Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (ΔG236) *(Deletion of Glycine 236 — Effector-Null Fc Variant)*

SKU#: AFV-16

Plasmid Information

- **Plasmid name:** pHC-IgG1-ΔG236
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert structure:** Signal peptide–MCS–C_H1–hinge–lower hinge(ΔG236)–C_H2(wild-type)-C_H3(wild-type)
- **Selection 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:** ΔG236 (single-residue deletion in the lower hinge)
- **Common name:** ΔG236 Fc Variant / Lower Hinge Deletion Variant
- **Engineering purpose:** Minimalist, single-residue Fc modification that selectively abolishes FcγR engagement and cellular effector functions (ADCC/ADCP) while preserving native C_H2 *N*-glycosylation, structural thermal stability, and baseline complement engagement.

Functional Profile

Property	Effect
FcγRI/IIa/IIb/IIIa binding	Severely Suppressed / Abolished ↓↓↓
ADCC	Severely Reduced / Minimal ↓↓↓
ADCP (Macrophage Phagocytosis)	Severely Reduced / Minimal ↓↓↓
C1q binding	Preserved / Baseline (WT-like loop integrity)
CDC	Preserved / Mildly Attenuated
FcRn binding & half-life	Normal / WT Baseline
Glycosylation & Stability	Intact (N297 glycan retained; high thermal stability T_m)

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

Deletion of Glycine 236 (**Δ**G236) operates via a targeted structural dislocation of the lower hinge:

- **Lower Hinge Contraction:** Glycine 236 forms the crucial structural pivot point at the lower hinge-C_H2 junction (EU residues 231–236). Deleting this single residue shortens the lower hinge loop, preventing the conformational flexibility required to fit into the extracellular ligand-binding cleft of FcγR receptors.

- **Preservation of C1q & *N*-Glycan Sites:** Because the deletion is restricted entirely to the lower hinge, downstream C_H2 structural loops—including the core ***N297*** glycosylation site and the C1q binding site (K322/P329/P331)—remain intact.

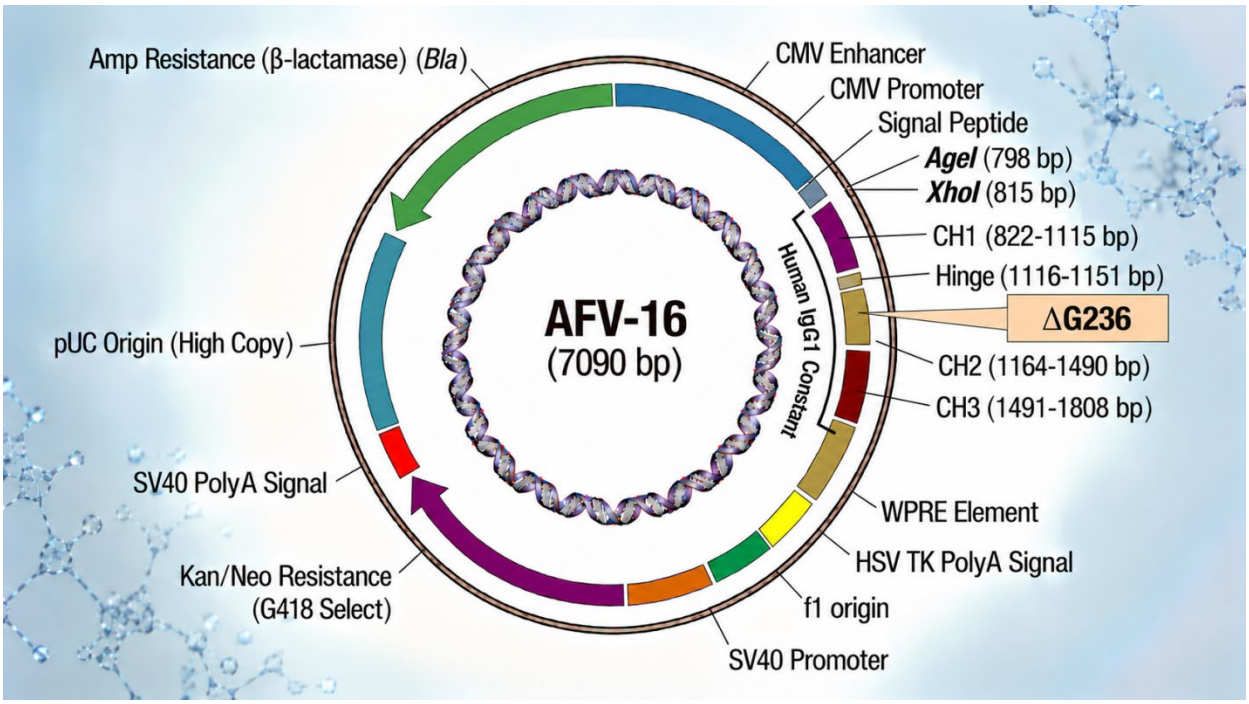
Phenotypic Effects

- Profound loss of binding across all activating (FcγRI, FcγRIIa, FcγRIIIa) and inhibitory (FcγRIIb) receptors
- Eliminates NK cell ADCC and macrophage ADCP cytotoxicity
- Maintains standard FcRn endosomal recycling and in vivo systemic half-life
- Preserves physiological *N*-glycosylation and native C_H2 domain thermal stability (*Tm* ≈ 70°C)

Applications

- Therapeutic formats requiring selective cellular silencing without aglycosylation
- Bispecific antibodies and receptor traps where FcγR cross-linking causes off-target toxicity
- Mechanistic controls comparing single-residue lower hinge modifications against multi-point mutants (LALA-PG, EN)
- Diagnostic and imaging reagents requiring FcRn-mediated extended persistence without cellular effector activation

Vector Map



Feature annotation:

- C_H1 — constant heavy 1
- **Hinge** — native IgG1 core hinge

- **Lower Hinge(ΔG236)** — single-residue lower hinge deletion site
- C_H2(WT) — wild-type C_H2 domain containing intact *N*297 glycosylation site
- C_H3(WT) — native IgG1 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)-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 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
- Handle using sterile technique
- Suitable for transient or stable mammalian expression (CHO, HEK293, NS0)

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 human IgG1 (ΔG236) variant offers a structurally elegant alternative to heavily mutated Fc-null designs. By utilizing a single deletion rather than introducing multiple non-native amino acid substitutions, ΔG236 minimizes immunogenicity risks while delivering potent selective FcγR silencing.

Comparison within Fusion BioLabs Effector-Null Family

Variant	Mechanism	Strength	Notes
human IgG1 (WT)	Wild-Type baseline	Baseline	Human WT Control. Native FcγR and C1q binding; fully active baseline control.
mouse IgG2a (WT)	Wild-Type baseline	Baseline	Murine WT Control. Native mouse FcγR and complement binding; active control for syngeneic models.
human IgG1 (EN) E233P / L234V / L235A / ΔG236+ A327G	Lower hinge & C_H2 chimeric sequence substitution	★★★★★	Ultra-Silent Chimeric Null. Cross-subclass IgG2/IgG4 sequence replacement; abolishes FcγR & CDC

/ A330S / P331S			engagement.
human IgG1 (LALA-PG) L234A / L235A / P329G	Lower hinge & C_H2 proline-sandwich null	★★★★★	Clinical Gold Standard. Complete, double-silent suppression of all human FcγRs and C1q/CDC.
mouse IgG2a (LALA-PG) L234A / L235A / P329G	Murine lower hinge & C_H2 proline-sandwich	★★★★★	In Vivo Murine Gold Standard. Complete silencing of murine FcγRs and complement in syngeneic mouse models.
human IgG1 (LALA) L234A / L235A	Lower hinge double alanine substitution	★★★☆☆	Classic Benchmark Control. Widely used legacy variant; retains minor residual FcγRI (CD64) binding.
human IgG1 (ΔG236) ΔG236	Lower hinge single residue deletion	★★★☆☆	Selective FcγR-Null. Dislocates FcγR binding site while preserving native N-glycan structure and C1q loops.
human IgG1 (N297Q) N297Q	C_H2 domain N-glycan site elimination	★★★★☆	Human Aglycosylated Control. Broad effector silencing via glycan removal; lowers thermal stability (Tm).
mouse IgG2a (N297Q) N297Q	Murine C_H2 domain N-glycan site elimination	★★★★☆	Murine Aglycosylated Control. Aglycosylated mouse control for in vivo preclinical rodent studies.

References

- *Brinkhaus M, Douwes RGJ, Bentlage AEH, Temming AR, de Taeye SW, Tammes Buirs M, Gerritsen J, Mok JY, Brasser G, Ligthart PC, van Esch WJE, Verheesen P, de Haard H, Rispens T, Vidarsson G. Glycine 236 in the Lower Hinge Region of Human IgG1 Differentiates FcγR from Complement Effector Function. J Immunol. 2020;205(12):3456-3467.*
- *Richards JO, Karki S, Lazar GA, Chen H, Dang W, Desjarlais JR. Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther. 2008;7(8):2517-2527.*

Document Version

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