Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG1 (LS: M428L/N434S) — LS Fc Variant

SKU#: AFV-27

Plasmid Information

- **Plasmid name:** pHC-IgG1-LS(M428L/N434S)
- **Backbone:** Human IgG1 heavy chain expression vector
- **Insert:** Signal peptide–MCS –CH1–hinge–CH2–CH3(M428L/N434S)
- **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:** M428L + N434S (CH3 domain)
- **Common name:** LS Fc Variant
- **Engineering purpose:** Enhance FcRn binding and extend serum half-life.

Functional Profile

Property	Effect
FcγRIIIa binding	Neutral
ADCC	Neutral
CDC	Neutral
FcRn binding	**Increased ↑↑**
Serum half-life	**Extended ↑↑**
Effector potency	Normal

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 LS Fc variant introduces two synergistic CH3-domain mutations—**M428L** and **N434S**—that optimize the Fc–FcRn interaction surface.

- **M428L** improves hydrophobic packing at the FcRn interface.
- **N434S** refines hydrogen bonding and steric geometry for enhanced FcRn engagement.

Together, these mutations significantly increase FcRn affinity at acidic pH, improving endosomal recycling and extending serum half-life without altering FcγR or C1q interactions.

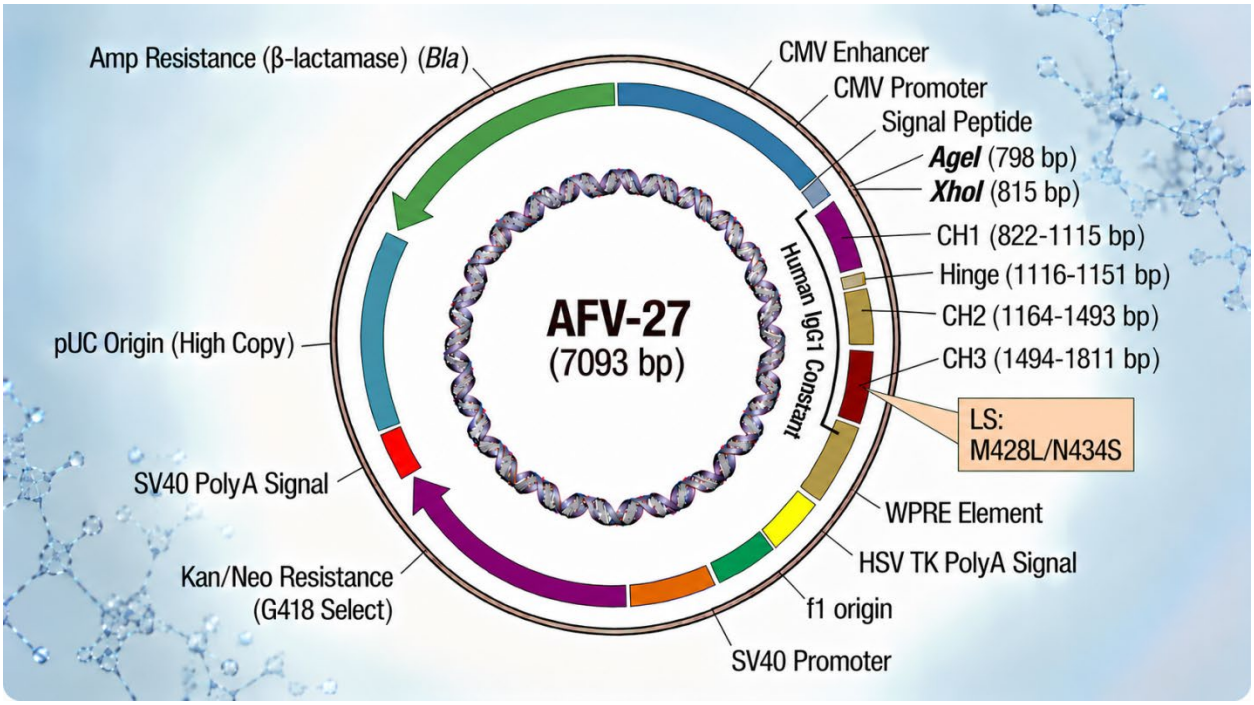
Phenotypic Effects

- Increased FcRn binding
- Extended serum half-life
- Preserved ADCC and CDC
- Maintains normal effector function profile
- Ideal for long-acting therapeutic antibodies

Applications

- Long-acting IgG1 therapeutics
- Antibodies requiring reduced dosing frequency
- Fc-fusion proteins needing improved exposure
- PK optimization studies
- Biologics where effector function must remain unchanged

Vector Map



Feature annotation:

- **CH1** — constant heavy 1
- **Hinge** — native IgG1 hinge
- **CH2** — native IgG1 CH2
- **CH3(M428L/N434S)** — FcRn-enhancing mutations

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**
- Avoid repeated freeze–thaw cycles
- Use sterile technique
- 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 LS Fc variant is widely used in clinical antibodies (e.g., durvalumab, atezolizumab) due to its strong FcRn affinity and extended half-life. It is a preferred backbone for long-acting biologics where effector functions must remain unchanged.

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)	FcRn-enhanced	★★★★	Benchmark PK Extension. Industry-standard

M252Y / S254T / T256E		★	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

- **Zalevsky J et al.** *Enhanced antibody half-life improves in vivo activity.* **Nat Biotechnol.** 2010;28:157–159.

Document Version

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