AFV-34 | Human IgG1 dsFab–KiH Bispecific Antibody Expression System

Five purchasable configurations | Precloned Avastin/Lc06 reference inserts | Customer-specific variable-region cloning

Catalog number: AFV-34 | Family 5: Isotype-Stabilized & Structural Engineering | Datasheet revision: 5.0

1. Product Description

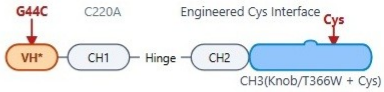
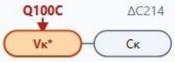
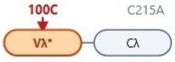
AFV-34 is a six-backbone mammalian expression platform combining Knobs-into-Holes (KiH) heavy-chain heterodimerization with a disulfide-engineered Fab (dsFab) on Arm 1. Customers select one of four four-plasmid configurations (KK, KL, LK, LL), or the complete six-vector kit (AFV-34-6V). Each plasmid is supplied with a precloned Avastin or Lc06 reference variable-region insert; the inserts can be replaced with customer-specific sequences.

Unlike AFV-35 CrossMab, AFV-34 uses engineered VH–VL cysteine pairing on Arm 1. When replacing reference inserts, the Arm 1 VH and VL require the appropriate dsFab-specific substitutions. Arm 2 uses a conventional Fab with a KiH-hole heavy chain and does not require dsFab-specific VH/VL cysteine engineering. Precloned reference inserts may be used for cloning development or reference studies; functional control performance requires validation.

2. Six Vector Backbones and Functions

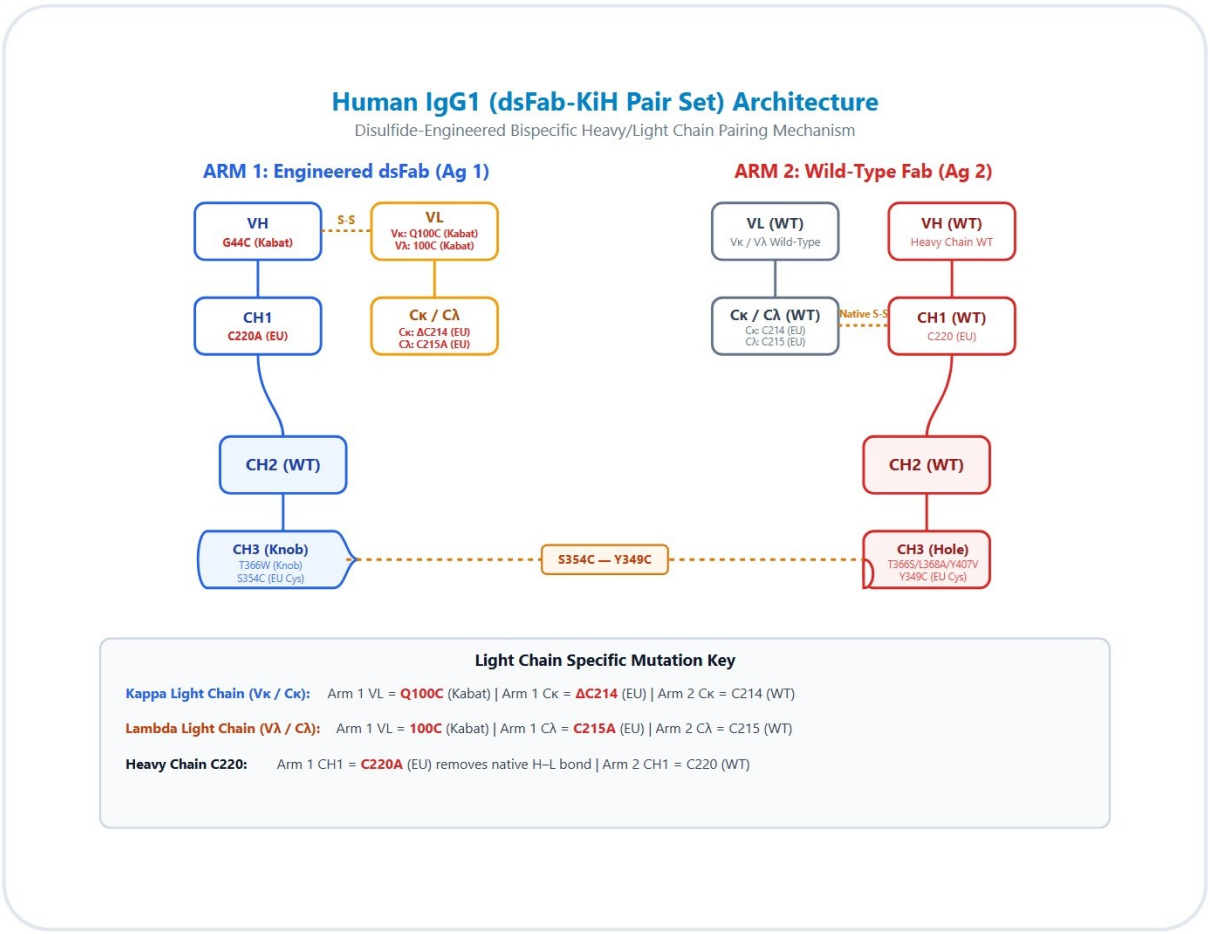
Vector	Arm / role	Design features
pHC-hIgG1-dsFab-Knob	Arm 1 heavy	Engineered VH G44C (Kabat); CH1 C220A; CH3 T366W/S354C
pLC-hIgκ-dsFab	Arm 1 κ light	Engineered Vκ Q100C (Kabat); Cκ ΔC214
pLC-hIgλ2-dsFab	Arm 1 λ2 light	Engineered Vλ cysteine at position 100 (Kabat, verify starting residue); human Cλ2 C215A
pHC-hIgG1-KiH-Hole	Arm 2 heavy	Unmodified Fab constant-domain pairing; CH3 T366S/L368A/Y407V/Y349C
pLC-hIgκ-WT	Arm 2 κ light	Conventional Vκ–Cκ; native Cκ cysteine retained
pLC-hIgλ2-WT	Arm 2 λ2 light	Conventional Vλ–human Cλ2; native Cλ2 cysteine retained

Figure 1. Six-backbone vector architecture and engineering summary.

VECTOR NAME	FUNCTIONAL ROLE	CHAIN / ISOTYPE	ENGINEERING & KEY MUTATIONS
pHC-hIgG1-dsFab-Knob	Arm 1 Heavy Chain	Human IgG1	
pLC-hIgk-dsFab	Arm 1 κ Light Chain	Human Igκ	
pLC-hIgλ-dsFab	Arm 1 λ Light Chain	Human Igλ	
pHC-hIgG1-KiH-Hole	Arm 2 Heavy Chain	Human IgG1	
pLC-hIgk-WT	Arm 2 κ Light Chain	Human Igκ	
pLC-hIgλ-WT	Arm 2 λ Light Chain	Human Igλ	

3. dsFab–KiH Engineering Mechanism

Figure 2. Arm 1 engineered VH–VL disulfide and Arm 2 conventional Fab, paired through KiH CH3 domains.



Arm 1: dsFab + Knob

VH G44C (Kabat) is paired with an engineered cysteine at VL position 100 (Kabat; Vκ Q100C in the Avastin example). CH1 C220A and Cκ ΔC214 or human Cλ2 C215A disrupt the native CH1–CL disulfide in the engineered Fab. CH3 carries T366W (knob) and S354C.

Arm 2: Conventional Fab + Hole

VH and VL retain their target-specific sequences without the AFV-34 dsFab cysteine substitutions; the CH1–CL native disulfide is retained. The heavy-chain CH3 hole carries T366S/L368A/Y407V and Y349C. S354C and Y349C are designed to form an inter-heavy-chain disulfide.

Mutation numbering: Kabat for variable regions and EU for constant regions. The VL position 100 residue and precise mutagenesis design must be checked against each antibody sequence; the notation “100C” alone does not identify the original amino acid.

4. Plasmid Selection Guide

Option	Arm 1 heavy	Arm 1 light	Arm 2 heavy	Arm 2 light
KK	dsFab-Knob	κ-dsFab	KiH-Hole	κ-WT
KL	dsFab-Knob	κ-dsFab	KiH-Hole	λ2-WT
LK	dsFab-Knob	λ2-dsFab	KiH-Hole	κ-WT
LL	dsFab-Knob	λ2-dsFab	KiH-Hole	λ2-WT

SKU	Arm 1 / Arm 2	Included vectors	DNA supplied

AFV-34-KK	κ / κ	dsFab-Knob HC; κ-dsFab LC; KiH-Hole HC; κ-WT LC	20 µg (4 × 5.0 µg)
AFV-34-KL	κ / human λ2	dsFab-Knob HC; κ-dsFab LC; KiH-Hole HC; λ2-WT LC	20 µg (4 × 5.0 µg)
AFV-34-LK	human λ2 / κ	dsFab-Knob HC; λ2-dsFab LC; KiH-Hole HC; κ-WT LC	20 µg (4 × 5.0 µg)
AFV-34-LL	human λ2 / human λ2	dsFab-Knob HC; λ2-dsFab LC; KiH-Hole HC; λ2-WT LC	20 µg (4 × 5.0 µg)
AFV-34-6V	All κ and human λ2 options	All six expression plasmids	30 µg (6 × 5.0 µg)

Five purchasable options are offered: AFV-34-KK, -KL, -LK and -LL each contain four plasmids (5.0 µg per plasmid; 20 µg total). AFV-34-6V includes all six plasmids (5.0 µg each; 30 µg total). The first letter denotes Arm 1 engineered dsFab κ (K) or human λ2 (L); the second denotes Arm 2 conventional κ (K) or human λ2 (L).

5. Cloning and Control-Vector Use

A. Customer-specific variable-region cloning

Each supplied plasmid contains a precloned reference insert (Avastin or Lc06, as identified in the corresponding map). To create a customer-specific construct, replace the reference variable-region insert in the appropriate heavy- or light-chain vector. The intended AgeI/XhoI restriction-cloning strategy requires double digestion of the relevant plasmid and a compatible insert. Verify restriction sites, digestion pattern, reading frame, signal peptide junction and the exact reference sequence before cloning. For Arm 1, engineer VH G44C (Kabat) and a compatible VL cysteine at position 100 (e.g., Avastin Vκ Q100C); confirm the residue and numbering in each customer sequence. Arm 2 VH/VL do not require these dsFab-specific substitutions.

B. Reference and control constructs

All six supplied AFV-34 plasmids are intended to carry the reference inserts shown in Figures 3–8: Avastin VH on the engineered dsFab-Knob heavy chain; Lc06 VH on the KiH-Hole heavy chain; Avastin Vκ on engineered κ and conventional κ light chains; and Lc06 Vλ on engineered human λ2 and conventional human λ2 light chains. These are insert-containing cloning/reference constructs, not empty vectors. They may support assay development and comparisons; a validated positive control claim requires experimental confirmation of expression, assembly and binding.

C. Cloning-site annotations from the provided maps

The reference plasmid maps show AgeI at 798 bp and insert-specific XhoI positions: Avastin heavy chain 1175 bp, Lc06 heavy chain 1193 bp, Avastin κ light chain 1134 bp, and Lc06 human λ2 light chain 1134 bp. Additional NheI or BsiWI sites appear on some maps. These coordinates apply to the illustrated insert-containing constructs, not necessarily to every customer-specific insert. Confirm each cloning site and absence of conflicting internal sites by sequence analysis before AgeI/XhoI double digestion.

6. Expression, Purification and QC

As a starting point, co-transfect the four selected heavy- and light-chain plasmids at a 1:1:1:1 molar ratio (HC1:LC1:HC2:LC2), then optimize for plasmid length, expression balance and product assembly. A 1:1:1:1 mass ratio is not identical to an equimolar ratio when plasmid lengths differ. Compatible mammalian transient-expression systems include ExpiCHO and Expi293 workflows, subject to their manufacturers' protocols.

Purify the assembled IgG using an appropriate affinity-capture and polishing workflow. Evaluate expression, aggregate content and assembly with SEC, reducing/non-reducing SDS-PAGE and, where available, intact or peptide-

level mass spectrometry. These assays alone may not establish correct light-chain pairing without suitably discriminating analytical evidence.

7. Six Illustrated Plasmid Maps

Figures 3–8 show the six insert-containing reference plasmids supplied across the AFV-34 configurations. Each map identifies the reference variable region, backbone engineering and annotated restriction sites. Map annotations should be checked against the production sequence files; they do not independently establish sequence-level validation.

Figure 3. Arm 1 engineered heavy chain — Avastin VH dsFab-Knob (7453 bp).

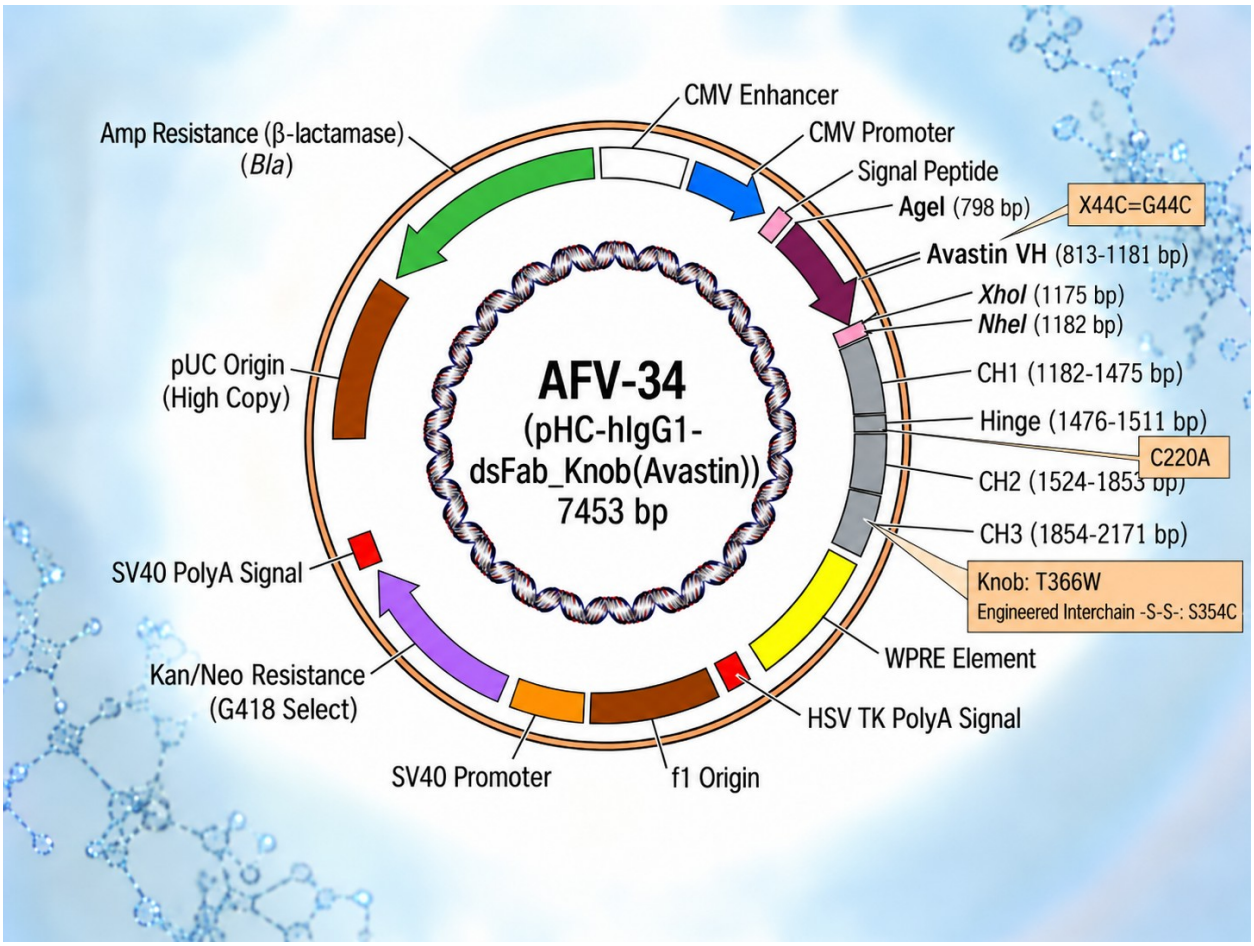


Figure 4. Arm 2 heavy chain — Lc06 VH KiH-Hole (7471 bp).

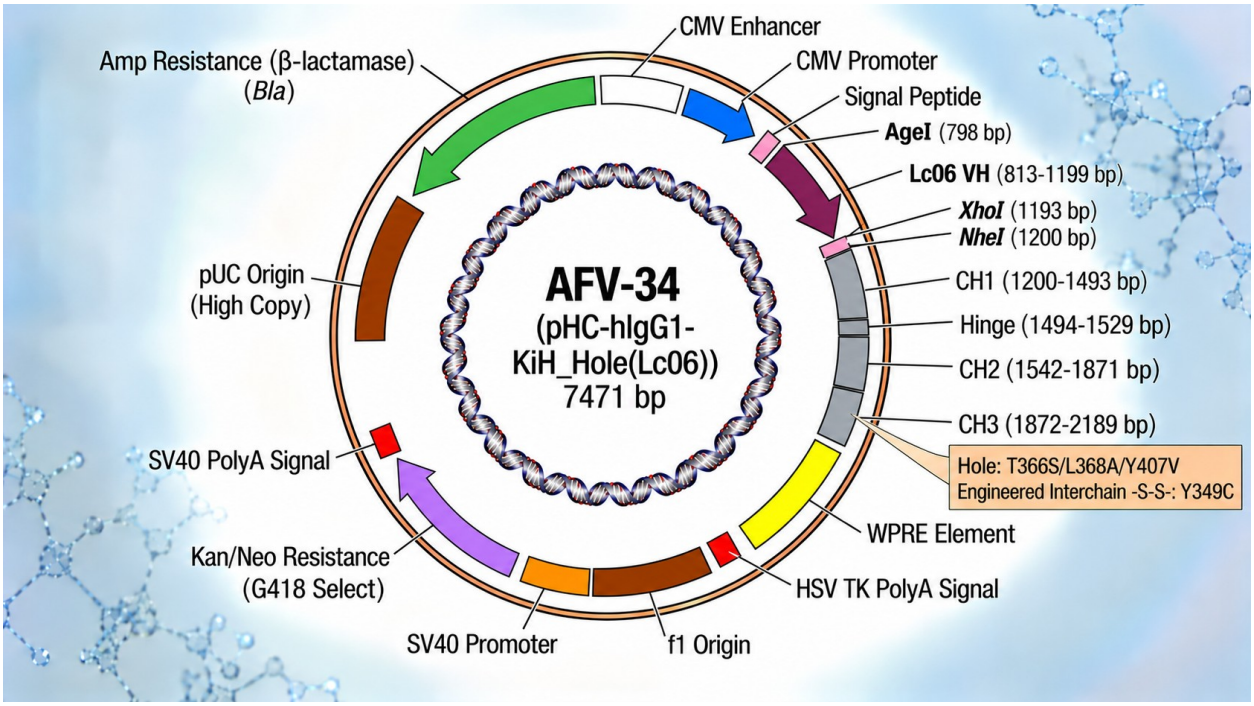


Figure 5. Arm 1 engineered κ light chain — Avastin Vκ dsFab (6739 bp).

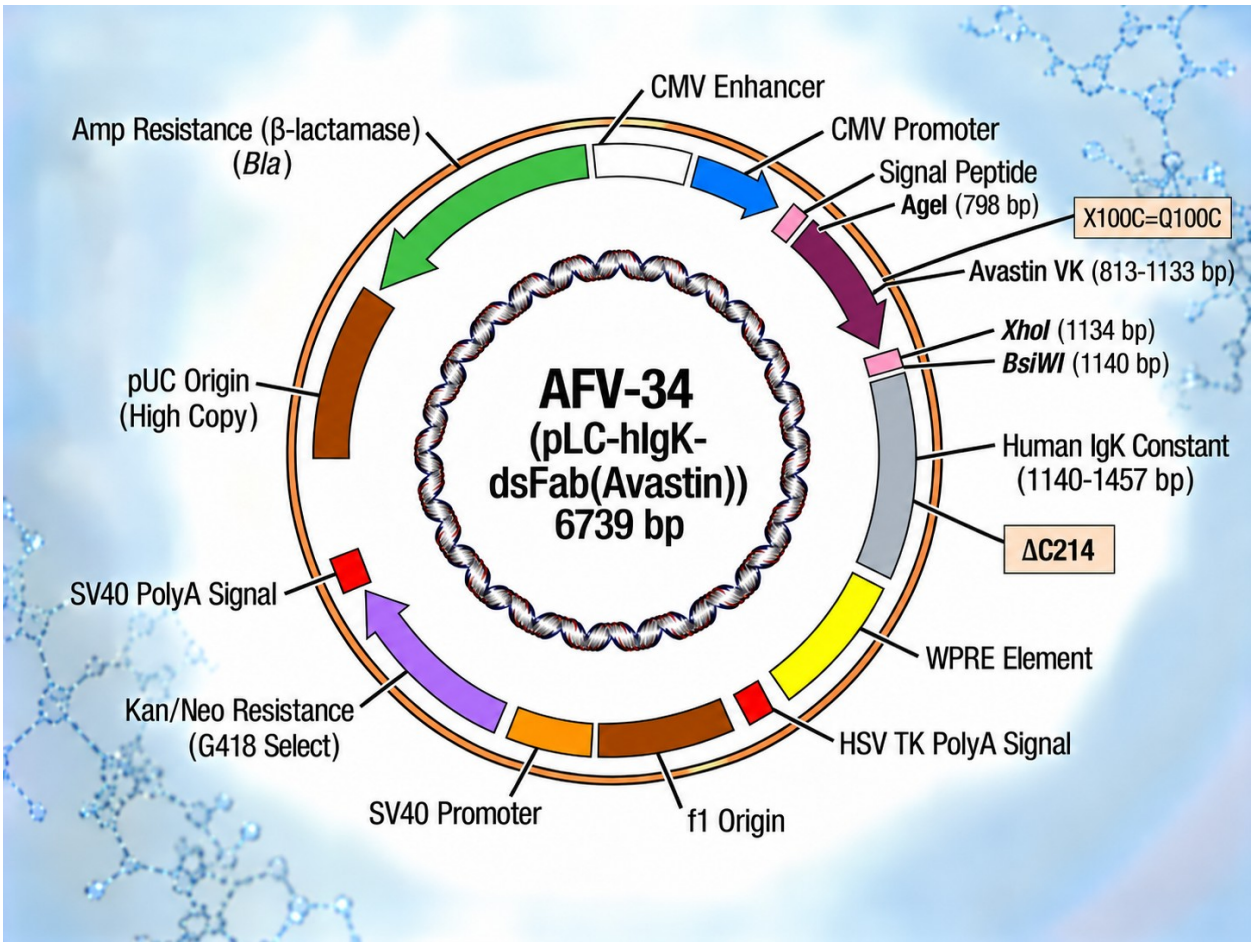


Figure 6. Conventional κ light chain — Avastin Vκ WT (6742 bp).

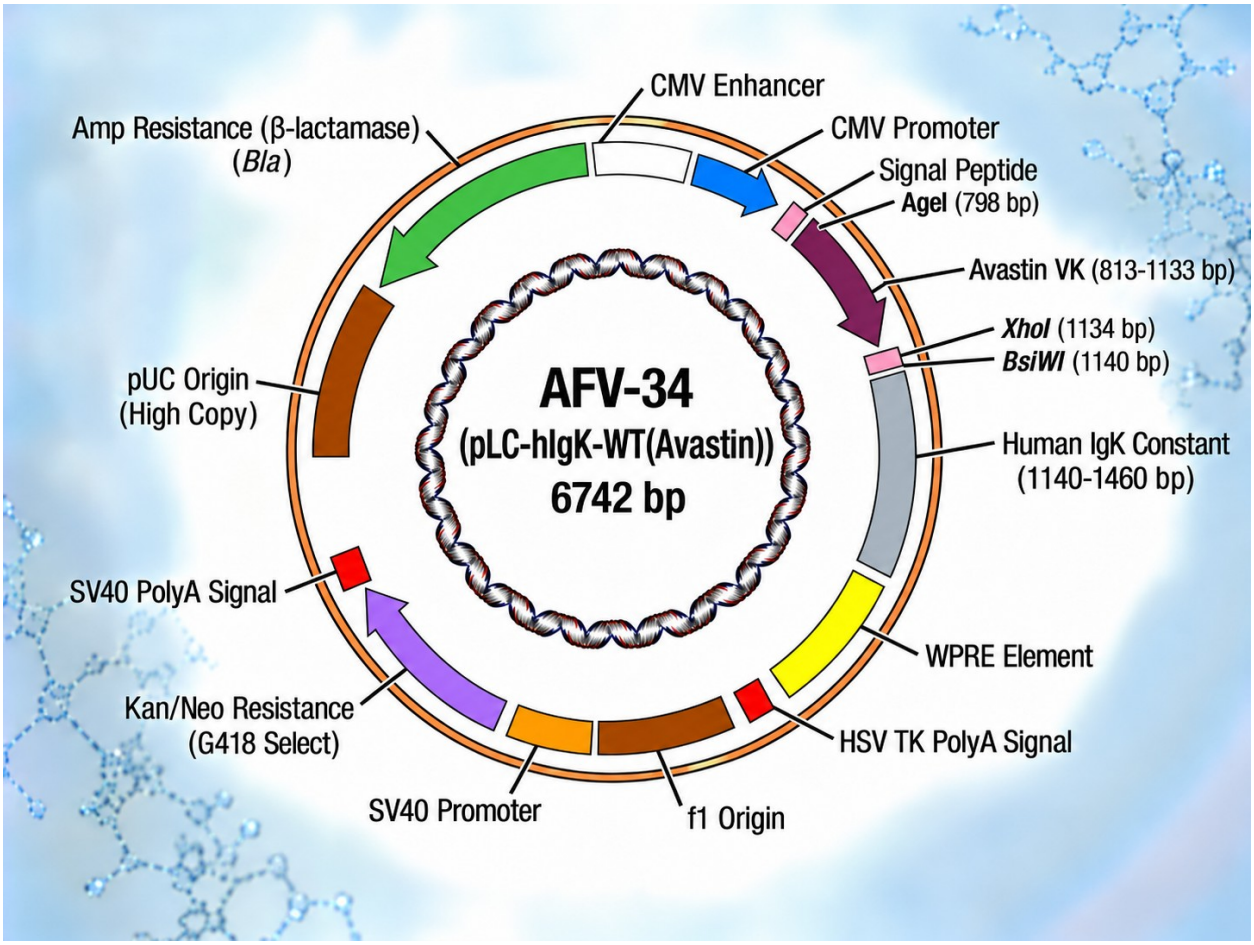


Figure 7. Arm 1 engineered human λ2 light chain — Lc06 Vλ dsFab (6739 bp).

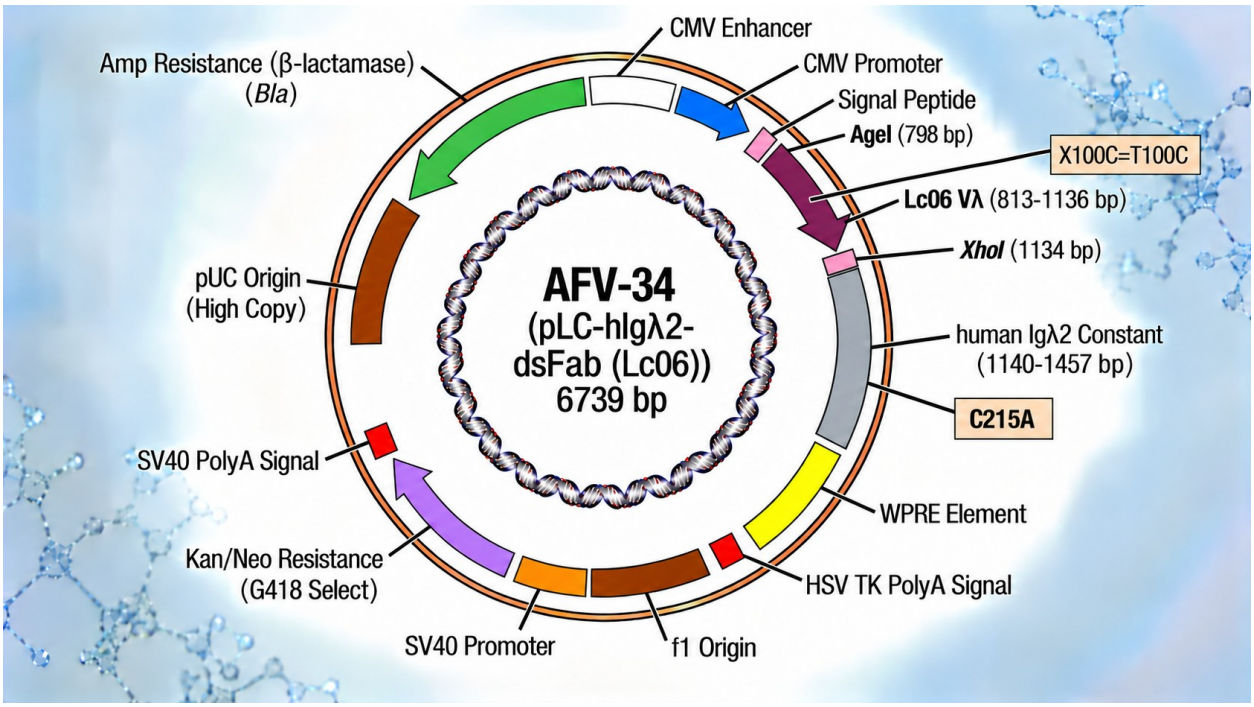
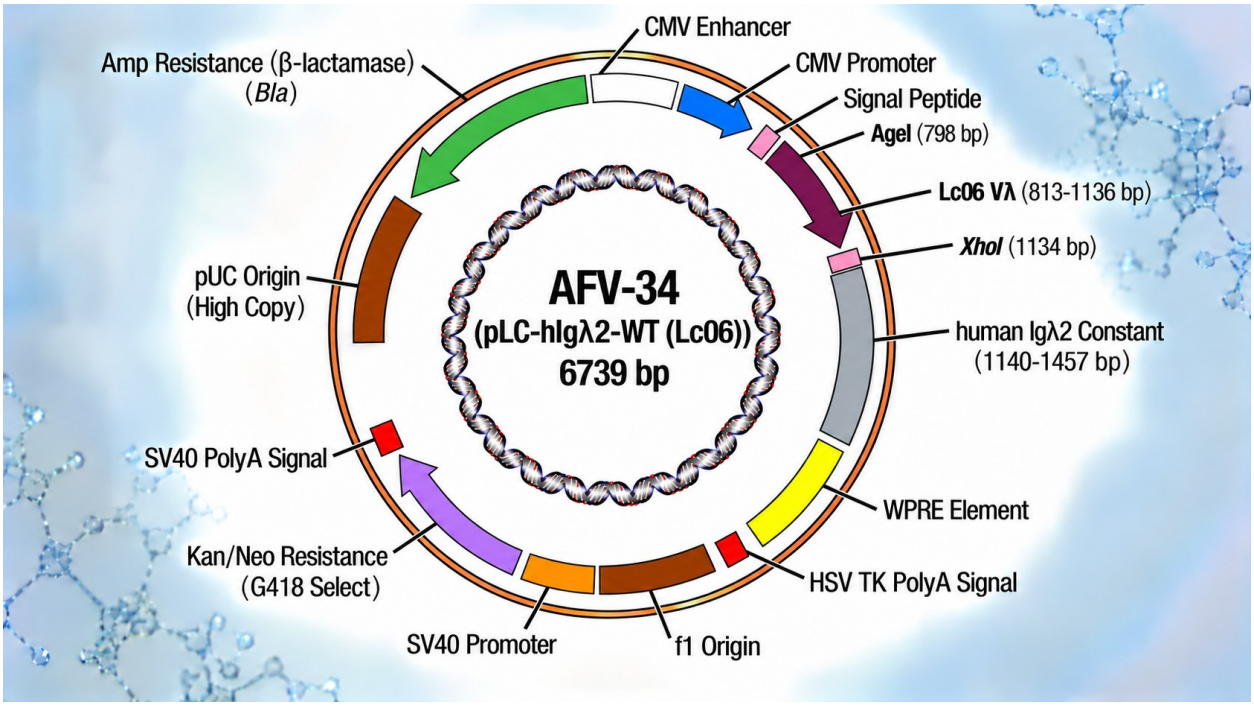


Figure 8. Conventional human λ2 light chain — Lc06 Vλ WT (6739 bp).



8. Storage, Applications and Limitations

Store plasmid DNA at −20 °C; minimize repeated freeze–thaw cycles. Applications include bispecific antibody discovery, comparative assembly studies, dsFab engineering, and reference/control expression experiments. For research use only; not for diagnostic or therapeutic use.

Important: dsFab disulfide formation, target binding, expression yield, heterodimer purity and light-chain fidelity depend on the antibody sequences and expression conditions. No guaranteed performance level is implied. All mutation and restriction-site claims should be reconciled to verified full-length plasmid sequences before product release.

9. Product Configurations, Contents

AFV-34-KK, AFV-34-KL, AFV-34-LK and AFV-34-LL: four plasmids per kit, 5.0 µg each (20 µg total). AFV-34-6V: all six plasmids, 5.0 µg each (30 µg total). All plasmids include the corresponding precloned Avastin/Lc06 reference variable-region insert. Research use only.