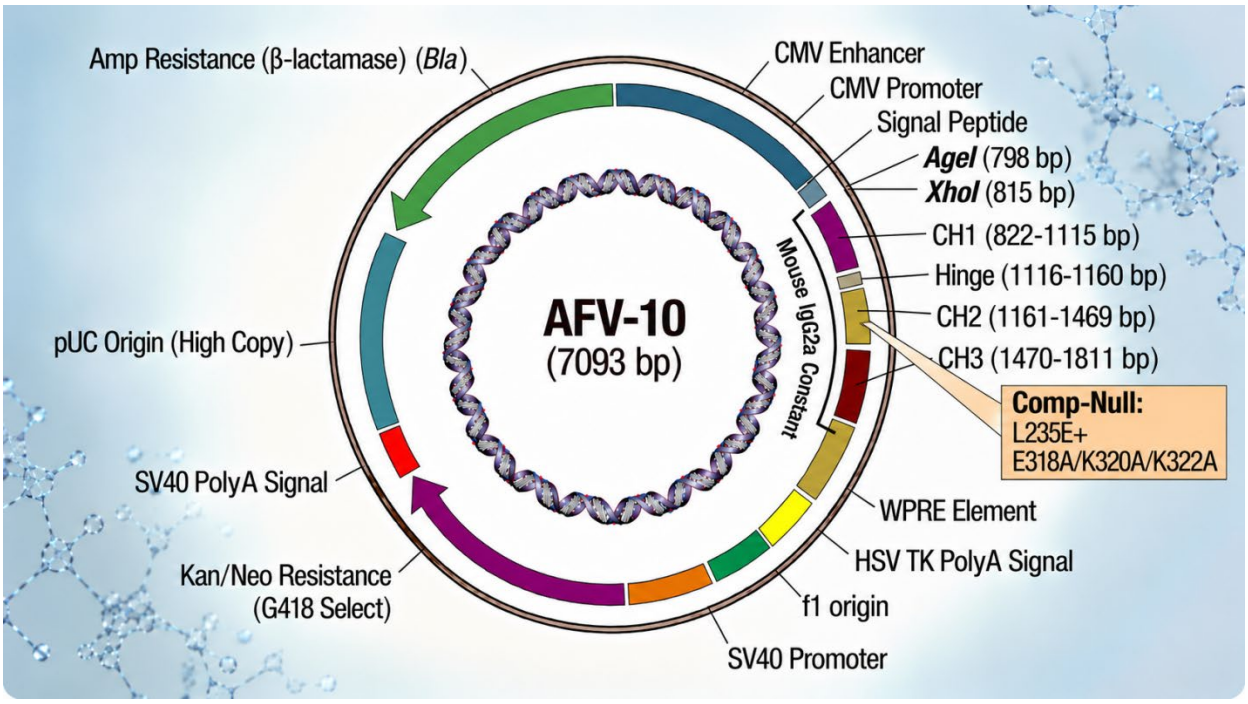
Phenotypic Effects

- Absolute loss of C1q binding affinity and complement-dependent cytotoxicity (CDC)
- Full preservation of native mouse FcγR engagement, mediating intact ADCC and ADCP
- Eliminates complement-mediated systemic activation or toxicity in syngeneic mouse models
- Preserves normal FcRn endosomal recycling and systemic clearance kinetics

Applications

- Syngeneic mouse *in vivo* studies requiring complete elimination of complement activation
- Mechanistic dissection separating FcγR-mediated cellular effector functions from complement activation
- Development of safety-optimized surrogate mouse antibodies for translational evaluation
- Murine equivalent/control for human CDC-null variants (e.g., human IgG1 K322A)

Vector Map



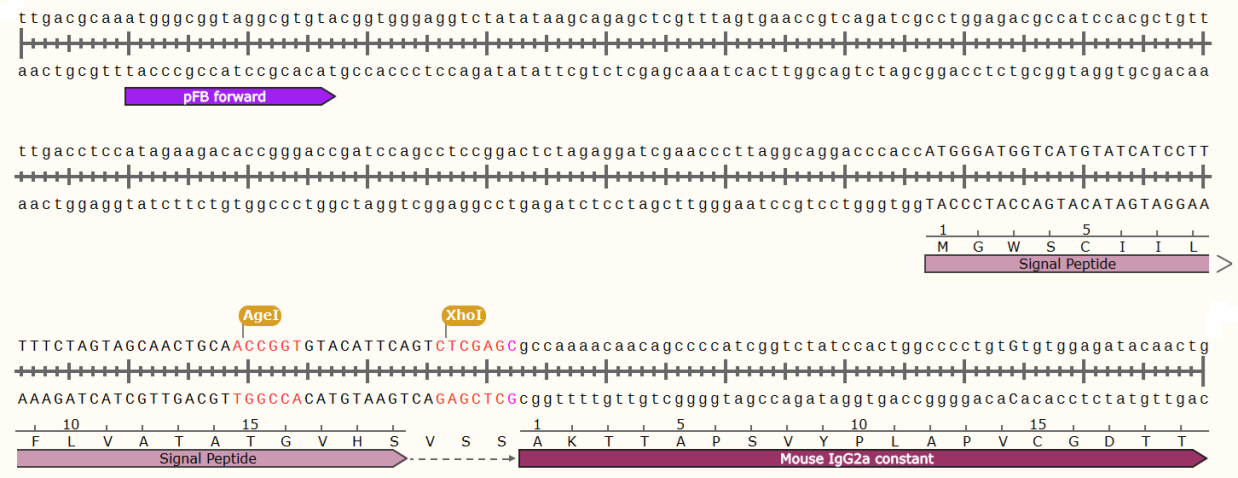
- **Feature annotation:**
  - C_H1 — constant heavy 1
  - **Hinge** — native mouse IgG2a hinge
  - C_H2(L235E/E318A/K320A/K322A) — complement-binding triad ablation mutation
  - C_H3(wild-type) — native mouse IgG2a C_H3 domain

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'- GGGCTGTTGTTTTGGCGCTCGAGACN_{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'- GGGCTGTTGTTTTGGCN_{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.

- CompNull serves as the flagship complement-null mouse Fc vector in the Fusion BioLabs collection. While wild-type mouse IgG2a is the most potent complement-fixing murine isotype, the L235E + E318A/K320A/K322A combination provides multi-point disruption of the C1q interface, ensuring complete complement silence without compromising cellular effector functions or pharmacokinetics.

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 C_H2 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

- *Duncan AR, Woof JM, Partridge LJ, Burton DR, Winter G. Localization of the binding site for the human high-affinity Fc receptor on IgG. Nature. 1988; 332(6164):563-564.*
- *Duncan AR, Winter G. The binding site for C1q on IgG. Nature. 1988; 332(6166):738-740.*

Document Version

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