Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (EN) —*Multi-Residue Effector-Null Fc Variant*

SKU#: AFV-04

Plasmid Information

- **Plasmid name:** pHC-IgG1- EN
- **Fc variant name:** human IgG1 (EN)
- **Insert structure:** Signal peptide-MCS-C_H1 – hinge(E233P/L234V/L235A/ΔG236)-C_H2(A327G/A330S/P331S) – C_H3(WT)
- **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

The human IgG1 (EN) Fc variant is an ultra-silent, multi-residue chimeric effector-null backbone based on the Armour paradigm. By transplanting sequence elements from non-effector human IgG2 and IgG4 subclasses into the IgG1 lower hinge (E233P/L234V/L235A/ΔG236) and C_H2 complement loop (A327G/A330S/P331S), EN completely abolishes all FcγR engagement (FcγRI, IIa, IIb, IIIa) and C1q complement activation. It serves as Fusion BioLabs' premier ultra-silent chimeric option.

Functional Profile

Property	Effect
FcγRI/IIa/IIb/IIIa binding	Completely Abolished / Undetectable ↓↓↓↓
ADCC	Completely Abolished ↓↓↓↓
ADCP (Macrophage Phagocytosis)	Completely Abolished ↓↓↓↓
C1q binding	Completely Abolished ↓↓↓↓
CDC	Completely Abolished ↓↓↓↓
FcRn binding & half-life	Normal / WT Baseline
Glycosylation & Thermal Stability	Intact (*N*297 glycan retained; native CH2 stability Tm≈70∘C)

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 EN variant employs a dual-subdomain chimeric substitution strategy within the lower hinge and C_H2 domain:

- **Lower Hinge Silencing** (E233P/L234V/L235A/ΔG236): Replaces the IgG1 lower hinge with the corresponding IgG2 sequence motif (PVA) and deletes Glycine 236. This eliminates the flexible hydrophobic ridge required for high-affinity FcγRI (CD64) and low-affinity FcγRII/III binding.
- **C_H2 Loop Silencing** (A327G/A330S/P331S): Replaces IgG1 residues with IgG4/IgG2 equivalents in the Proline 331 loop. This disrupts the globular head C1q binding interface and prevents complement activation.
- **Combined Effect:** Dual-level disruption resulting in complete effector silencing while retaining full endosomal FcRn recycling and wild-type *N*297 glycosylation.

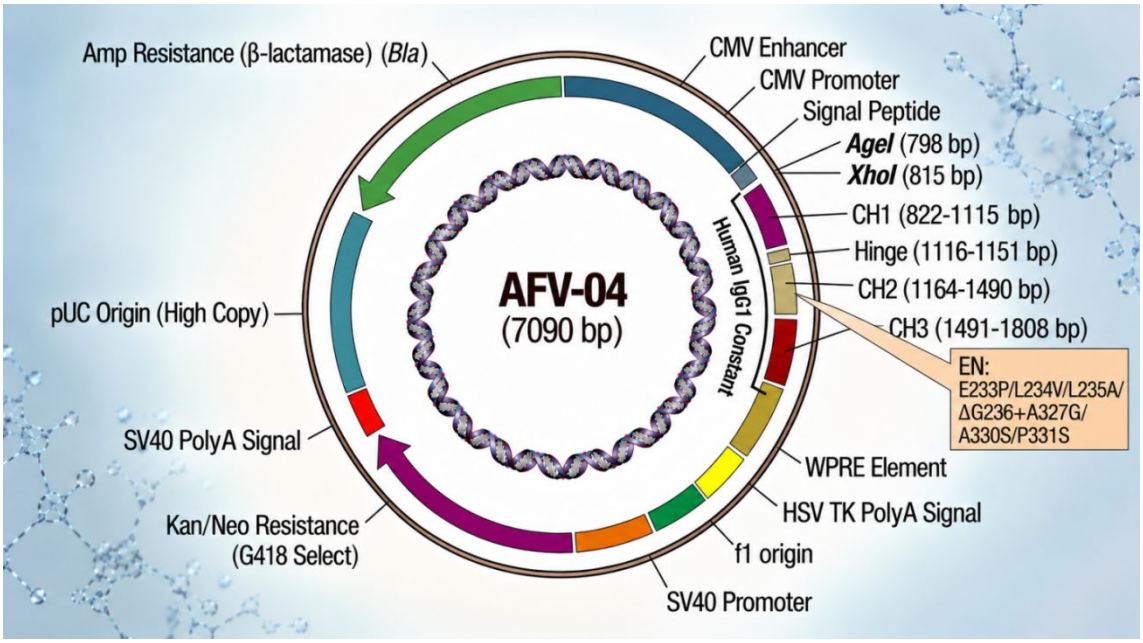
Phenotypic Effects

- Complete absence of NK cell-mediated ADCC, macrophage ADCP, and antibody-dependent cellular phagocytosis
- Total loss of C1q binding and classical complement cascade activation (CDC-null)
- Zero detectable binding across all human Fcγ receptor classes
- Preserves standard IgG1 pharmacokinetic half-life and endosomal recycling in vivo
- Retains native *N***297** glycosylation, biophysical solubility, and high production yield

Applications

- Safety-critical therapeutic antibodies where zero effector activity or Fc-mediated cytokine release is required
- Immune checkpoint blockers (anti-PD-1, anti-PD-L1, anti-TIGIT, anti-CTLA-4) targeting cell-surface receptors on delicate immune populations
- Agonist antibodies requiring pure target engagement without Fc receptor cross-linking
- Multispecific and bispecific formats requiring an ultra-silent, non-immunogenic Fc backbone
- Primary comparative reference for testing multi-residue sequence replacements against proline-sandwich variants (LALA-PG)

Vector Map



Feature annotation:

- C_H1 — constant heavy 1
- Hinge — native IgG1 core hinge
- Lower Hinge (E233P/L234V/L235A/ΔG236) — IgG2-derived lower hinge substitution and deletion
- C_H2(A327G/A330S/P331S) — C_H2 complement loop substitutions (retains native *N*297 glycan)
- 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)

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

- **Plasmid:** Store at −20 °C
- **Purified antibody:**
  - 2–8 °C for short-term storage
  - −80 °C for long-term storage
- Avoid repeated freeze–thaw cycles
- Use sterile technique for all handling

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.

- Human IgG1 (EN) is a highly reinforced, multi-substituted effector-null Fc variant. Because its mutations are derived from human IgG2 and IgG4 sequence motifs, it delivers complete functional silencing while minimizing potential immunogenicity.

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 / A330S / P331S	Lower hinge & C_H2 chimeric sequence substitution	★★★★★	Ultra-Silent Chimeric Null. Cross-subclass IgG2/IgG4 sequence replacement; abolishes FcγR & CDC 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

- *Armour KL, Clark MR, Hadley AG, Williamson LM. Recombinant human IgG molecules lacking Fcgamma receptor I binding and monocyte triggering activities. Eur J Immunol. 1999;29(8):2613-2624.*
- *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.*
- *Armour KL, van de Winkel JG, Williamson LM, Clark MR. Differential binding to human FcgammaRIIa and FcgammaRIIb receptors by human IgG wildtype and mutant antibodies. Mol Immunol. 2003;40(9):585-593.*

Document Version

- **Datasheet version:** 3.1
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