Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (N297Q)—Aglycosylated Effector-Null Fc Variant

SKU#: AFV-05

Plasmid Information

- **Plasmid name:** pHC-IgG1-Agly(N297Q)
- **Backbone:** Human IgG1 heavy-chain expression vector
- **Insert:** Signal peptide–MCS–C_H1–hinge(wild-type)–C_H2(N297Q)–C_H3(wild-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:** N297Q (CH2 domain *N*-glycosylation site removal)
- **Common name:** Aglycosylated Human IgG1 / `N297Q` Fc Variant
- **Engineering purpose:** Primary aglycosylated human Fc vector designed to eliminate the conserved *N*297 biantennary glycan. Disables FcγR and C1q binding via global conformational collapse of the C_H2 domain interface rather than primary sequence substitution.

Functional Profile

Property	Effect
FcγRI/IIa/IIb/IIIa binding	Abolished / Undetectable ↓↓↓↓
ADCC	Completely Abolished ↓↓↓↓
ADCP (Macrophage Phagocytosis)	Completely Abolished ↓↓↓↓
C1q binding	Severely Reduced / Minimal ↓↓↓
CDC	Severely Reduced / Minimal ↓↓↓
FcRn binding & half-life	Normal / WT Baseline
Glycosylation & Thermal Stability	Aglycosylated (N297 glycan absent); reduced CH2 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 mechanism within the C_H2 domain:

- **Glycan Elimination:** Replacing Asparagine with Glutamine at EU position 297 removes the consensus Asn-X-Ser/Thr motif, preventing *N*-linked glycosylation during post-translational processing.
- **Conformational Collapse:** The conserved *N*297 glycan acts as an internal molecular strut that maintains the open, rigid conformation of the two C_H2 domains. Without this glycan, the C_H2 domains collapse inward toward one another, physically closing off the binding sites required for FcγRs and globular C1q heads.

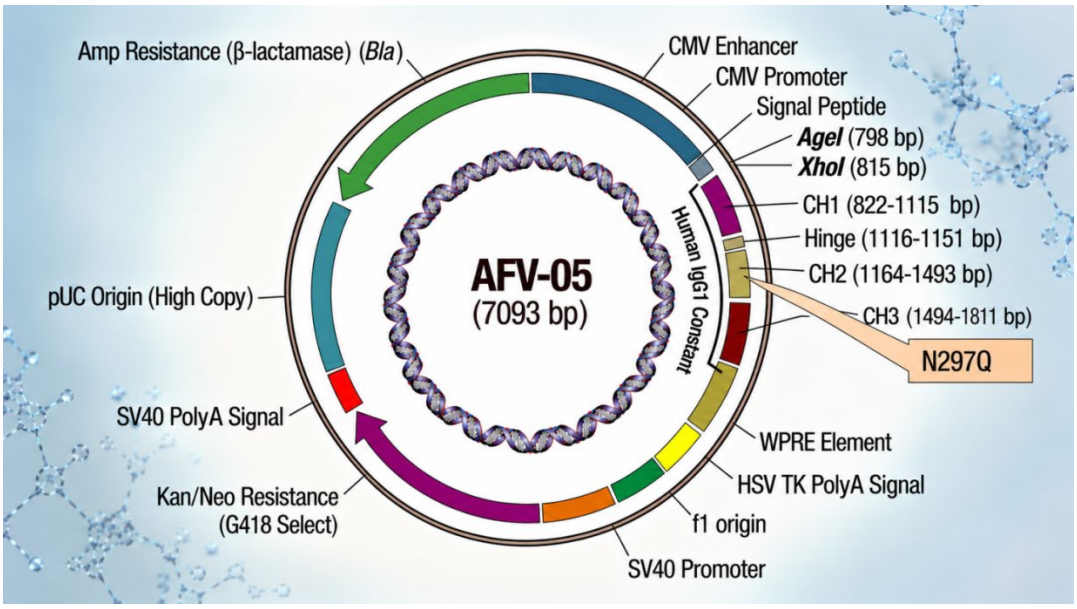
Phenotypic Effects

- Complete elimination of cell-mediated cytotoxicity (ADCC) and macrophage phagocytosis (ADCP)
- Severe attenuation of classical complement pathway activation (CDC)
- Preserves standard FcRn endosomal recycling and systemic serum half-life
- Produces homogeneous, non-glycosylated antibody preparations in mammalian systems
- Exhibits reduced C_H2 domain thermal stability (*Tm* drops from ~71°C down to ~ 60°C)

Applications

- Therapeutic antibody programs targeting cell surface receptors where effector function induces cytotoxicity (e.g., anti-PD-1, anti-PD-L1, anti-TIGIT)
- Agonist antibodies requiring pure target engagement without Fc-mediated receptor cross-linking
- Expression in expression systems lacking human-like glycosylation pathways
- Baseline control for comparing sequence-silenced null formats (LALA-PG, EN) against aglycosylated variants

Vector Map



Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge** — native IgG1 core and lower hinge
- **C_H2(N297Q)** — C_H2 domain containing the aglycosylating N297Q substitution
- **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

- Store plasmid at –20°C
- Purified antibody: Store at 2–8°C short-term, –80°C long-term
- 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.

- The N297Q variant remains a benchmark aglycosylated Fc control in therapeutic antibody discovery. While N297Q delivers complete effector silencing, users should note that the absence of the *N*297 glycan lowers the thermal melting temperature (*Tm*) of the C_H2 domain. For applications requiring maximum biophysical stability, fully glycosylated sequence-silenced variants such as human IgG1 (LALA-PG) or human IgG1 (ΔG236) 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) 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)	Murine lower hinge & C_H2	★★★★★	In Vivo Murine Gold Standard. Complete silencing of

L234A / L235A / P329G	proline-sandwich		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

- *Wright A, Morrison SL. Effect of altered CH2-associated carbohydrate structure on the functional properties and in vivo fate of chimeric mouse-human immunoglobulin G1. J Exp Med. 1994;180(3):1087-1096.*
- *Radaev S, Sun P. Recognition of immunoglobulins by Fcγ receptors. Mol Immunol. 2002; 38(14):1073-1083.*
- *Sazinsky SL, Ott RG, Silver NW, Tidor B, Ravetch JV, Wittrup KD. Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. Proc Natl Acad Sci U S A. 2008;105(51):20167-20172.*

Document Version

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