Fusion BioLabs Fc Engineering Vector Datasheet

Human IgG4 (S228P) — IgG4-Stabilized Core Hinge Isotype Vector

SKU#: AFV-09

Plasmid Information

- **Plasmid name:** pHC-IgG4-S228P(S228P)
- **Fc variant name:** human IgG4 (S228P)
- **Mutation set:** S228P (EU numbering) in core hinge
- **Insert structure:** Signal peptide–MCS –C_H1–hinge(S228P)–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

The human IgG4 (S228P) vector replaces the wild-type serine at residue 228 with a proline in the IgG4 core hinge (CPSC → CPPC). Wild-type IgG4 exhibits inter-heavy chain disulfide bond instability, leading to dynamic *in vivo* Fab-arm exchange (half-antibody formation). By restoring the IgG1-like core hinge proline, this variant permanently stabilizes inter-heavy chain disulfides, preventing Fab-arm exchange while maintaining characteristically low IgG4 effector activity.

Functional Profile

Property	Effect
Fab-Arm Exchange (in vivo)	Completely Abolished (0%)
Inter-chain Disulfide Integrity	High (>98% intact IgG4 monomer)
FcγRI (CD64) binding	Low / Retains native IgG4 profile
FcγRII/III binding	Extremely Low / Negligible
C1q binding / CDC	Undetectable / Null
FcRn binding & half-life	Normal / Baseline IgG circulation

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

- **Core Hinge Rigidification**: Wild-type IgG4 core hinge undergoes intra-chain disulfide folding, leading to non-covalent, reversible heavy-chain dissociation into half-molecules (H_1L_1).

- **S228P Substitution:** Re-introduces the rigid CPPC motif found in IgG1, forcing 100% inter-chain disulfide bond formation between heavy chains and locking the bivalent H_2L_2 antibody assembly.

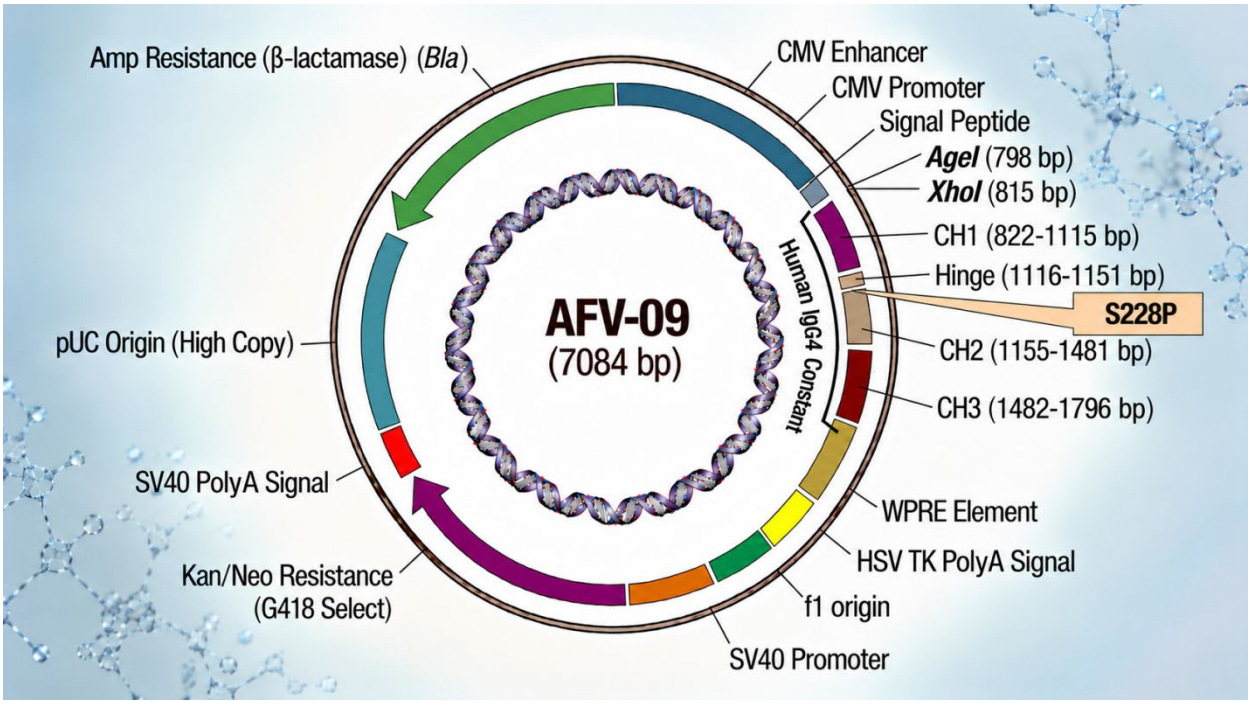
Phenotypic Effects

- Prevents *in vivo* Fab-arm exchange and bispecific recombination with endogenous host IgG4 antibodies
- Increases hinge structural stability and thermal tolerance
- Maintains native IgG4 profile with minimal effector function
- Significantly improves structural homogeneity during recombinant expression and long-term storage
- Eliminates half-antibody species in therapeutic or diagnostic production runs

Applications

- Clinical gold standard for non-depleting therapeutic antibodies (e.g., anti-PD-1 checkpoint blockers like pembrolizumab and nivolumab).
- Blocking receptor-ligand interactions without immune cell clearance or cell lysis.
- Monovalent or bivalent binding applications where structural stability is mandatory.

Vector Map



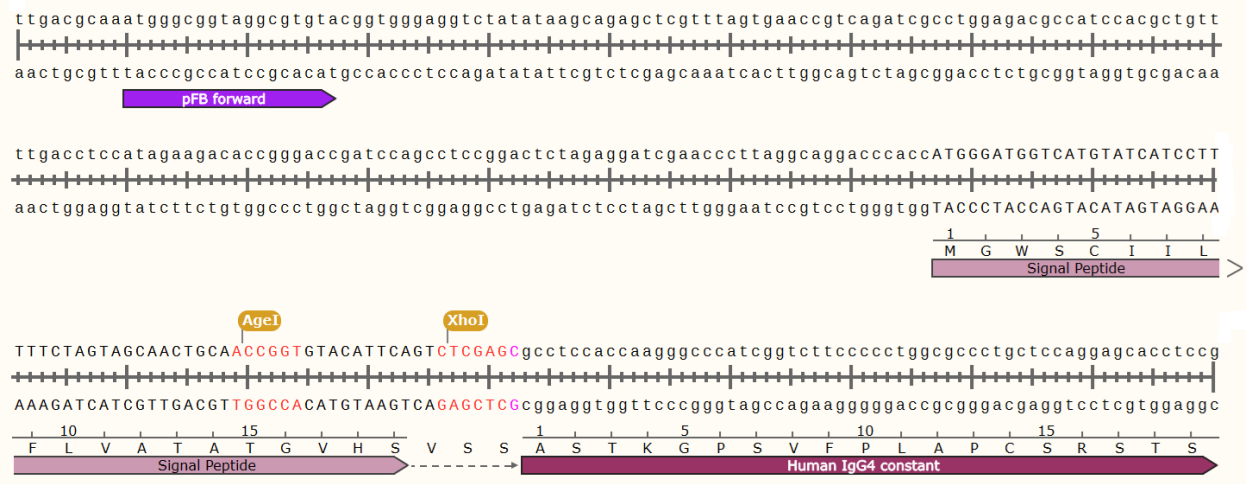
Feature annotation:

- **C_H1** — constant heavy 1
- **Hinge(S228P)** — stabilized IgG4 core hinge region
- **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)

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

- 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.

- This Fc variant is widely used in therapeutic IgG4 antibodies (such as pembrolizumab and nivolumab), where structural stability and low effector activity are essential.

References

- *Angal S, King DJ, Bodmer MW, Turner A, Lawson AD, Roberts G, Pedley B, Adair JR. A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody. Mol Immunol. 1993;30(1):105-108.*
- *Silva JP, Vetterlein O, Jose J, Peters S, Kirby H. The S228P mutation prevents in vivo and in vitro IgG4 Fab-arm exchange as demonstrated using a combination of novel quantitative immunoassays and physiological matrix preparation. J Biol Chem. 2015; 290(9):5462-5469.*

Document Version

- Datasheet version: 3.1
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