# Fusion BioLabs Fc Engineering Vector Datasheet

## Human IgG1 (I253A) — FcRn-Reduced Fc Variant

SKU#: AFV-30

## Plasmid Information

- **Plasmid name:** pHC-IgG1-I253A(I253A)
- **Backbone:** Human IgG1 heavy-chain expression vector
- **Insert:** Signal peptide–MCS–CH1–hinge–$C_H2$(I253A)–$C_H3$
- **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:** I253A ($C_H2$ domain)
**Common name:** FcRn-Reduced Fc Variant
**Engineering purpose:** Reduce FcRn binding to shorten serum half-life and accelerate antibody clearance.

## Functional Profile

| Property | Effect |
|---|---|
| **FcγR binding** | Normal |
| **ADCC** | Normal |
| **CDC** | Normal |
| **FcRn binding** | Reduced ↓ |
| **Serum half-life** | Shortened ↓ |
| **PK profile** | Faster clearance |

*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 **I253A mutation** disrupts a key hydrophobic residue in the $C_H2$ domain that contributes to FcRn binding at acidic pH. By replacing isoleucine with alanine, the Fc loses an important contact point required for efficient FcRn engagement. This reduces recycling through the FcRn pathway, resulting in **accelerated serum clearance** while preserving effector functions and structural integrity.

## Phenotypic Effects

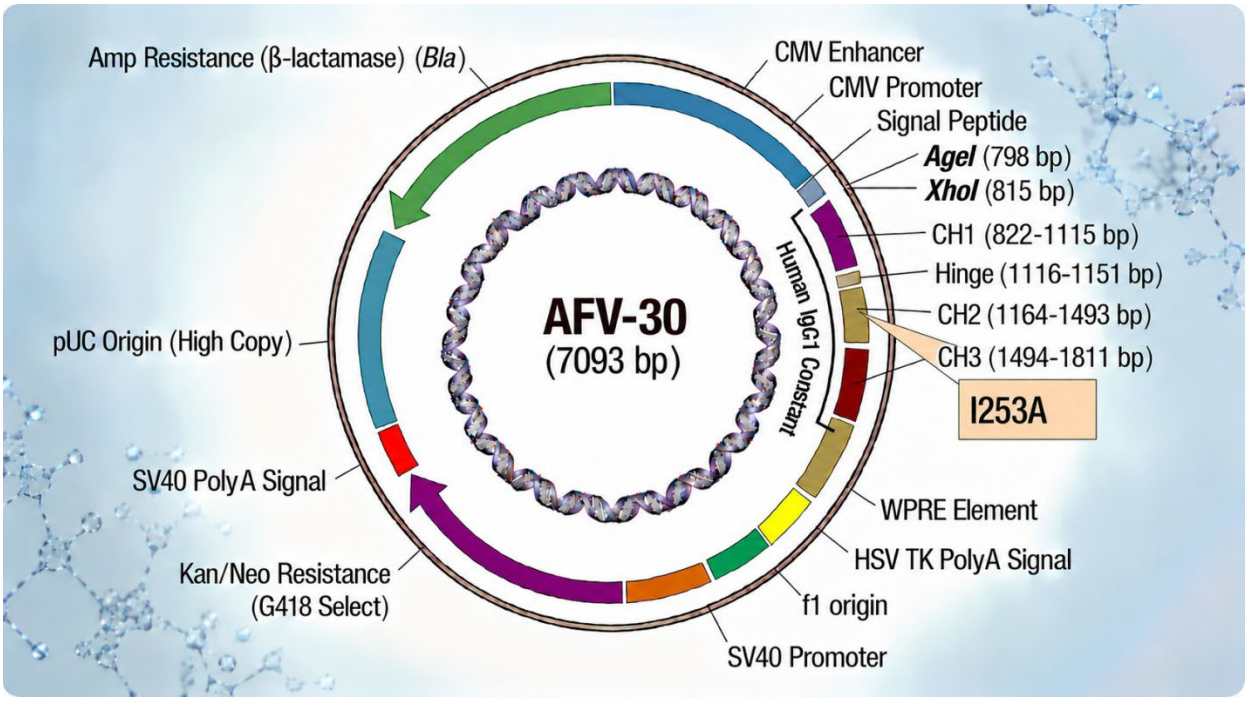
- Reduced FcRn binding affinity

- Shortened serum half-life
- Faster systemic clearance
- Maintains normal ADCC and CDC
- Useful for antibodies requiring rapid turnover or controlled PK

## Applications

- Antibodies requiring rapid clearance (e.g., imaging agents)
- Mechanistic studies of FcRn biology
- PK/PD tuning in therapeutic antibody development
- Preclinical evaluation of short-acting antibody formats
- Antibodies where prolonged exposure is undesirable

## Vector Map




**Feature annotation:**

- **$C_H1$** — constant heavy 1
- **Hinge** — native IgG1 hinge
- **$C_H2$(I253A)** — FcRn-reducing mutation
- **$C_H3$** — constant heavy 3

## 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'- CTAGTAGCAACTGCAACCGGTGTACATTCA$N_{VHF}$(15-18)

Reverse Primer: 5'- GGCCCTTGGTGGAGGCGCTCGAGAC$N_{VHR}$(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 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'- CTAGTAGCAACTGCAACCGGTGTACATTCA$N_{VHF}$(15-18) -3'

Reverse Primer: 5'- GGCCCTTGGTGGAGGC$N_{VHR}$(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 I253A Fc variant is widely used in applications requiring **short-acting antibodies**, including diagnostic imaging, targeted payload delivery, and mechanistic FcRn studies. It provides predictable PK reduction without altering effector function.

## 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


- *Kim JK, Tsen MF, Ghetie V, Ward ES. Localization of the site of the murine IgG1 molecule that is involved in binding to the murine intestinal Fc receptor. Eur J Immunol. 1994;24(10):2429-2434.*
- *Kim JK, Firan M, Radu CG, Kim CH, Ghetie V, Ward ES. Mapping the site on human IgG for binding of the MHC class I-related receptor, FcRn. Eur J Immunol. 1999;29(9):2819-2825.*
- *Martin WL, West AP Jr, Gan L, Bjorkman PJ. Crystal structure at 2.8 A of an FcRn/heterodimeric Fc complex: mechanism of pH-dependent binding. Mol Cell. 2001;7(4):867-877.*


## Document Version

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