Fusion BioLabs Short Protocol for Fc-Engineered Antibody Expression Vectors

1. Product Description

Fusion BioLabs Fc Engineering Portfolio		
Family 1: Effector-Null Family		
SKU	Fc Engineering Vector	Description
AFV-12	Human IgG1 (LALA: L234A/L235A)	Human IgG1 (LALA)
AFV-13	Human IgG1 (LALA PG: L234A/L235A/P329G)	Human IgG1 (LALA PG)
AFV-14	Mouse IgG2a(LALA PG: L234A/L235A/P329G)	Mouse IgG2a (LALA PG)
AFV-04	Human IgG1 (EN: E233P/L234V/L235A/ΔG236+A327G/A330S/P331S)	Human IgG1 (EN)
AFV-05	Human IgG1 (N297Q)	Human IgG1 (N297Q)
AFV-15	Mouse IgG2a (N297Q)	Mouse IgG2a (N297Q)
AFV-16	Human IgG1 (ΔG236)	Human IgG1 (ΔG236)
Family 2: ADCC/ADCP-Enhanced Family		
SKU	Fc Engineering Vector	Description
AFV-17	Human IgG1 (GASDALIE:G236A/S239D/A330L/I332E)	Human IgG1 (GASDALIE)
AFV-18	Human IgG1 (ADE:G236A/S239D/I332E)	Human IgG1 (ADE)
AFV-02	Human IgG1 (DLE:S239D/A330L/I332E)	Human IgG1 (DLE)
AFV-01	Human IgG1 (DE: S239D/I332E)	Human IgG1 (DE)
AFV-19	Human IgG1 (G236A)	Human IgG1 (G236A)

Fusion BioLabs Fc Engineering Portfolio		
Family 3: CDC / Complement Modulated Family		
SKU	Fc Engineering Vector	Description
AFV-20	Human IgG1 (WS: K326W/E333S)	Human IgG1 (WS)
AFV-21	Human IgG1 (HexaBody: E345K/E430G)	Human IgG1 (HexaBody)
AFV-22	Human IgG1 (HexaBody-Extended: E345K/E430G/E431K)	Human IgG1 (HexaBody-Extended)
AFV-24	Human IgG2 (K322A)	Human IgG2 (K322A)
AFV-23	Human IgG1 (K322A)	Human IgG1 (K322A)
AFV-25	Human IgG1 (SEHF: S267E / H268F)	Human IgG1 (SEHF)
AFV-10	Mouse IgG2a (Comp-Null: L235E+ E318A/K320A/K322A)	Mouse IgG2a (Comp-Null)
Family 4: FcRn / PK-Modulated Family		
SKU	Fc Engineering Vector	Description
AFV-26	Human IgG1 (LA: M428L/N434A)	Human IgG1 (LA)
AFV-27	Human IgG1 (LS: M428L/N434S)	Human IgG1 (LS)
AFV-06	Human IgG1 (YTE: M252Y/S254T/T256E)	Human IgG1 (YTE)
AFV-28	Human IgG1 (QL:T250Q/M428L)	Human IgG1 (QL)
AFV-29	Human IgG1 (YTE+KF: M252Y/S254T/T256E/H433K/N434F)	Human IgG1 (YTE+KF)
AFV-30	Human IgG1 (I253A)	Human IgG1 (I253A)
AFV-31	Human IgG1 (N434A)	Human IgG1 (N434A)
AFV-32	Human IgG1 (V308P)	Human IgG1 (V308P)
Family 5: Isotype-Stabilized & Structural Engineering Family		

Fusion BioLabs Fc Engineering Portfolio		
SKU	Fc Engineering Vector	Description
AFV-09	Human IgG4 (S228P)	Human IgG4 (S228P)
AFV-33	Human IgG4 (S228P / L235E)	Human IgG4 (S228P / L235E)

2. PROTOCOL

2.1 Obtaining VH and VL sequences

You could obtain VH and VL sequences from either **gene synthesis** or **PCR amplification** from your template:

For gene synthesis, a 5'-end with sequence (5'-TAGTAGCAACTGCAACCGGTGTACATTCA-3') and 3'-end with the following sequence (different, see table below) should be appended to your VH or VL (Vk or Vλ) ends.

Note: There is no need to add signal sequence to your VH and VL fragment.

SKU	Isotype	Whole VH/VL Sequence
AFV-XX*	**hIgG1**	**5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgcctccaccaagggc-3'**
AFV-24	hIgG2	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgcctccaccaagggc-3'
AFV-09, AFV-33	hIgG4	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgcctccaccaagggc-3'
AEV-09	hIgκ	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vk-GTCTCGAGCgaactgtggctgcac-3'
AEV-10	hIgλ2	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vλ-TTGCTCGAGggtcagcccaaggct-3'
AFV-10, AFV-14, AFV-15	mIgG2a	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgccaaaacaacagcc-3'
AEV-19	mIgκ	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vk-CGTCTCGAGcgggctgatgctgca-3'
AEV-23	mIgλ1	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vλ-GTCTCGAGCggccagcccaagtct-3'

AFV-XX*: any human IgG1 Fc-engineering expression vector.

For PCR amplification, the Forward Primer and Reverse Primer should be as following. The optimized annealing temperature should be 53-58°C. For best in-frame insert, the resulting amplicons must be sequenced before or after the cloning into the expression vector.

Forward sequencing primer (**pFB forward**): 5'-ATGGGCGGTAGGCGTGTA-3' (included in the Kit).

Note: Forward Primer's $N_{(12-18)}$ is from your VH or VL coding region (no need adding signal peptide sequence); Reverse Primer's $N_{(12-18)}$ is the terminal coding sequence of your VH or VH.

SKU	Isotype	Forward Primer	Reverse Primer
AFV-XX*	hIgG1	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-gcccttggtggaggcGCTCGAGACN$_{(12-18)}$-3'
AFV-24	hIgG2	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-gcccttggtggaggcGCTCGAGACN$_{(12-18)}$-3'
AFV-09, AFV-33	hIgG4	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-gcccttggtggaggcGCTCGAGACN$_{(12-18)}$-3'
AEV-09	hIgκ	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-gtgcagccacagttcGCTCGAGACN$_{(12-18)}$-3'
AEV-10	hIgλ2	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-agccttgggctgaccCTCGAGCAAN$_{(12-18)}$-3'
AFV-10, AFV-14, AFV-15	mIgG2a	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-ggctgttgttttggcGCTCGAGACN$_{(12-18)}$-3'
AEV-19	mIgκ	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-tgcagcatcagcccGCTCGAGACGN$_{(12-18)}$-3'
AEV-23	mIgλ1	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN$_{(12-18)}$-3'	5'-agacttgggctggccGCTCGAGACN$_{(12-18)}$-3'

AFV-XX*: any human IgG1 Fc-engineering expression vector.

2.2 Cloning into AFV-XX (Fc-engineered heavy chain expression vector) and AEV-XX (Wild-type light chain expression vector)

Once the VH and VL sequences have been obtained, the VH and VL could be cloned into the AFV-XX heavy chain expression vector, and the AEV-XX light chain expression vector, respectively. Two methods are available:

Restriction Enzyme Cloning

There is 5'-end AgeI and 3'-end XhoI for all AFV-XX and AEV-XX expression vector. All of our antibody expression vector are compatible

with high throughput platform.

1) Digestion setup

Component	50 µl reaction
VH or VL Inserts / AFV-XX vector)	1 µg / 5 µg
Restriction buffer (10x)	5 µl (1x)
AgeI	5 units
XhoI	20 units
Nuclease-free H_2O	to 50 µl

- Incubate at 37°C for 1-3 hours.
- Run agarose gel to purify the digested inserts and vector backbone.

2) T4 DNA ligation

Component	20 µl reaction
T4 DNA ligation buffer (10x)	2 µl
Vector DNA	80 ng
Insert DNA	15 ng
T4 DNA ligase	400 units
Nuclease-free H_2O	to 20 µl

- Mix gently and microfuge briefly, and incubate at 16°C or 4°C overnight or room temperature for 30 min.
- Transformation: chill on ice and transform 5 µl of the reaction into 50 µl competent cells.

Cloning through homologous assembly

There are many convenient kits for this method from different supply. We recommend NEBuilderHiFi DNA Assembly Kit (Cat# E2621S).

Component	5 µl reaction in PCR tube
Vector DNA	45 ng
Insert DNA	4.5 ng
NEBuilder HiFi DNA Assembly Master Mix	2.5 µl
Nuclease-free H_2O	to 5 µl

- Mix gently and microfuge briefly, move the PCR tube to previously set PCR program: 50°C, 15 minutes, 4°C, 5 minutes.
- Store PCR reaction tube on ice or at -20°C for subsequent transformation.

- Transformation: chill on ice and transform 2.5 µl of the reaction into 25 µl competent cells.

2.3 Antibody Production

Cotransfect mammalian cells, such as CHO and 293 cells, with the sequencing confirmed expression plasmid pair, AFV-XX encoding the Fc-engineered heavy chain, and AEV encoding the wild-type light chain. Typically, we recommend using a ratio of 2:3 of AFV-XX: AEV-XX plasmids.

Note: Antibody production after transfection, you may take an aliquot of growth medium and perform SDS-PAGE, target protein-specific ELISA, or bioassay of choice to determine that your cells are producing your antibody of interest.

2.4 Antibody Purification

The resulting Fc-engineered human IgG1, human IgG2, human IgG4, and mouse IgG2a antibody can be purified from the CHO supernatant or HEK293 supernatant using the appropriate Protein A, Protein G, Protein L or antigen-coupled resin for affinity chromatography.

Notes:In humans, lambda 2 is often a prominent constant/subfamily focus. Whereas, lambda 1 (Vλ1) is the most frequently expressed and predominant lambda light chain subtype in natural antibody responses in mice.

2.5 Functional Characterization of Fc-Engineered Antibodies

Following antibody expression and purification, Fc-engineered antibodies may be evaluated using functional assays appropriate to the intended Fc modification. Wild-type antibodies of the same isotype should be included as reference controls whenever possible.

2.5.1 Antibody-Dependent Cellular Cytotoxicity (ADCC) Assay

ADCC activity may be assessed using antigen-positive target cells and FcγRIIIa-expressing effector cells or a validated ADCC reporter assay. Compare Fc-engineered antibodies with the corresponding wild-type antibody to determine relative effector activity.

2.5.2 Complement-Dependent Cytotoxicity (CDC) Assay

CDC activity may be evaluated using antigen-positive target cells in the presence of an appropriate complement source. Cell viability or complement-mediated cell lysis can be measured and compared with wild-type antibody controls.

2.5.3 Antibody-Dependent Cellular Phagocytosis (ADCP) Assay

ADCP activity may be assessed using antigen-expressing target cells and suitable phagocytic effector cells. Phagocytosis can be quantified using fluorescence-based methods or validated reporter assays.

2.5.4 Fcγ Receptor and C1q Binding Assays

Binding of Fc-engineered antibodies to Fcγ receptors and complement component C1q may be characterized using ELISA, biolayer interferometry (BLI), or surface plasmon resonance (SPR).

2.5.5 FcRn Binding and pH-Dependent Interaction

FcRn-modulated antibodies may be evaluated for FcRn binding under acidic conditions (typically pH 6.0) and release under near-neutral conditions (approximately pH 7.4).

Note: These assays are optional downstream characterization procedures. Assay conditions should be optimized for the specific antibody, antigen, cell system, and experimental objective. Fc-engineering vectors do not include the additional reagents required for these functional assays.