Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG4 (PE) — IgG4-Stabilized & Effector-Silenced Isotype Vector

SKU#: AFV-33

Plasmid Information

- **Plasmid name:** pHC-IgG4-PE(S228P/L235E)
- **Fc variant name:** human IgG4 (S228P/L235E)
- **Mutation set:** S228P (Core Hinge)+L235E(Lower Hinge)
- **Insert structure:** Signal peptide–MCS–C_H1–hinge(S228P)–lower hinge(L235E)-C_H2(WT)–C_H3(WT)
- **Backbone:** Human IgG4 heavy-chain expression vector
- **Selection 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

Human IgG4 (PE) combines hinge stabilization (S228P) with lower-hinge silencing (L235E). While standard IgG4 (S228P) eliminates Fab-arm exchange, it retains residual affinity for FcγRI (CD64). The addition of L235E abolishes this residual binding, creating an ultra-silent IgG4 backbone that prevents both structural recombination and unwanted immune activation.

Functional Profile

Property	Effect
Fab-Arm Exchange (*in vivo*)	Completely Abolished (0%)
FcγRI (CD64) binding	Completely Abolished ↓↓↓↓
FcγRII/III binding	Undetectable
ADCC / ADCP	Completely Null
C1q binding / CDC	Undetectable
FcRn binding & recycling	Unaltered / Normal Half-life

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

- **S228P Substitution:** Re-introduces the rigid CPPC motif into the core hinge, establishing permanent inter-heavy chain disulfide linkages and preventing half-antibody formation.

- **L235E Substitution:** Introduces a negative charge and steric perturbation into the lower hinge region, disrupting the hydrophobic interaction required for high-affinity FcγRI (CD64) engagement without affecting FcRn endosomal recycling loops.

Phenotypic Effects

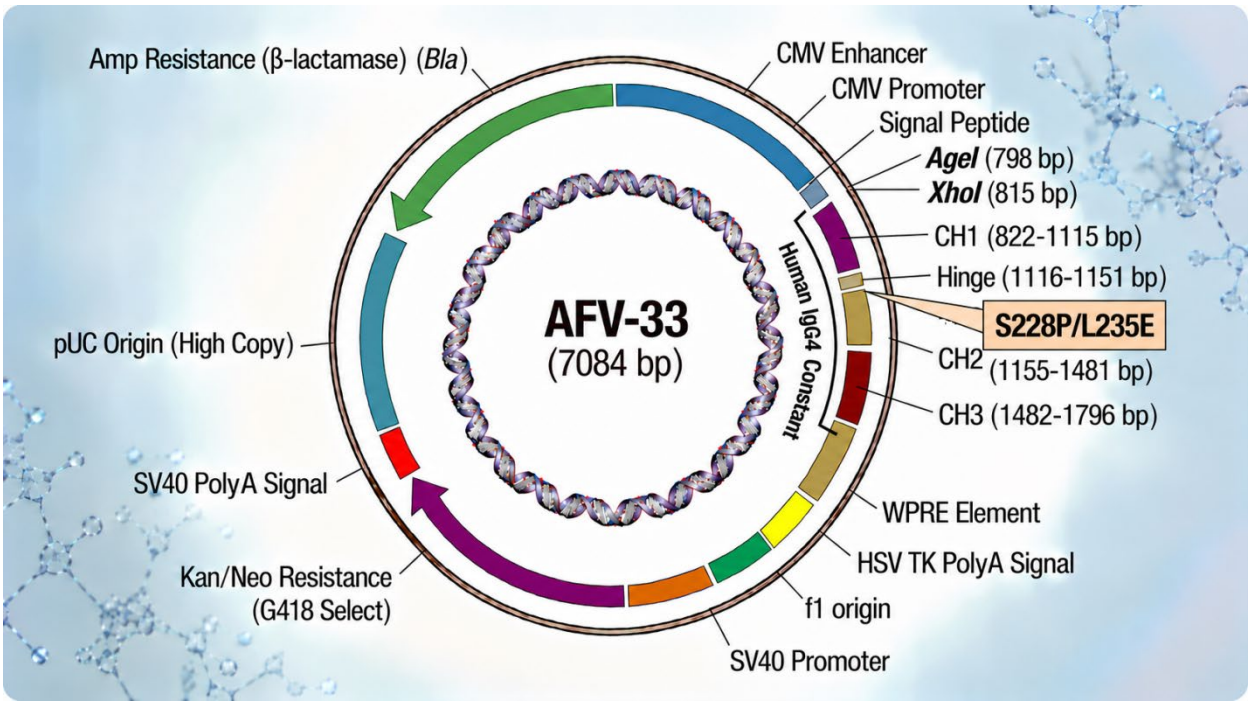
- Complete protection against *in vivo* Fab-arm exchange and dynamic exchange with host IgGs
- Elimination of residual FcγRI-mediated monocyte/macrophage activation and cytokine release
- Zero detectable NK cell-mediated ADCC or macrophage-mediated ADCP
- Total absence of complement activation or C1q binding
- Maintains full serum half-life via intact FcRn interactions
- High structural integrity and batch homogeneity during recombinant mammalian expression

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Applications

- Safety-critical therapeutic antibodies where complete elimination of FcγRI binding on an IgG4 framework is required.
- Receptor-targeted antagonist or neutralizer antibodies where Fc-mediated receptor cross-linking or target-cell destruction must be completely avoided.
- Immune checkpoint modulators targeting immune cells sensitive to FcγR-mediated hyper-activation.

Vector Map



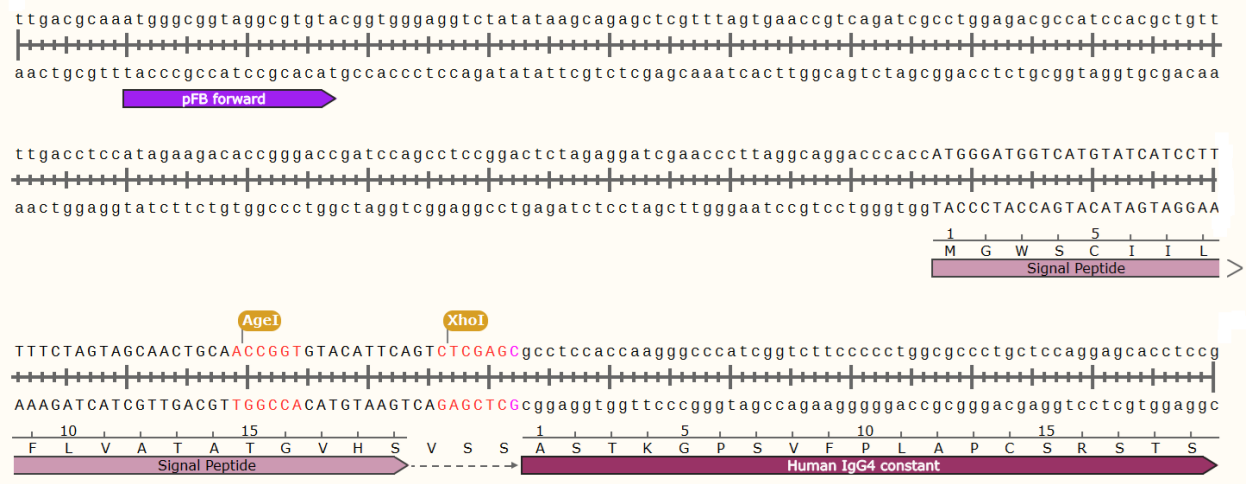
Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge(S228P)** — stabilized IgG4 core hinge region (CPPC)
- **Lower Hinge(L235E)** — effector-silencing lower hinge substitution
- **C_H2** — constant heavy 2 (WT)
- **C_H3** — constant heavy 3 (WT)

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

- 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
- Optimized for transient or stable expression in mammalian expression systems (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 human IgG4 (PE) backbone is widely utilized in therapeutic development as a reinforced alternative to wild-type IgG4 or single-mutant IgG4 (S228P). By addressing both inter-chain disulfide stability and residual FcγRI engagement, it delivers a clinically validated, ultra-silent profile.

References

- *Alegre ML, Collins AM, Pulito VL, Brosius RA, Olson WC, Zivin RA, Knowles R, Thistlethwaite JR, Jolliffe LK, Bluestone JA. Effect of a single amino acid mutation on the activating and immunosuppressive properties of a "humanized" OKT3 monoclonal antibody. J Immunol. 1992; 148(11):3461-3468.*
- *Reddy MP, Kinney CA, Chaikin MA, Payne A, Fishman-Lobell J, Tsui P, Dal Monte PR, Doyle ML, Brigham-Burke MR, Anderson D, Reff M, Newman R, Hanna N, Sweet RW, Truneh A.*

Elimination of Fc receptor-dependent effector functions of a modified IgG4 monoclonal antibody to human CD4. J Immunol. 2000;164(4):1925-1933.

Document Version

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