Fusion BioLabs Short Protocol for Fc-engineered Antibody Expression Vector Kit

1. Product Description

	SKU	Heavy chain vector	Fc effector	SKU	Light chain vector	Description
Human IgG1	AFV-01	pFB-CHIg-hG1e1	Increased ADCC	AEV-09	pFB-CLIg-hk	Human Igk Mammalian Expression Vector
	AFV-02	pFB-CHIg-hG1e2	Increased ADCC	AEV-10	pFB-CLIg-hl	Human Igλ2 Mammalian Expression Vector
	AFV-03	pFB-CHIg-hG1e3	Increased CDC and ADCC	*Note: Antibody light chain expression vector needed for antibody production*		
	AFV-04	pFB-CHIg-hG1e4	Reduced CDC and ADCC			
	AFV-05	pFB-CHIg-hG1e5	Reduced CDC and ADCC			
	AFV-06	pFB-CHIg-hG1e6	Increased half-life			
	AFV-07	pFB-CHIg-hG1e7	Increased half-life			
	AFV-08	pFB-CHIg-hG1e8	Increased half-life			
Human IgG4	AFV-09	pFB-CHIg-hG4e1	Reduced Fab-arm exchange			
Mouse IgG2a	AFV-10	pFB-CHIg-mG2ae1	Reduced CDC and ADCC	AEV-19	pFB-CLIg-mk	Mouse Igk Mammalian Expression Vector
				AEV-23	pFB-CLIg-ml1	Mouse Igλ1 Mammalian Expression Vector
				AEV-24	pFB-CLIg-ml2	Mouse Igλ2 Mammalian Expression Vector

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.

hIgG1	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgcctccaccaagggc-3'
hIgG4	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgcctccaccaagggc-3'
hIgk	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vk-GTCTCGAGCgaactgtggctgcac-3'
hIgl	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vλ-TTGCTCGAGggtcagcccaaggct-3'
mIgG2a	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-VH-GTCTCGAGCgccaaaacaacagcc-3'
mIgk	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vk-CGTCTCGAGcgggctgatgctgca-3'
mIgl1	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vλ-GTCTCGAGCggccagcccaagtct-3'
mIgl2	5'-TAGTAGCAACTGCAACCGGTGTACATTCA-Vλ-GTCTCGAGCggtcagcccaagtcc-3'

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 (**pCMV5F**): 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.

	Forward Primer	**Reverse Primer**
hIgG1	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-gcccttggtggaggcGCTCGAGACN(12-18)-3'
hIgG4	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-gcccttggtggaggcGCTCGAGACN(12-18)-3'
hIgk	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-gtgcagccacagttcGCTCGAGACN(12-18)-3'
hIgl	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-agccttgggctgaccCTCGAGCAAN(12-18)-3'
mIgG2a	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-ggctgttgttttggcGCTCGAGACN(12-18)-3'
mIgk	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-tgcagcatcagcccGCTCGAGACGN(12-18)-3'

mIgl1	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-agacttgggctggccGCTCGAGACN(12-18)-3'
mIgl2	5'-TAGTAGCAACTGCAACCGGTGTACATTCAN(12-18)-3'	5'-ggacttgggctgaccGCTCGAGACN(12-18)-3'

2.2 Cloning into pFB-CHIg (heavy chain expression vector) and pFB-CLIg (light chain expression vector)

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

Restriction Enzyme Cloning

There is 5'-end AgeI and 3'-end XhoI for all pFB-CHIg and pFB-CLIg 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 / pFB-CHIg or pFB-CLIg 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 NEBuilder HiFi 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, pFB-CHIg encoding the heavy chain, and pFB-CLIg encoding the light chain. Typically, we recommend using a ratio of 2:3 of pFB-CHIg: pFB-CLIg 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 IgG4, and mouse IgG2a antibody can be affinity chromatography purified from the CHO supernatant or HEK293 supernatant using the appreciate Protein A, Protein G, Protein L or antigen-coupled resin.