Fusion BioLabs Fc Engineering Vector Datasheet

Mouse IgG2a (LALA-PG: L234A/L235A/P329G) — *In Vivo* Murine Effector-Null Fc Variant

SKU#: AFV-14

Plasmid Information

- **Plasmid name:** pMC-IgG2a-LALA-PG(L234A/L235A/P329G)
- **Backbone:** Mouse IgG2a heavy-chain expression vector
- **Insert:** Signal peptide–MCS –C_H1–hinge–lower hinge(L234A/L235A)-C_H2(P329G)–C_H3(wilde-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:** L234A + L235A (lower hinge) + P329G (C_H2 domain)
- **Common name:** Mouse IgG2a LALA-PG / Murine Effectorless Fc Variant
- **Engineering purpose:** Next-generation sequence-silenced murine Fc vector designed to completely eliminate binding to all mouse Fcγ receptors (FcγRI, IIb, III, IV) and murine complement C1q. Serves as the primary double-silent surrogate vector for syngeneic mouse model studies, providing direct translational equivalence to human IgG1 (LALA-PG).

Functional Profile

Property	Effect
Mouse FcγRI/IIb/III/IV binding	Completely Abolished / Undetectable ↓↓↓↓
ADCC (Murine NK / Granulocyte)	Completely Abolished ↓↓↓↓
ADCP (Murine Macrophage)	Completely Abolished ↓↓↓↓
Mouse C1q binding	Completely Abolished ↓↓↓↓
CDC (Murine Complement)	Completely Abolished ↓↓↓↓
mFcRn binding & half-life	Normal / WT Baseline
Glycosylation & Stability	Intact (N297 glycan retained; native thermal stability Tm)

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 (LALA-PG) variant utilizes the "proline sandwich" engineering strategy across the lower hinge and C_H2 domain:

- **L234A / L235A (Lower Hinge):** Double alanine substitution in the lower hinge region disrupts the primary hydrophobic and ionic interaction surfaces required for binding to mouse FcγRI, FcγRIIb, FcγRIII, and FcγRIV.

- **P329G (C_H2 Domain):** Proline-to-glycine substitution disrupts the conserved hydrophobic sandwich formed with W381 and W382 of FcγR extracellular domains, while simultaneously abolishing C1q globular head binding.

Together, these three mutations eliminate both cellular receptor activation and classical complement fixation in mice while keeping the *N*-linked glycan at position 297 intact.

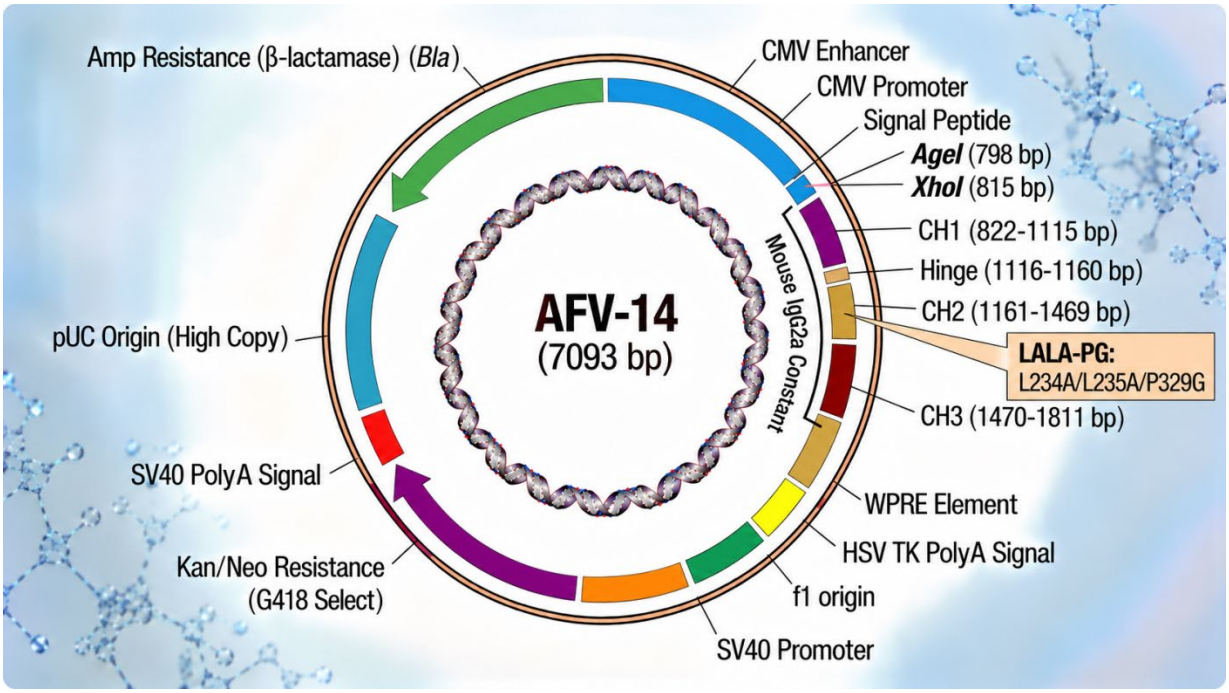
Phenotypic Effects

- Total ablation of antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) in mouse immune systems
- Prevents target cell depletion, immune complex-mediated platelet activation, and cytokine release syndrome (CRS) in rodent models
- Maintains standard murine FcRn endosomal recycling and normal in vivo serum pharmacokinetics
- Preserves physiological glycosylation and biophysical stability without risking the structural destabilization associated with aglycosylated variants (*N*297*Q*)

Applications

- Syngeneic mouse model studies evaluating receptor agonists or blocking antibodies where effector functions cause target cell depletion or toxicity
- In vivo surrogate antibody generation translating human IgG1 (LALA-PG) candidates into mouse-derived models
- Neutralizing cytokine or checkpoint receptor blocking antibodies (e.g., anti-PD-1, anti-CTLA-4, anti-CD40) requiring zero Fc receptor cross-linking
- In vivo negative control antibody construction for murine target validation

Vector Map



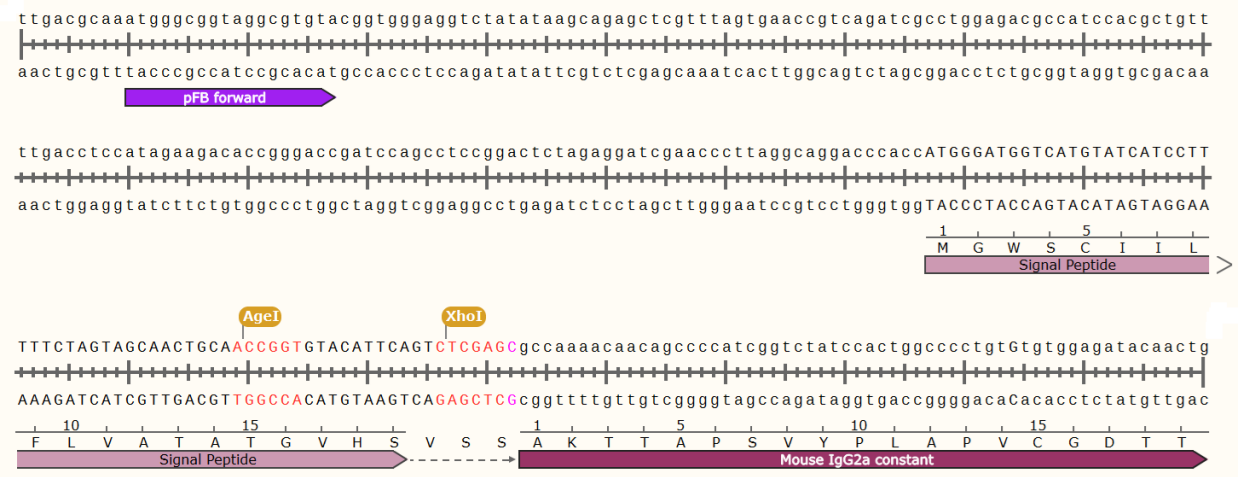
Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — native mouse IgG2a core hinge
- **Lower Hinge(L234A/L235A)** — mouse lower hinge FcγR-silencing mutations
- **C_H2(P329G)** — C_H2 domain proline-sandwich mutation (abolishes C1q and residual FcγR binding)
- **C_H3(WT)** — 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
- 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.

- Mouse IgG2a is the functional mouse counterpart to human IgG1 in terms of high effector activity. The mouse IgG2a (LALA-PG) vector is essential for preclinical rodent studies where complete effector silencing is required. Unlike legacy single/double mutants or aglycosylated variants, LALA-PG provides zero detectable binding to mouse FcγRIV and C1q while retaining wild-type thermal stability and expression yields.

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

- *Schlothauer T, Herter S, Koller CF, Grau-Richards S, Steinhart V, Spick C, Kubbies M, Klein C, Umaña P, Mössner E. Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. Protein Eng Des Sel. 2016;29(10):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. Effector-attenuating Substitutions That Maintain Antibody Stability and Reduce Toxicity in Mice. J Biol Chem. 2017;292(9):3900-3908.*

Document Version

- **Datasheet version:** 3.1
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