Fusion BioLabs Fc Engineering Vector Datasheet

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

SKU#: AFV-12

Plasmid Information

- **Plasmid name:** pHC-IgG1- LALA
- **Fc variant name:** human IgG1 (LALA)
- **Mutation set:** L234A + L235A (lower hinge/C_H2)
- **Insert structure:** Signal peptide–MCS – C_H1 – hinge – lower hinge(L234A/L235A) – C_H2(WT)-C_H3(WT)
- **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) Fc variant introduces two alanine substitutions (L234A/L235A) into the lower hinge region to disrupt the canonical hydrophobic interaction interface required for FcγR engagement and complement C1q recruitment. It significantly reduces immune effector functions while preserving native endosomal FcRn recycling, structural stability (Tm ≈ 70°C), and normal IgG1 pharmacokinetics. LALA is the foundational benchmark legacy effector-null Fc variant widely used in therapeutic antibody discovery.

Functional Profile

Property	Effect
FcγRI (CD64) binding	Residual Low-Affinity Binding Retained ↓↓↓
FcγRIIa/IIb/IIIa binding	Severely Reduced / Minimal ↓↓↓
ADCC	Strongly Attenuated / Minimal ↓↓↓
ADCP (Macrophage Phagocytosis)	Strongly Attenuated / Minimal ↓↓↓
C1q binding	Strongly Reduced ↓↓
CDC	Strongly Reduced ↓↓
FcRn binding & half-life	Normal / WT Baseline
Glycosylation & Thermal Stability	Intact (N297 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 double substitution acts directly upon the upper contact interface of the Fc region:

- Lower Hinge Disruption: Residues L234 and L235 form a vital hydrophobic contact patch within the lower hinge (EU 231–236). Replacing these hydrophobic leucine residues with alanine severely destabilizes binding across the FcγR family.
- C1q Depletion: The mutations partially collapse the adjacent hinge-C_H2 junction, reducing initial C1q attachment and subsequent complement cascade progression.
- Residual Binding Note: Because the proline-sandwich residue (P329) remains wild-type, LALA retains minor residual binding to high-affinity FcγRI (CD64), which can trigger subtle background activation under high receptor density.

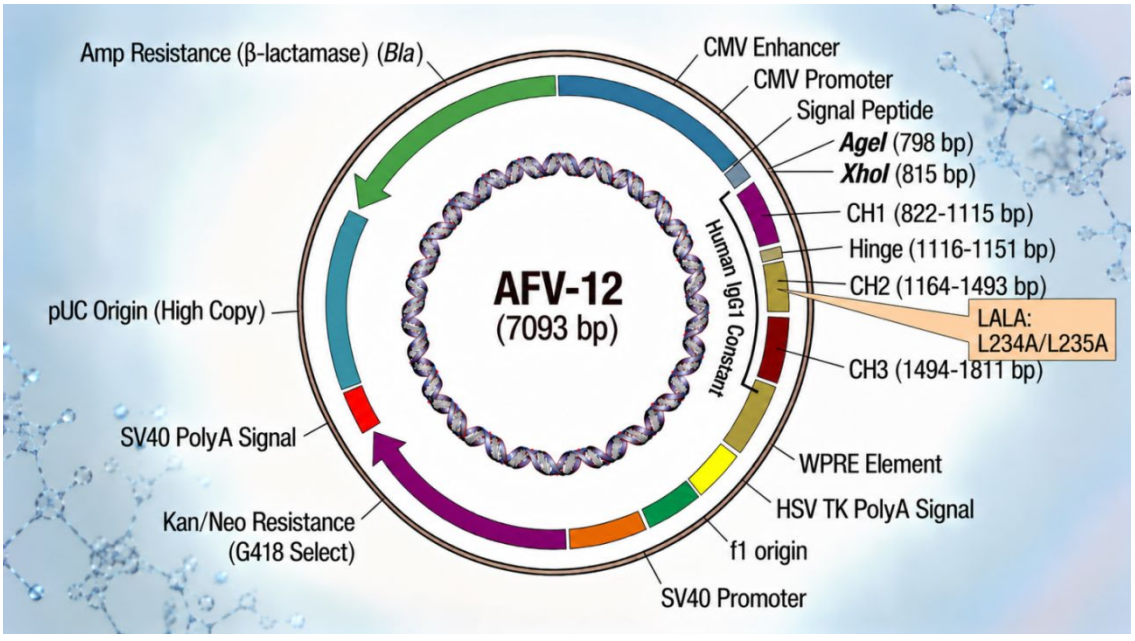
Phenotypic Effects

- Substantial reduction of NK cell-mediated ADCC and macrophage-mediated ADCP
- Strong attenuation of complement fixation and classical pathway activation (CDC)
- Retains low-level baseline interaction with FcγRI
- Preserves standard FcRn recycling dynamics and serum half-life
- Maintains high thermal melting temperature (*Tm*), structural integrity, and production yields identical to wild-type human IgG1

Applications

- Standard legacy baseline control for evaluating effector-attenuated antibody candidates
- Checkpoint inhibitors (PD-1, PD-L1, TIGIT) requiring reduced effector engagement
- Blockade and neutralization therapies where full effector activity is undesirable
- Comparative benchmark against reinforced double-silent variants (LALA-PG, EN)

Vector Map



Feature annotation:

- C_H1 — constant heavy 1
- Hinge — native IgG1 core hinge
- Lower Hinge/ C_H2 (L234A/L235A) — double alanine substitutions at EU positions 234 and 235 (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 9^{th}--8^{th} to 4^{th} 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.

- While IgG1 (LALA) remains a widely published and historically validated effector-null backbone, developers seeking total elimination of residual FcγRI binding or CDC should consider the reinforced **human IgG1 (LALA-PG)** or **human IgG1 (EN)** variants.

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 / Armour)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

- *Xu D, Alegre ML, Varga SS, Rothermel AL, Collins AM, Pulito VL, Hanna LS, Dolan KP, Parren PW, Bluestone JA, Jolliffe LK, Zivin RA. In vitro characterization of five humanized OKT3 effector function variant antibodies. Cell Immunol. 2000;200(1):16-26.*
- *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 Dec;75(24):12161-12168.*

Document Version

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