Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (LALA-PG: L234A/L235A/P329G)— *Effector-Null Fc Variant*

SKU#: AFV-13

Plasmid Information

- **Plasmid name:** pHC-IgG1-LALA-PG
- **Fc variant name:** human IgG1 (LALA-PG)
- **Mutation set:** L234A+ L235A(lower hinge) + P329G(C_H2 domain)
- **Insert structure:** Signal peptide–MCS–C_H1–hinge–lower hinge(L234A/L235A) – C_H2(P329G)-C_H3(wild-type)
- **Backbone:** Human IgG1 heavy chain expression vector
- **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 (LALA-PG) Fc variant combines **two lower hinge mutations (L234A/L235A)** with a **C_H2 domain mutation (P329G)** to completely eliminate all Fcγ receptor binding (FcγRI, IIa, IIb, IIIa) and complement C1q activation, while preserving native FcRn interaction, structural stability, and normal IgG1 pharmacokinetics. It is widely recognized as the clinical gold-standard **effector-null Fc backbone** in therapeutic antibody development.

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 C_H2 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 LALA-PG variant operates via a double-silent synergistic mechanism located within the lower hinge and C_H2 domain:

- **L234A/L235A (Lower Hinge):** Double alanine substitution in the lower hinge region disrupts the primary hydrophobic backbone interface required for FcγR engagement, severely diminishing binding across all receptor classes.
- **P329G (C_H2 Domain):** Mutating Proline 329 to Glycine breaks the conserved "proline sandwich" formed between Fc and tryptophan residues (W381/W382) on FcγRs, while concurrently destabilizing the core C1q binding loop.
- **Combined Effect:** Complete effector silencing (FcγR-null + C1q-null). Eliminates residual FcγRI (CD64) binding observed in legacy LALA double mutants while maintaining full endosomal FcRn recycling and native *N*-glycosylation.

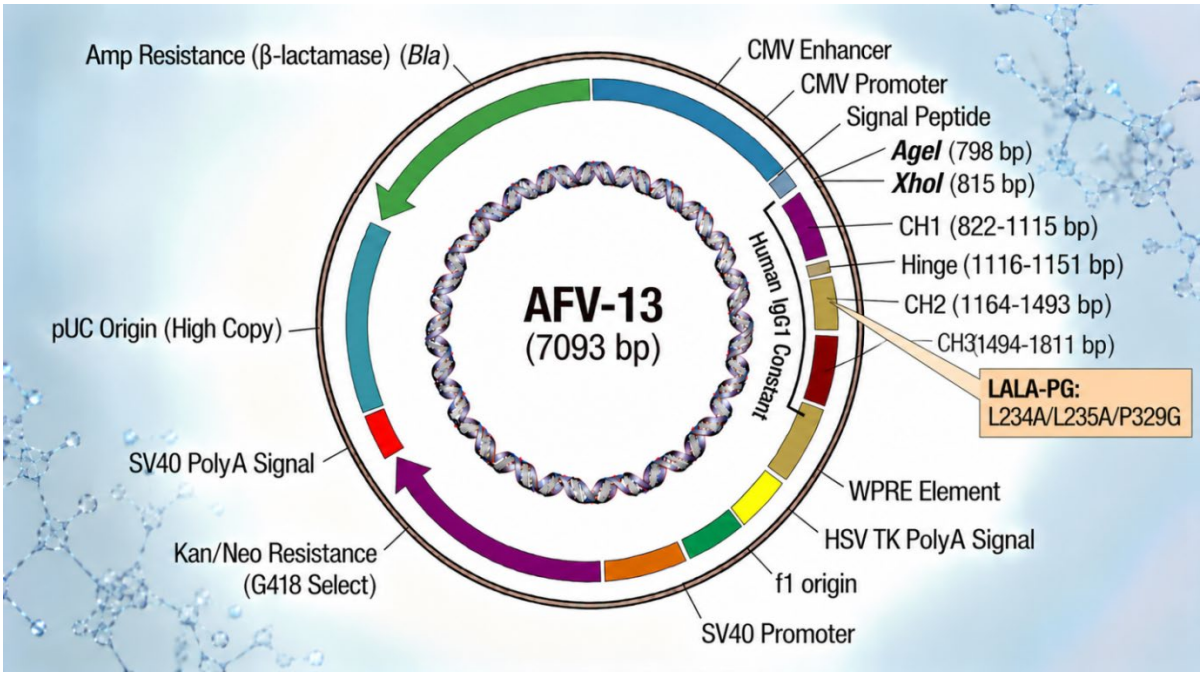
Phenotypic Effects

- Complete absence of NK cell-mediated ADCC and macrophage-mediated ADCP
- Total ablation of classical complement pathway activation and C1q fixation (CDC-null)
- Zero detectable binding to all human Fcγ receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa)
- Normal pharmacokinetic profile and serum persistence matching wild-type IgG1
- Maintains full biophysical stability (*Tm*), yield, and manufacturability without needing aglycosylation

Applications

- Immune checkpoint inhibitors (PD-1, PD-L1, TIGIT, CTLA-4) requiring non-depleting receptor blockade
- Agonist antibodies requiring target engagement without Fc-mediated receptor cross-linking or platelet activation
- Multispecific and bispecific antibody constructs requiring zero Fc-mediated toxicity
- Safety-optimized therapeutic antibodies targeting immune cell populations
- Primary clinical-grade reference control for effector-null assays

Vector Map



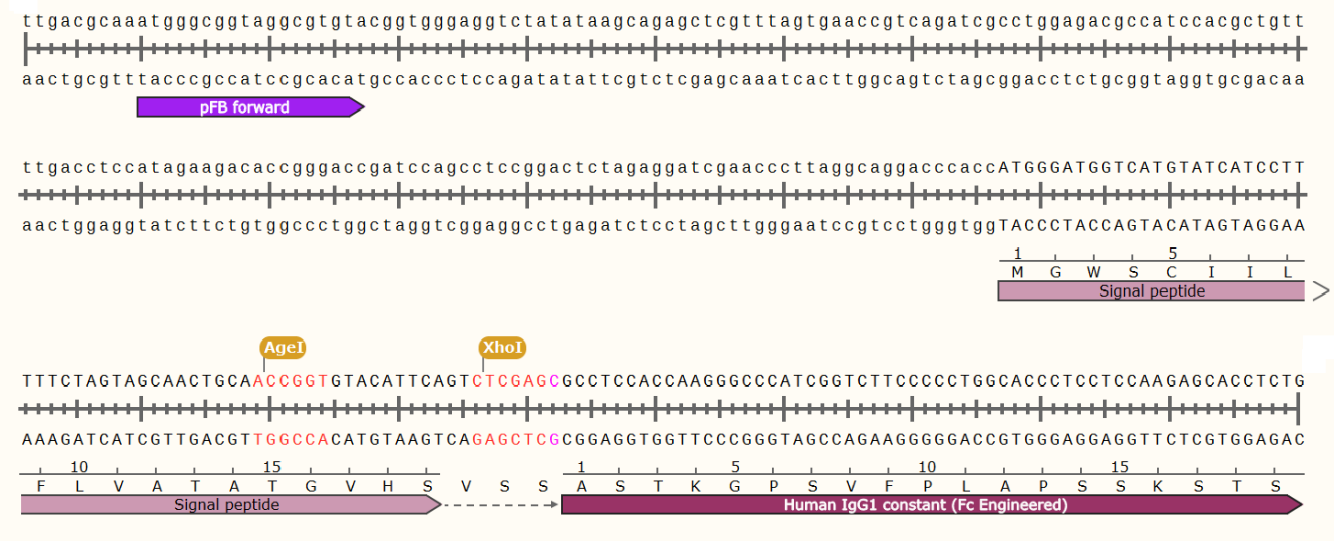
Feature annotation:

- C_H1 — constant heavy 1
- Hinge — native IgG1 core hinge
- Lower Hinge (L234A/L235A) — lower hinge alanine substitutions
- C_H2(P329G) — C_H2 domain proline-sandwich mutation (abolishes C1q and residual FcγR binding; contains *N297* glycan in C_H2)
- CH3(WT) — native human IgG1 CH3 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.

- LALA-PG is considered the premier industry-standard effector-null Fc backbone. By incorporating P329G into the classic LALA framework, it achieves total immunogenic and functional silencing while retaining native *N*-glycosylation (*N*297) and high biophysical stability (*Tm* ≈ 70°C).

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

- *Schlothauer T, Herter S, Koller CF, Grau-Richards S, Steinhart V, Spick C, Kubbies M, Klein C, Umaña P, and Mössner E (2016) Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. Protein Eng. Des. Sel. 29, 457–466.*
- *Lo M, Kim HS, Tong RK, Bainbridge TW, Vernes JM, Zhang Y, Lin YL, Chung S, Dennis MS, Zuchero YJ, Watts RJ, Couch JA, Meng YG, Atwal JK, Brezski RJ, Spiess C, Ernst JA (2017) Effector-attenuating Substitutions That Maintain Antibody Stability and Reduce Toxicity in Mice. J Biol Chem. 292(9):3900-3908.*

Document Version

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