Fusion BioLabs Fc Engineering Vector Datasheet

Mouse IgG2a (N297Q) — Aglycosylated Fc Variant

SKU#: AFV-15

Plasmid Information

- **Plasmid name:** pMC-IgG2a-Agly(N297Q)
- **Fc variant name:** mouse IgG2a (N297Q)
- **Common name:** Aglycosylated Mouse IgG2a / mIgG2a-N297Q Fc Variant
- **Mutation set:** N297Q(C_H2 domain *N*-glycosylation site removal)
- **Insert structure:** Signal peptide–MCS–C_H1–hinge(WT)–C_H2(N297Q)–C_H3(WT)
- **Backbone:** Mouse IgG2a heavy-chain expression vector
- **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

Engineering purpose: Primary aglycosylated mouse Fc vector engineered to eliminate the conserved N297 biantennary glycan in mouse IgG2a. Disables murine FcγR (I, IIb, III, IV) and complement C1q binding via structural collapse of the internal C_H2 domain interface rather than primary lower-hinge sequence alteration.

Functional Profile

Property	Effect
Murine FcγRI/IIb/III/IV binding	Abolished / Undetectable ↓↓↓↓
ADCC (Mouse Effector Cells)	Completely Abolished ↓↓↓↓
ADCP (Macrophage Phagocytosis)	Completely Abolished ↓↓↓↓
C1q binding (Murine Complement)	Severely Reduced / Minimal ↓↓↓
CDC	Severely Reduced / Minimal ↓↓↓
FcRn binding & half-life	Normal / WT Baseline
Glycosylation & Thermal Stability	Aglycosylated (*N*297 glycan absent); reduced C_H2 thermal stability (*Tm*≈60∘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 N297Q substitution operates through a glycosylation-dependent conformational collapse within the murine C_H2 domain:

- **Glycan Elimination:** Mutating Asparagine to Glutamine at position 297 removes the consensus Asn-X-Ser/Thr motif, preventing *N*-linked carbohydrate addition during post-translational modification.

- **Conformational Collapse:** The conserved *N*297 glycan acts as a molecular spacer keeping the two C_H2 domains in an open, receptive orientation. In its absence, the C_H2 domains collapse inward, occluding the binding sites for murine FcγRs and C1q head groups.

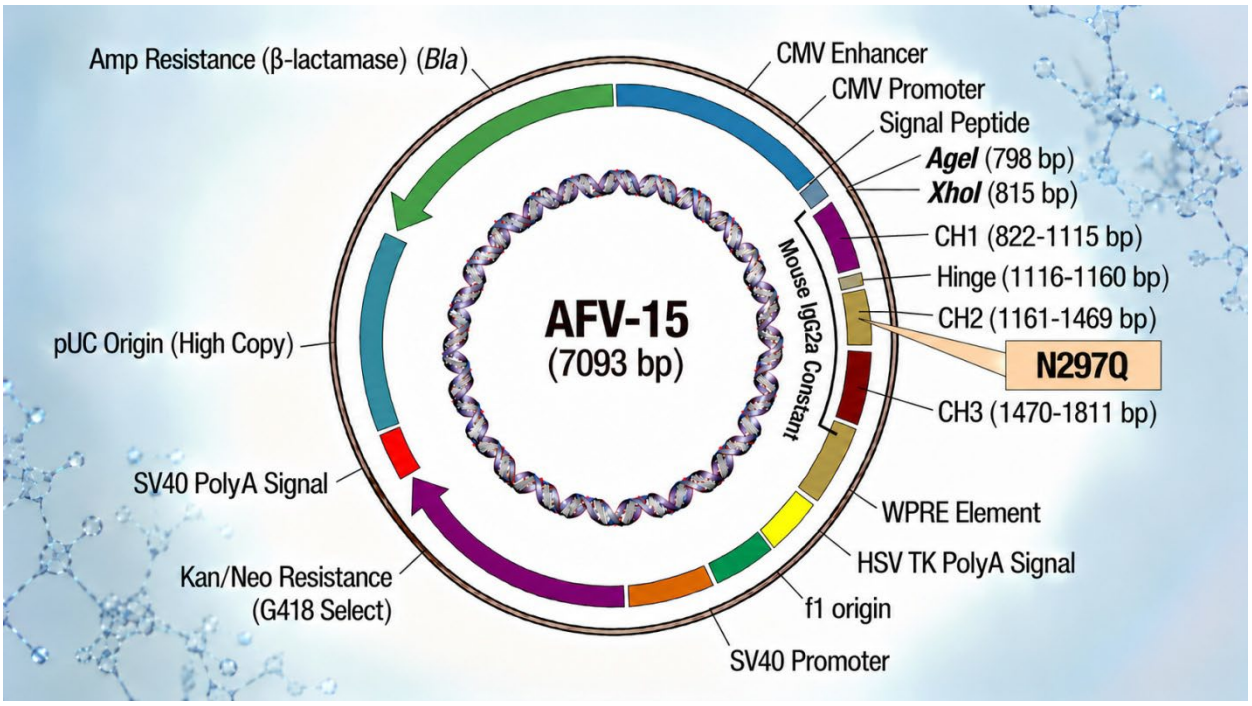
Phenotypic Effects

- Complete loss of binding to all murine Fcγ receptors (FcγRI, FcγRIIb, FcγRIII, FcγRIV)
- Elimination of NK cell-mediated ADCC, neutrophil activation, and macrophage ADCP in mouse models
- Severe attenuation of classical complement activation (CDC)
- High structural homogeneity (completely free of glycoform microheterogeneity)
- Preserves endosomal FcRn recycling dynamics and systemic pharmacokinetic half-life in rodents
- Displays reduced C_H2 domain thermal melting temperature (*Tm* reduced by ~ 10-12°C)

Applications

- Preclinical in vivo syngeneic mouse models requiring zero Fc-mediated effector background
- Murine surrogate antibodies targeting immune cell receptors (e.g., anti-mPD-1, anti-mPD-L1, anti-mTIGIT) where Fc activation induces toxic cell depletion
- Receptor-blocking antibodies and ligand traps in rodent disease models
- Mechanistic studies evaluating the contribution of Fc glycosylation vs. peptide sequence silencing
- Agonist surrogate antibodies requiring pure target cross-linking without FcγR co-engagement

Vector Map



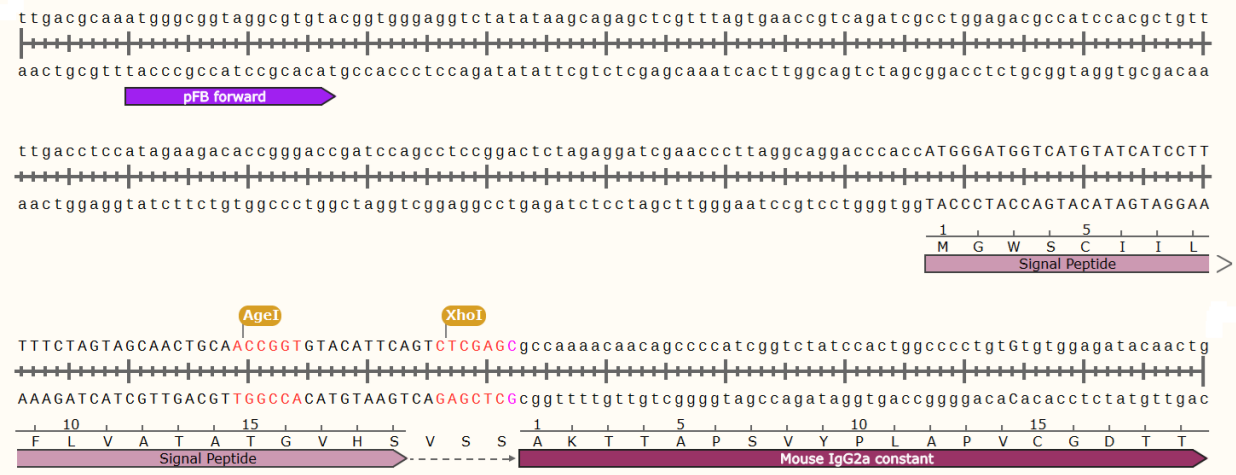
Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — mouse IgG2a hinge
- **C_H2(N297Q)** — murine C_H2 domain containing the aglycosylating N297Q mutation
- **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

- 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
- Suitable for transient or stable mammalian expression (CHO, HEK293)

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 mIgG2a-N297Q Fc variant serves as the standard aglycosylated mouse control for preclinical target validation. While N297Q offers complete effector silencing in rodent models, researchers should note that aglycosylation lowers the thermal stability (*Tm*) of the C_H2 domain. For in vivo preclinical studies requiring maximum biophysical stability, sequence-silenced variants such as mouse IgG2a (LALA-PG) are recommended.

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)	Lower hinge & C_H2 chimeric	★★★★★	Ultra-Silent Chimeric Null. Cross-subclass IgG2/IgG4

E233P / L234V / L235A / ΔG236+ A327G / A330S / P331S	sequence substitution		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

- *Tao MH, Morrison SL. Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. J Immunol. 1989;143(8):2595-2601.*
- *Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science. 2005;310(5753):1510-1512.*
- *Baudino L, Shinohara Y, Nimmerjahn F, Furukawa J, Nakata M, Martínez-Soria E, Petry F, Ravetch JV, Nishimura S, Izui S. Crucial role of aspartic acid at position 265 in the CH2 domain for murine IgG2a and IgG2b Fc-associated effector functions. J Immunol. 2008;181(9):6664-6669.*

Document Version

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