AFV-35

Human IgG1 CrossMab CH1–CL + Knobs-into-Holes (KiH) Bispecific Antibody Expression Vector Sets

Catalog: AFV-35 | Family 5: Isotype-Stabilized & Structural Engineering

Available configurations: AFV-35-KK, AFV-35-KL, AFV-35-LK, AFV-35-LL (four plasmids × 5.0 µg = 20.0 µg per kit), or AFV-35-6V (complete six-plasmid set × 5.0 µg = 30.0 µg).

1. Product Description

AFV-35 is a modular mammalian expression-vector platform for engineering full-length human IgG1 bispecific antibodies. Knobs-into-Holes (KiH) mutations in the heavy-chain CH3 domains promote preferential heterodimerization. In one antigen-binding arm, CrossMab CH1–CL swaps the heavy-chain CH1 domain with the light-chain constant domain (Cκ or human Cλ2), supporting cognate light-chain pairing. The other arm retains a conventional Fab architecture.

The complete kit contains six plasmid backbones to support conventional-arm κ or human Cλ2 light chains and CrossMab-arm Cκ or human Cλ2 constant-domain choices. Each individual antibody uses two heavy-chain and two light-chain expression vectors selected from the six. Antibody-specific VH and VL sequences must be cloned unless supplied as pre-inserted constructs; purified antibody is not included.

2. Platform Vector Specifications & Supplied Quantities

#	Vector backbone	Length	DNA per vector	Functional architecture
1	pHC-hIgG1-KiH-knob	7,093 bp	5.0 µg	VH–CH1–Hinge–CH2–CH3; T366W + S354C
2	pLC-hIgκ-WT	6,765 bp	5.0 µg	Vκ–Cκ; conventional Arm 1 LC
3	pLC-hIgλ2-WT	6,748 bp	5.0 µg	Vλ–Cλ2; conventional Arm 1 LC
4	pHC-hIgG1-CrossMab-Cκ-hole	7,120 bp	5.0 µg	VH–Cκ–Hinge–CH2–CH3; T366S/L368A/Y407V + Y349C
5	pHC-hIgG1-CrossMab-Cλ2-hole	7,117 bp	5.0 µg	VH–Cλ2–Hinge–CH2–CH3; T366S/L368A/Y407V + Y349C
6	pLC-hIg-CrossMab	6,394 bp	5.0 µg	Universal Vκ–CH1 or Vλ–CH1; engineered Arm 2 LC

Total kit DNA: 30.0 µg (six individually supplied plasmids, 5.0 µg each). Buffer and DNA concentration should be stated on the lot-specific certificate or product label; not independently established by the supplied maps.

3. Orderable Configurations & Four-Plasmid Selection Matrix

Arm 1 LC / Arm 2 LC	Arm 1: conventional + knob	Arm 2: CrossMab + hole
AFV-35-KK — Cκ / Cκ	KiH-knob HC + pLC-hIgκ-WT	Cκ-hole HC + pLC-hIg-CrossMab (Vκ–CH1)
AFV-35-KL — Cκ / Cλ2	KiH-knob HC + pLC-hIgκ-WT	Cλ2-hole HC + pLC-hIg-CrossMab (Vλ–CH1)
AFV-35-LK — Cλ2 / Cκ	KiH-knob HC + pLC-hIgλ2-WT	Cκ-hole HC + pLC-hIg-CrossMab (Vκ–

		CH1)
AFV-35-LL — Cλ2 / Cλ2	KiH-knob HC + pLC-hIgλ2-WT	Cλ2-hole HC + pLC-hIg-CrossMab (Vλ–CH1)

Optional complete set — AFV-35-6V: pHC-hIgG1-KiH-knob; pLC-hIgκ-WT; pLC-hIgλ2-WT; pHC-hIgG1-CrossMab-Cκ-hole; pHC-hIgG1-CrossMab-Cλ2-hole; pLC-hIg-CrossMab. Six plasmids × 5.0 µg = 30.0 µg total. The complete set enables selection of any of the four configurations.

The universal pLC-hIg-CrossMab backbone accepts κ-derived or λ-derived VL inserts and generates VL–CH1. It is the same backbone in all four combinations; the inserted VL sequence differs by target antibody. Variation naming: first letter = conventional Arm 1 LC; second letter = CrossMab Arm 2 LC. K = κ; L = human Cλ2. The universal CrossMab LC vector is included in every four-plasmid variation.

4. CrossMab CH1–CL Engineering Mechanism

Arm 1 (conventional): VH–CH1–Hinge–CH2–CH3 (KiH knob) pairs with Vκ–Cκ or Vλ–Cλ2.

Arm 2 (engineered): VH–Cκ or VH–Cλ2 replaces CH1 in the hole heavy chain; the corresponding VL–CH1 replaces conventional VL–CL in the light chain. The variable domains remain on their respective chains; the constant domains are exchanged.

KiH knob heavy chain: CH3 T366W and S354C. KiH hole heavy chain: CH3 T366S/L368A/Y407V and Y349C. The S354C–Y349C pair is designed to support an engineered inter-heavy-chain disulfide. Pairing preference is design intent, not a guaranteed purity percentage.

5. Vector Cloning & Expression Guidance

Insert antibody-specific VH and VL sequences into the corresponding expression plasmids, confirm the correct reading frames and junctions by sequencing, and choose the appropriate four-vector combination using Section 3. A 1:1:1:1 molar DNA input is a possible starting point for optimization, not an equal-mass recipe because plasmid lengths differ. Optimize transfection, expression, assembly, and purification empirically for each antibody and host cell line.

Cloning-site annotations for all six vectors: pHC-hIgG1-KiH-knob, pHC-hIgG1-CrossMab-Cκ-hole, pHC-hIgG1-CrossMab-Cλ2-hole, and pLC-hIg-CrossMab each show AgeI (798 bp) and XhoI (815 bp). The conventional WT light-chain vectors have longer annotated insert regions: pLC-hIgκ-WT between AgeI (798 bp) and XhoI (1,157 bp), and pLC-hIgλ2-WT between AgeI (798 bp) and XhoI (1,143 bp). Plasmid-map annotations do not replace sequence-level verification.

6. Suggested Characterization & Quality Control

Verify insert identity by DNA sequencing; evaluate expression and assembly using appropriate analytical methods such as reducing/non-reducing electrophoresis, size-exclusion chromatography and intact or subunit mass analysis where available. Verify bispecific target binding and chain pairing with assays appropriate to the actual antibody. No fixed expression yield, pairing efficiency, or purity guarantee is claimed.

7. Storage, Scope & Use

Store plasmid DNA under the conditions specified on the shipment label or lot-specific documentation; avoid unnecessary freeze–thaw cycles. Kit supplies expression plasmid backbones, not pre-cloned antibody variable regions, cells, transfection reagents, or purified antibody unless separately stated. For research use only; not for diagnostic or therapeutic use.

8. Engineering & Kit Architecture Figures

AFV-35 | HUMAN IgG1 (CROSSMAB + KiH SET)

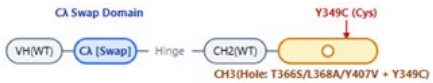
VECTOR NAME	FUNCTIONAL ROLE	CHAIN / ISOTYPE	ENGINEERING & DOMAIN ARCHITECTURE
pHC-hIgG1-KiH-Knob	Arm 1 Heavy Chain	Human IgG1	
pLC-hIgk-WT	Arm 1 κ Light Chain	Human Igκ	
pLC-hIgλ-WT	Arm 1 λ Light Chain	Human Igλ	
pHC-hIgG1-CrossMab-Ck-Hole	Arm 2 Heavy Chain (κ-Swap)	Human IgG1 / Igκ	
pHC-hIgG1-CrossMab-Cλ-Hole	Arm 2 Heavy Chain (λ-Swap)	Human IgG1 / Igλ	
pLC-hIg-CrossMab	Arm 2 Light Chain (Universal)	Human Ig (CH1 Swap)	

Figure 1. Six-vector functional roles and domain architectures. Interpret λ labels as human Cλ2 in this AFV-35 kit.

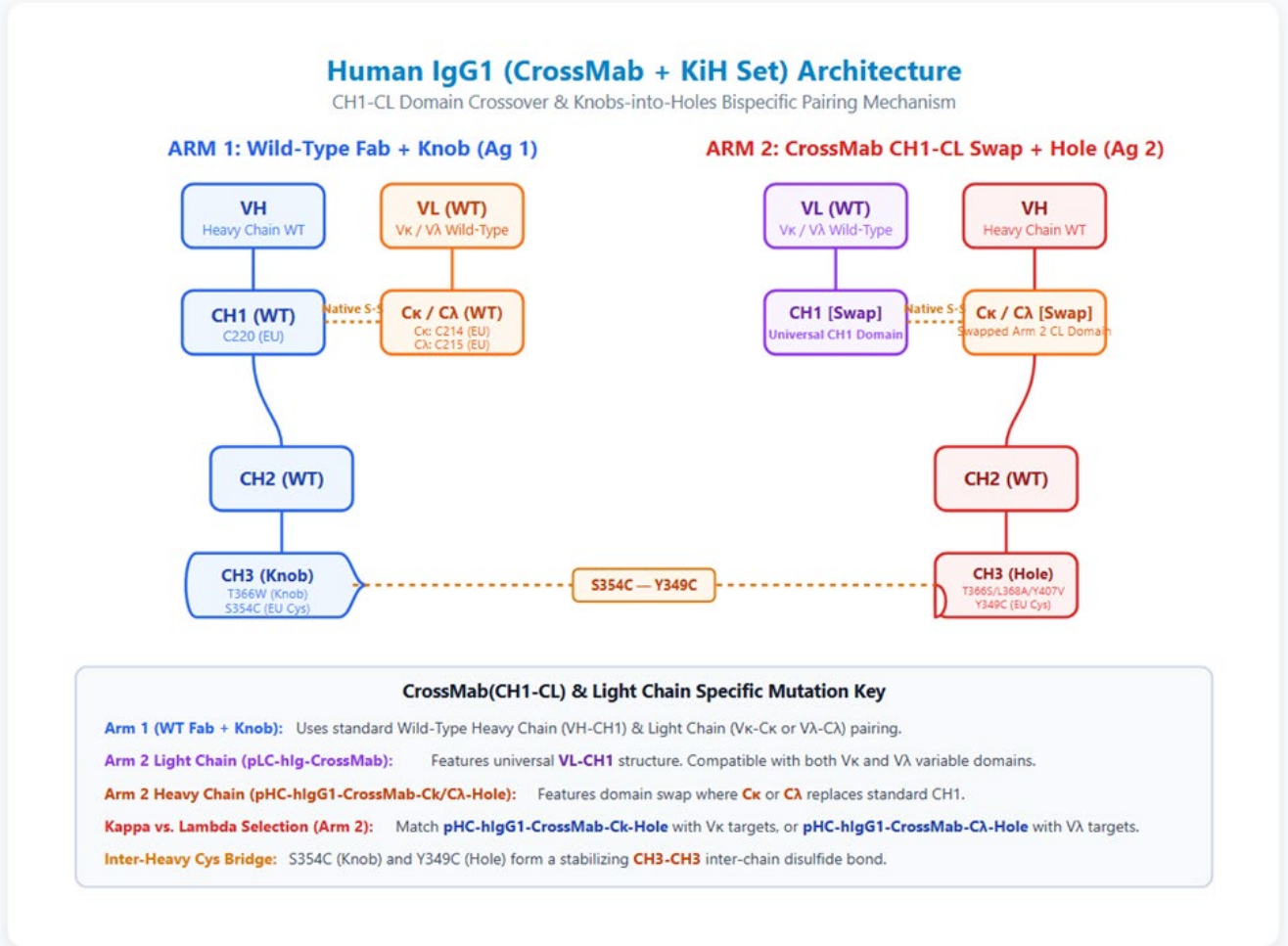
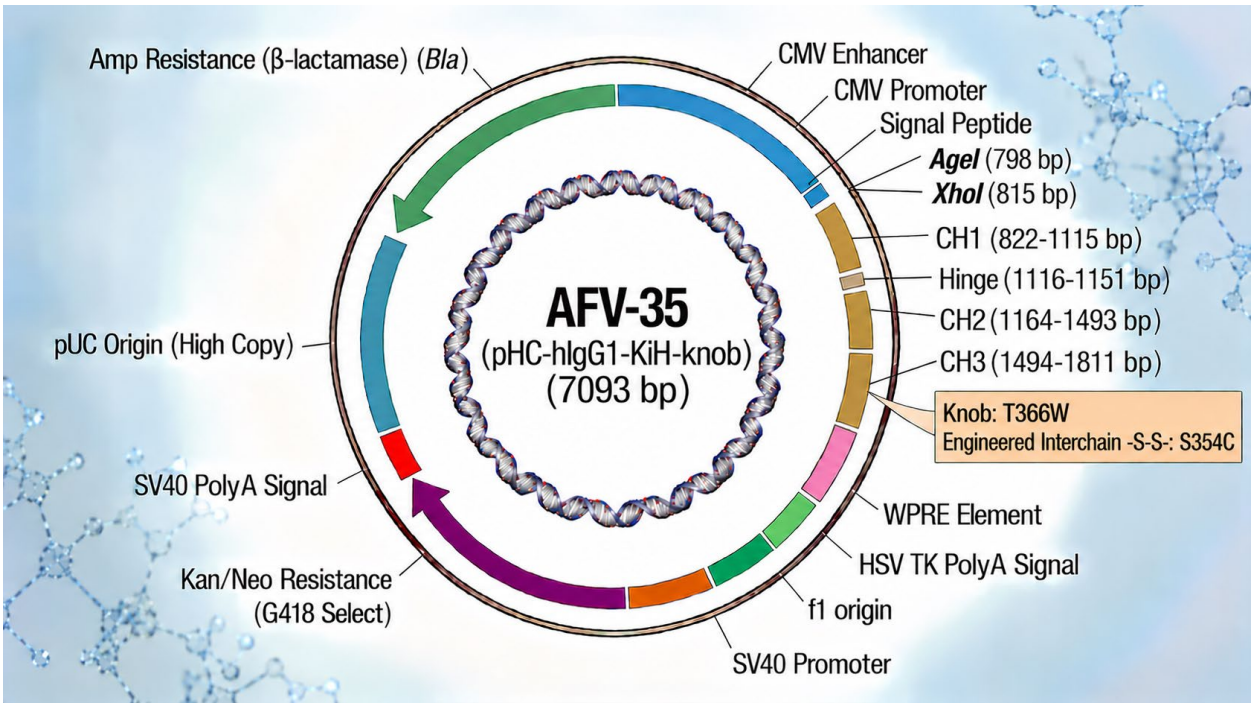


Figure 2. CrossMab CH1–CL constant-domain swap and KiH assembly mechanism. The λ constant-domain option in this kit is human Cλ2.

9. Plasmid Map Reference

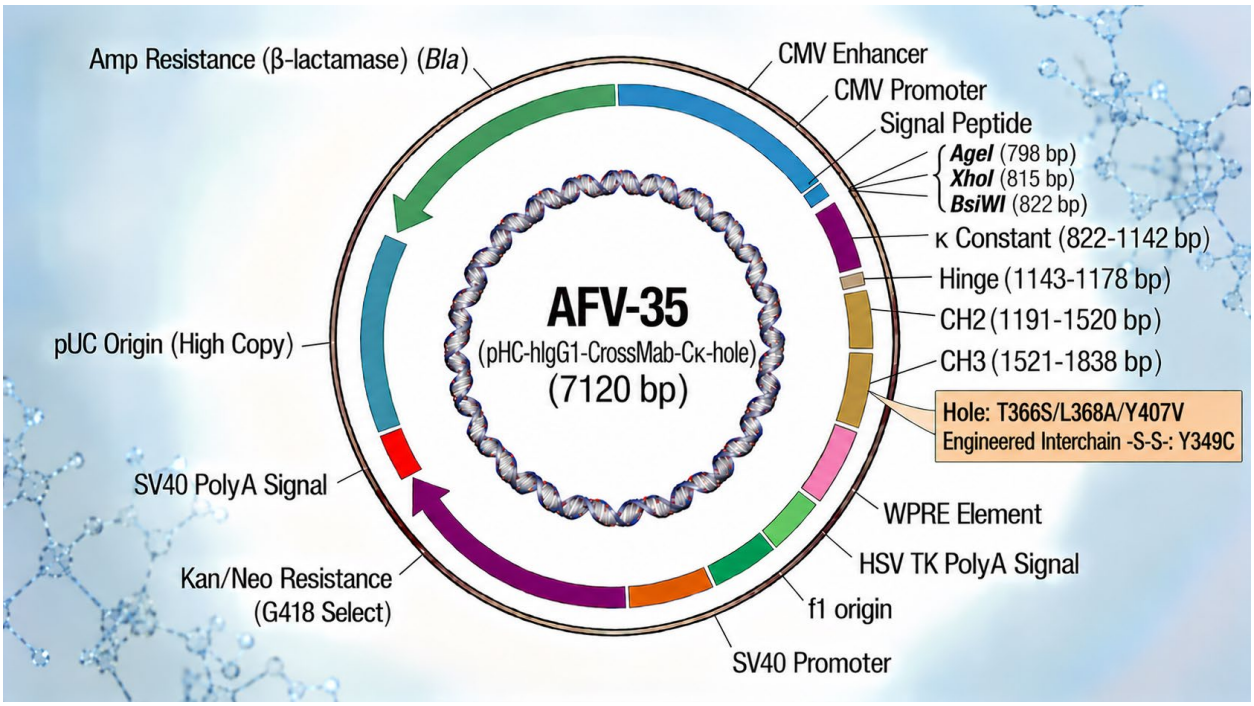
All six plasmid maps document the full AFV-35 platform. A selected four-plasmid variation contains only the four specified vectors; AFV-35-6V contains all six. Each map is shown below.

KiH-knob heavy chain



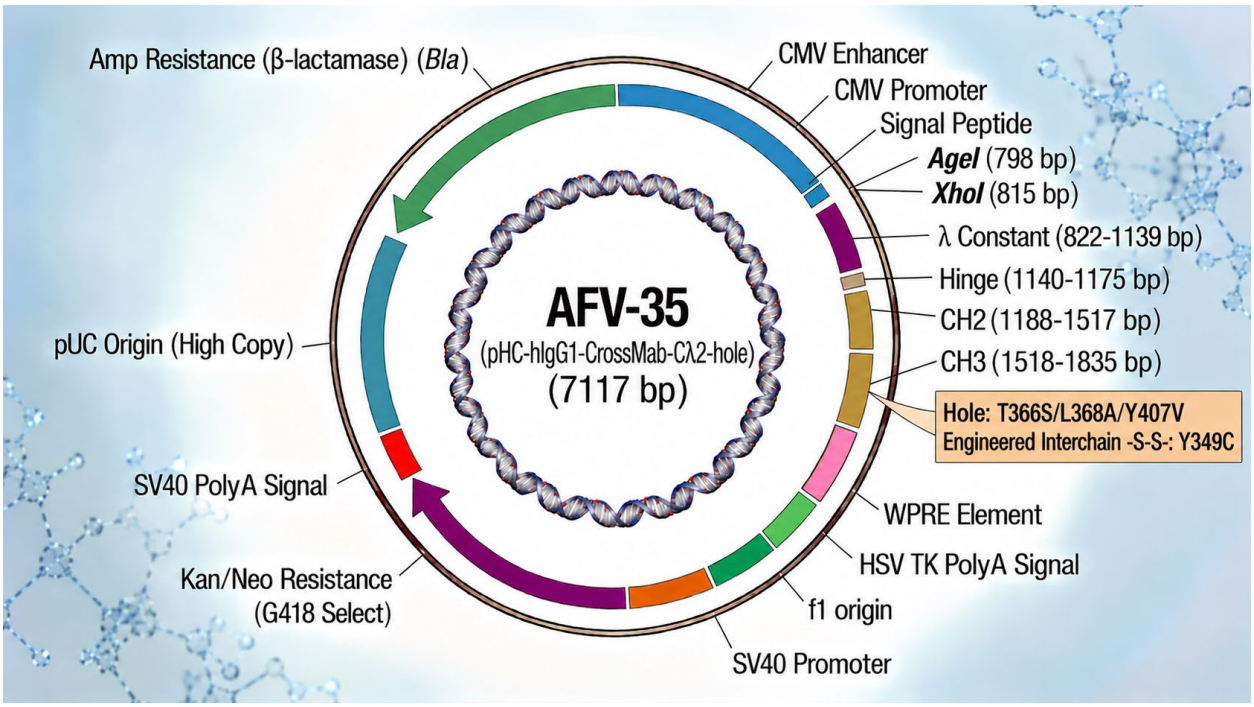
AFV-35 plasmid map — KiH-knob heavy chain

CrossMab Cκ-hole heavy chain



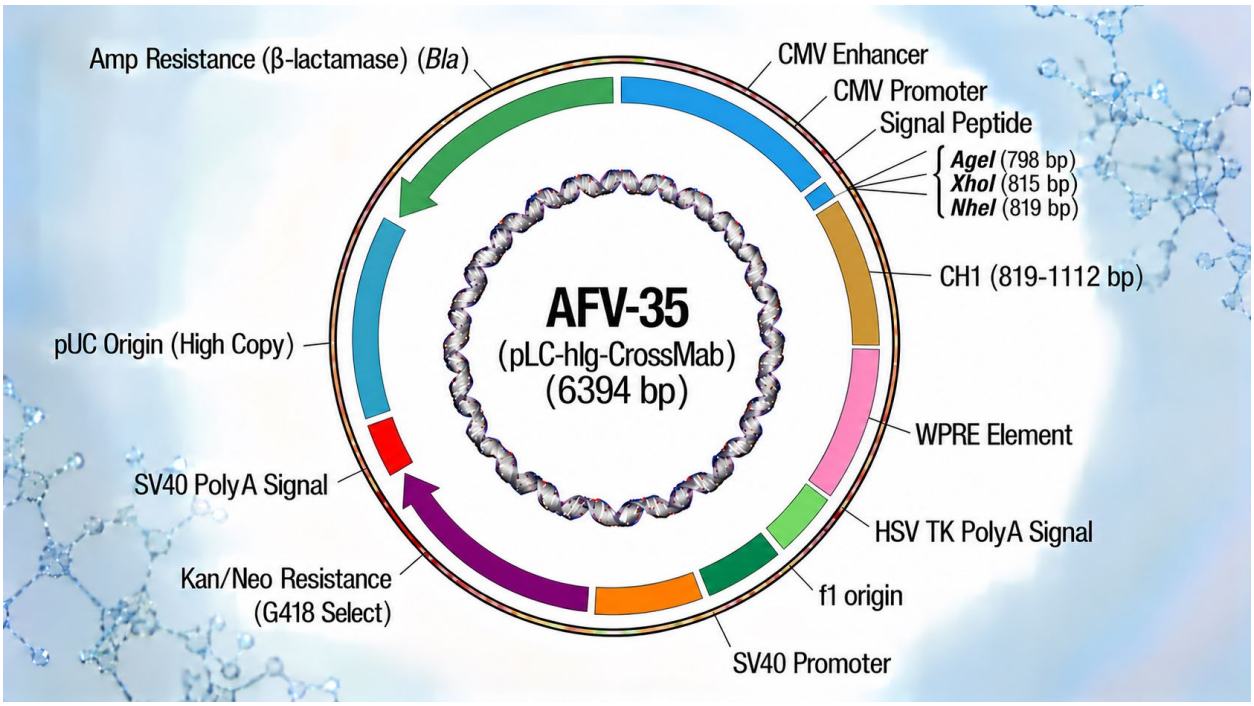
AFV-35 plasmid map — CrossMab Cκ-hole heavy chain

CrossMab Cλ2-hole heavy chain



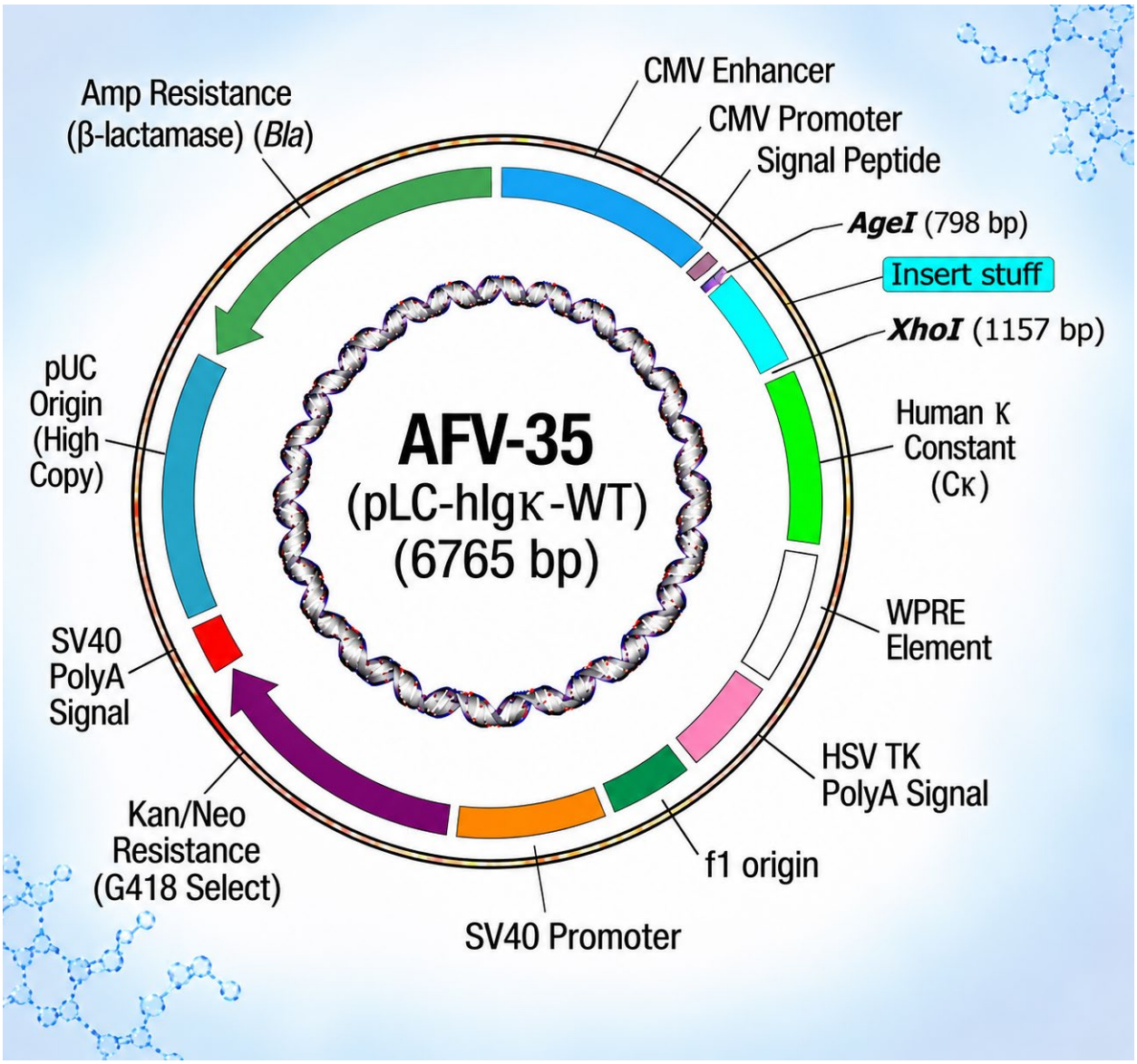
AFV-35 plasmid map — CrossMab Cλ2-hole heavy chain

Universal CrossMab light chain



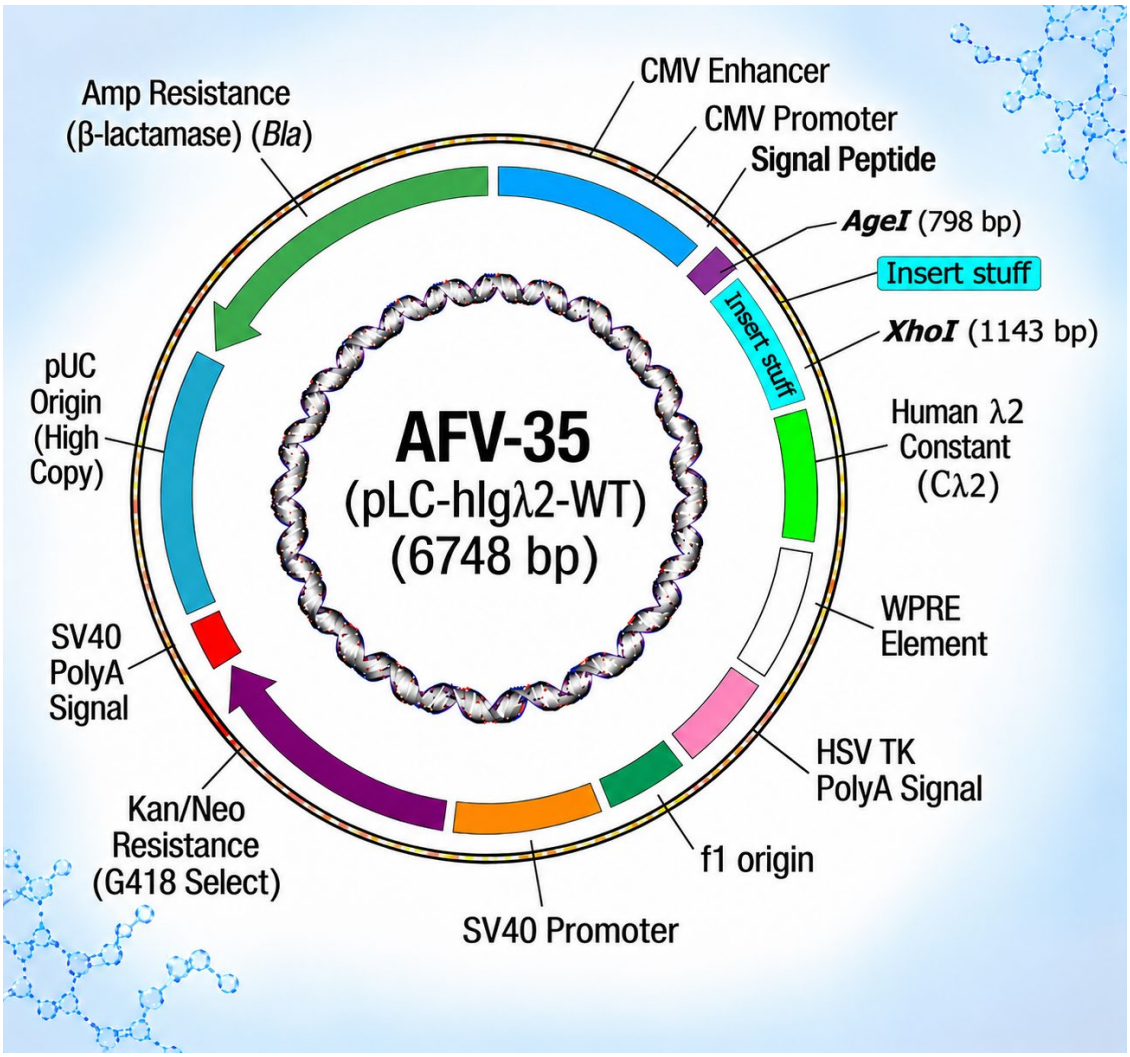
AFV-35 plasmid map — Universal CrossMab light chain

WT κ light chain



AFV-35 plasmid map — WT κ light chain

WT human Cλ2 light chain



AFV-35 plasmid map — pLC-hIgλ2-WT (6,748 bp); AgeI (798 bp) to XhoI (1,143 bp) insert region.

Document revision: Product-variation edition 3.1 • 05 November 2026. Parent catalog number: AFV-35. Variation SKUs: AFV-35-KK, AFV-35-KL, AFV-35-LK, AFV-35-LL, AFV-35-6V.