Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (T250Q/M428L) — QL Fc Variant

SKU#: AFV-28

Plasmid Information

- **Plasmid name:** pHC-IgG1-QL(T250Q/M428L)
- **Backbone:** Human IgG1 heavy-chain expression vector
- **Insert:** Signal peptide–MCS–C_H1–hinge–C_H2(T250Q)–C_H3(M428L)
- **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: T250Q (C_H2) + M428L (C_H3)
Common name: QL Fc Variant
Engineering purpose: Enhance FcRn binding through dual-site engineering to extend serum half-life.

Functional Profile

Property	Effect
FcγR binding	Normal
ADCC	Normal
CDC	Normal
FcRn binding	Increased ↑
Serum half-life	Extended ↑
PK profile	Long-acting

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 **QL Fc variant** introduces two complementary FcRn-enhancing mutations:

1. T250Q (C_H2 domain)
   - Increases FcRn affinity at acidic pH
   - Enhances recycling efficiency
2. M428L (C_H3 domain)
   - Strengthens FcRn interaction at the C_H3 interface
   - Improves endosomal rescue from degradation

Together, these mutations provide robust half-life extension while preserving normal FcγR and C1q interactions.

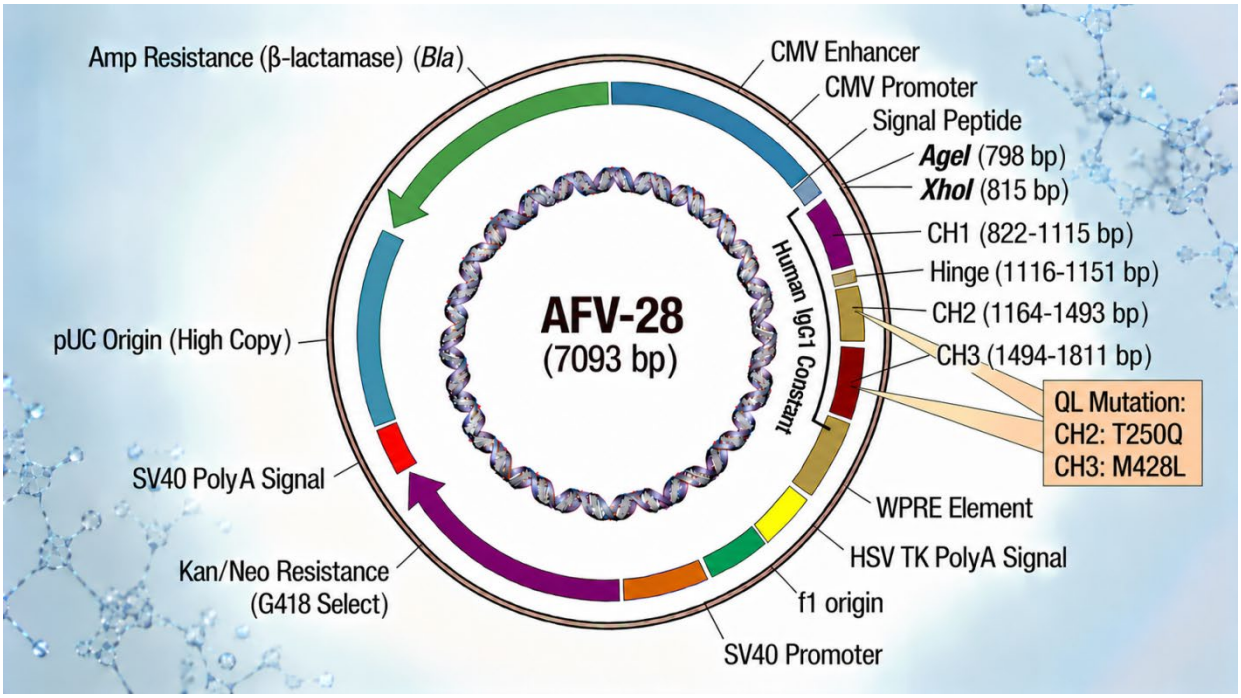
Phenotypic Effects

- Increased FcRn binding affinity
- Extended serum half-life (1.5–3× depending on species)
- Maintains normal ADCC and CDC
- Preserves structural integrity and antigen binding
- Suitable for long-acting therapeutic antibodies

Applications

- Long-acting therapeutic antibodies
- PK/PD optimization
- FcRn mechanistic studies
- Antibodies requiring preserved effector function with extended duration
- Preclinical development of extended-half-life biologics

Vector Map



Feature annotation:

- C_H1 — constant heavy 1
- Hinge — native IgG1 hinge
- C_H2(T250Q) — FcRn-enhancing mutation
- C_H3(M428L) — FcRn-enhancing mutation

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)-3'

Reverse Primer: 5'- GGCCCTTGGTGGAGGCGCTCGAGACN$_{VHR}$(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 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

- Store plasmid at –20°C
- Avoid repeated freeze–thaw cycles
- Use sterile technique when handling
- Suitable for transient or stable mammalian expression

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 QL Fc variant provides a balanced half-life extension strategy, enhancing FcRn binding without altering effector function. It is a versatile engineering module for therapeutic antibodies requiring extended duration with preserved immune activity.

Comparison within Fusion BioLabs FcRn / PK-Modulated Fc Variant Family

Variant	Mechanism	Strength (PK Extension)	Primary Application & Notes
human IgG1 (WT) (Wild-Type)	Native FcRn binding	★★☆☆☆	Baseline Control. Standard physiological baseline (~21-day half-life in humans).
human IgG1 (N434A) N434A	Single-point FcRn-enhanced	★★★☆☆	Single-point mutation providing a moderate 1.5–2× PK boost with low immunogenicity risk and easy manufacturing integration.
human IgG1 (LA) M428L / N434A	FcRn-enhanced	★★★★☆	Strong PK Extension. Dual mutation combining M428L and N434A; enhances

			acidic FcRn binding with minimal neutral binding.
human IgG1 (LS) M428L / N434S	FcRn-enhanced	★★★★★	Very Strong PK Extension. Extends human serum half-life up to 3–5× (>70–90 days; e.g., tixagevimab/ cilgavimab).
human IgG1 (YTE) M252Y / S254T / T256E	FcRn-enhanced	★★★★★	Benchmark PK Extension. Industry-standard benchmark delivering ~3–4× PK boost in humans (e.g., nirsevimab / Beyfortus®).
human IgG1 (QL) T250Q / M428L	FcRn-enhanced	★★★★☆	Moderate-to-Strong PK Extension. Increases acidic FcRn affinity with favorable intellectual property freedom to operate.
human IgG1 (YTE + KF) M252Y/S254T/T256E + H433K/N434F	Hyper-FcRn-enhanced / FcRn Blocker	★★★★★	FcRn Antagonist / Autoimmune Control. Binds tightly across pH 6.0 and 7.4 to block FcRn recycling and clear endogenous IgGs.
human IgG1 (V308P) V308P	Controlled/Accelerated Clearance	★★☆☆☆	Tunable Fast-Clearance Variant. Modulates FcRn dissociation kinetics to yield accelerated clearance (2–5-day half-life); ideal for ADCs and immuno-PET imaging to limit non-target toxicity.
human IgG1 (I253A)I253A	FcRn-null / Abrogated	★☆☆☆☆	Ultra-Fast Clearance Control. Complete knockout of the core FcRn binding triad; rapidly cleared within hours.

References

- *Hinton PR, Johlfs MG, Xiong JM, Hanestad K, Ong KC, Bullock C, Keller S, Tang MT, Tso JY, Vásquez M, Tsurushita N. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem. 2004;279(8):6213-6216.*
- *Hinton PR, Xiong JM, Johlfs MG, Tang MT, Keller S, Tsurushita N. An engineered human IgG1 antibody with longer serum half-life. J Immunol. 2006;176(1):346-356.*
- *Datta-Mannan A, Witcher DR, Lu J, Wroblewski VJ. Influence of improved FcRn binding on the subcutaneous bioavailability of monoclonal antibodies in cynomolgus monkeys. MAbs. 2012; 4(2):267-273.*

Document Version

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