



# Fusion BioLabs Short Protocol for Human Fab Phage Display Library Construction Kit

## pAPD-h-Fab: Human Fab phage display library construction kit

Catalog#: APD-02

### Product Overview

Fusion BioLabs offers a range of library primer sets and phagemid vector combination for antibody phage display and peptide phage display construction. With customizable features and robust performance, our primer sets and phagemid vectors are designed for facilitating phage display library generation as fast as within one week.

**pAPD-h-Fab** are the phagemid vectors for construction of a fragment antigen-binding (Fab) library for **human** antibodies. Here are the key steps involved in constructing such a library:

- Amplify V genes from cDNA reverse transcript from RNA isolated from peripheral blood lymphocytes (PBL) or lymphoid tissue of non-immunized or immunized donors using PCR primers corresponding to known  $V_H$ ,  $V_K$ , and  $V_\lambda$  gene sequences.
- Amplify CH1 fragment, C $\kappa$  fragment and C $\lambda$  fragment using **pAPD-h-Fab** as template.
- Combine  $V_H$  repertoires and CH1 fragment,  $V_K$  repertoires and C $\kappa$  fragment, and  $V_\lambda$  repertoires and C $\lambda$  fragment to create  $V_H$ -C $H1$ ,  $V_K$ -C $\kappa$  and  $V_\lambda$ -C $\lambda$  constructs respectively, using a simple two-fragment PCR assembly procedure.
- Overlap assembly  $V_K$ -C $\kappa$  and  $V_H$ -C $H1$ , and  $V_\lambda$ -C $\lambda$  and  $V_H$ -C $H1$  to make Fab repertoires respectively.
- Restriction enzyme digestion **pAPD-h-Fab** vector and Fab repertoires with SfiI.
- Ligation of digested and purified repertoires into digested and purified **pAPD-h-Fab** vector to make human Fab libraries.

### Key Features

**High expression efficiency:** Engineered for efficient expression and display of antibody fragment Fab on the surface, allowing for easy screening and selection of target molecules.

**Flexibility and versatility:** One vector for both antibody library construction and downstream antibody fragment expression. No need subcloning into expression vector for downstream application.

### Specifications

Antibiotic Resistance	Ampicillin (Amp <sup>R</sup> )
Constitutive or Inducible System	Inducible for downstream expression
Delivery Type	Transformation
Product Type	Bacterial Expression Vector
Cloning Method	Restriction Enzyme SfiI

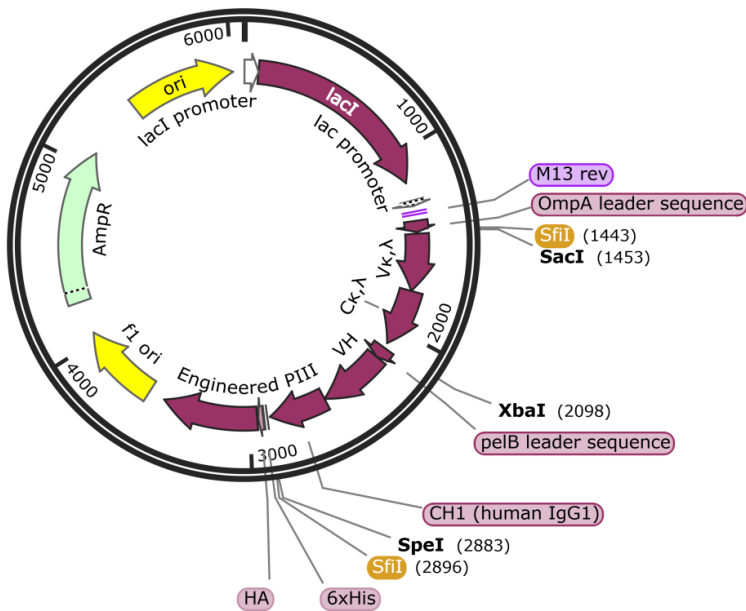


### Contents & Storage

Primer Set		
Vial 1	200 $\mu$ l, 10 $\mu$ M	Primer mix (F+R) for V <sub>H</sub> repertoires amplification
Vial 2	200 $\mu$ l, 10 $\mu$ M	Primer mix (F+R) for V <sub>K</sub> repertoires amplification
Vial 3	200 $\mu$ l, 10 $\mu$ M	Primer mix (F+R) for V <sub><math>\lambda</math></sub> repertoires amplification
Vial 4	200 $\mu$ l, 10 $\mu$ M	Primer mix (F+R) for CH1 fragment amplification
Vial 5	200 $\mu$ l, 10 $\mu$ M	Primer mix (F+R) for C <sub>K</sub> fragment amplification
Vial 6	200 $\mu$ l, 10 $\mu$ M	Primer mix (F+R) for C <sub><math>\lambda</math></sub> fragment amplification
Vial 7	200 $\mu$ l, 10 $\mu$ M	Assembly primers for V <sub>H</sub> -C <sub>H1</sub> repertoires construction
Vial 8	200 $\mu$ l, 10 $\mu$ M	Assembly primers for V <sub>K</sub> -C <sub>K</sub> repertoires construction
Vial 9	200 $\mu$ l, 10 $\mu$ M	Assembly primers for V <sub><math>\lambda</math></sub> -C <sub><math>\lambda</math></sub> repertoires construction
Vial 10	200 $\mu$ l, 10 $\mu$ M	Assembly primers for Fab repertoires construction
pAPD-h-Fab cloning vectors for human Fab library construction and templates for CH1, C <sub>K</sub> and C <sub><math>\lambda</math></sub> fragment amplification		
Vial 11	10.0 $\mu$ g in Tris-EDTA buffer	

- Store at -20°C. Primer sets and vectors are guaranteed stable for 12 months when properly stored.

### Vector for library Construction



**Phagemid vector for human Fab library construction**

6069 bp



## Protocols

1. PCR amplification of the  $V_H$  repertoires,  $V_k$  repertoires and  $V_\lambda$  repertoires using following condition.

- Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
$V_H$ repertoires	Vial 1	1.0 ml (100 $\mu$ l /rxn x 10 rxns)
$V_k$ repertoires	Vial 2	0.8 ml (100 $\mu$ l /rxn x 8 rxns)
$V_\lambda$ repertoires	Vial 3	0.8 ml (100 $\mu$ l /rxn x 8 rxns)

- PCR setup and PCR protocol

Component	Amount	PCR Protocol	
10 x PCR buffer, -Mg <sup>2+</sup>	10 $\mu$ l	Initial denaturation	94°C for 2 min
50 mM MgCl <sup>2</sup>	3 $\mu$ l	30 PCR cycles	Denature 94°C for 30 sec
10 mM dNTPs	2 $\mu$ l		Anneal 56°C for 30 sec
10 $\mu$ M Primer mix (F+R)	4 $\mu$ l		Extend 72°C for 90 sec
cDNA template (500-8000 ng/ $\mu$ l)	1 $\mu$ l	Final Extension	72°C for 10 min
Taq DNA Polymerase (10 units/ $\mu$ l)	0.4 $\mu$ l	Hold	4°C, indefinitely
Water, nuclease-free	79.6 $\mu$ l	Note: Recommended for our PCR condition. Optimization maybe needed.	

- Combine each repertoires reaction in 2.0 ml nuclease-free tube, respectively.
  - Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
  - Run the sample on a 2% agarose gel and an appropriate molecular weight marker.  
*Expect results: 350-400 bp products for each type of repertoire.*
  - Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
  - Quantitate yields using a NanoDrop Spectrophotometer at 260 nm  
*Expect results: Approximately 3-6  $\mu$ g of each repertoire. If yields are too low, repeat the PCR and combine the end product for each type of repertoire.*
2. PCR amplification of the human  $C_H1$ ,  $C_k$  and  $C_\lambda$  fragments using [cloned human Fab vectors \(Vial 11\)](#) as templates via following condition.



- Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
C <sub>H</sub> 1 fragment	Vial 4	0.6 ml (100 µl /rxn x 6 rxns)
C <sub>k</sub> fragment	Vial 5	0.6 ml (100 µl /rxn x 6 rxns)
C <sub>λ</sub> fragment	Vial 6	0.6 ml (100 µl /rxn x 6 rxns)

- PCR setup and PCR protocol

Component	Amount	PCR Protocol		
10 x PCR buffer, -Mg <sup>2+</sup>	10 µl	Initial denaturation		
50 mM MgCl <sup>2</sup>	3 µl	25 PCR cycles	94°C for 2 min	
10 mM dNTPs	2 µl		Denature	94°C for 30 sec
10 µM Primer mix (F+R)	4 µl		Anneal	56°C for 30 sec
PCR template (Vial 11, 250 ng/µl)	0.2 µl	Extend	72°C for 60 sec	
Taq DNA Polymerase (10 units/µl)	0.4 µl	Final Extension		
Water, nuclease-free	79.6 µl	Hold	72°C for 10 min	
			4°C, indefinitely	
		Note: Recommended for our PCR condition. Optimization maybe needed.		

- Combine each repertoire's reaction in 2.0 ml nuclease-free tube, respectively.
  - Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
  - Run the sample on a 2% agarose gel and an appropriate molecular weight marker.  
*Expect results: 350 bp for C<sub>H</sub>1 fragment, 420 bp for C<sub>k</sub> fragment and C<sub>λ</sub> fragment.*
  - Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
  - Quantitate yields using a NanoDrop Spectrophotometer at 260 nm  
*Expect results: Approximately 3-6 µg of each repertoire. If yields are too low, repeat the PCR and combine the end product for each type of fragment.*
3. Overlapping Extension PCR to generate V<sub>k</sub>-C<sub>k</sub>-linker repertoires, V<sub>λ</sub>-C<sub>λ</sub>-linker repertoires, and -linker-V<sub>H</sub>-C<sub>H</sub>1 repertoires according to the below template combination and assembly primers.
- Assembly primer set and total OE-PCR reaction volume

Overlapping extension PCR	Template combination	Assembly primers	Volume
-linker-V <sub>H</sub> -C <sub>H</sub> 1 repertoires	V <sub>H</sub> repertoires & C <sub>H</sub> 1 fragment	Vial 7	1.0 ml (10 rxns)



V <sub>k</sub> -C <sub>k</sub> -linker repertoires	V <sub>k</sub> repertoires & C <sub>k</sub> fragment	Vial 8	1.0 ml (10 rxns)
V <sub>λ</sub> -C <sub>λ</sub> -linker repertoires	V <sub>λ</sub> repertoires & C <sub>λ</sub> fragment	Vial 9	1.0 ml (10 rxns)

- OE-PCR setup and PCR protocol

Component	Amount	PCR Protocol	
10 x PCR buffer, -Mg <sup>2+</sup>	10 μl	Initial denaturation	
50 mM MgCl <sup>2</sup>	3 μl	20 PCR cycles	Denature
10 mM dNTPs	2 μl		Anneal
10 μM Assembly primers	4 μl		Extend
Template combination ( <b>100 ng each</b> )	x μl	Final Extension	
Taq DNA Polymerase (10 units/μl)	0.4 μl	Hold	
Water, nuclease-free	to 100 μl	Note: Recommended for our PCR condition. Optimization maybe needed.	

- Combine each repertoires reaction in 2.0 ml nuclease-free tube, respectively.
- Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
- Run the sample on a 2% agarose gel and an appropriate molecular weight marker.  
*Expect results: ~750-800 bp for -linker-V<sub>H</sub>-C<sub>H</sub>1 repertoires, V<sub>k</sub>-C<sub>k</sub>-linker repertoires and V<sub>λ</sub>-C<sub>λ</sub>-linker repertoires products.*
- Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
- Quantitate yields using a NanoDrop Spectrophotometer at 260 nm  
*Expect results: At least 2-4 μg of each repertoire. If yields are too low, repeat the overlapping extension PCR and combine the end product for each type of repertoire.*

4. Overlapping Extension PCR to generate either Fab repertoires with kappa light chain (k-Fab repertoires: Sfi-V<sub>k</sub>-C<sub>k</sub>-linker-V<sub>H</sub>-C<sub>H</sub>1-Sfil) or Fab repertoires with lambda light chain (λ-Fab repertoires: Sfi-V<sub>λ</sub>-C<sub>λ</sub>-linker-V<sub>H</sub>-C<sub>H</sub>1-Sfil) according to the below template combination and assembly primers.

- Assembly primer set and total OE-PCR reaction volume
- 

Overlapping extension PCR	Template combination	Assembly primers	Volume
---------------------------	----------------------	------------------	--------



k-Fab repertoires	V <sub>k</sub> -C <sub>k</sub> -linker repertoires & -linker-VH-C <sub>H</sub> 1 repertoires	Vial 10	1.0 ml (10 rxns)
λ-Fab repertoires	V <sub>λ</sub> -C <sub>λ</sub> -linker repertoires & -linker-VH-C <sub>H</sub> 1 repertoires	Vial 10	1.0 ml (10 rxns)

- OE-PCR setup and PCR protocol

Component	Amount	PCR Protocol	
10 x PCR buffer, -Mg <sup>2+</sup>	10 μl	Initial denaturation	
50 mM MgCl <sup>2</sup>	3 μl	25 PCR cycles	Denature
10 mM dNTPs	2 μl		Anneal
10 μM Assembly primers	4 μl		Extend
Template combination ( <b>100 ng each</b> )	x μl	Final Extension	
Taq DNA Polymerase (10 units/μl)	0.4 μl	Hold	
Water, nuclease-free	to 100 μl	Note: Recommended for our PCR condition. Optimization maybe needed.	

- Combine each repertoires reaction in 2.0 ml nuclease-free tube, respectively.
  - Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
  - Run the sample on a 2% agarose gel and an appropriate molecular weight marker.  
*Expect results: ~1500 bp for Fab repertoires (either k-Fab repertoires or λ-Fab repertoires) products.*
  - Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
  - Quantitate yields using a NanoDrop Spectrophotometer at 260 nm  
*Expect results: At least 10-20 μg of each purified Fab repertoire. If yields are too low, repeat the overlapping extension PCR and combine the end product for each type of repertoire.*
5. Restriction digestion of Fab repertoires and pAPD-h-Fab cloning vectors by SfiI at 50°C for overnight or 6 h.

Components	Fab repertoires	pAPD-h-Fab vectors
DNA	10 μg	10 μg
10X digestion buffer	20 μl	20 μl
SfiI (20 units/μl)	10 μl	10 μl
Water, nuclease-free	to 200 μl	to 200 μl

- Add gel loading buffer to each tube, mix, and briefly centrifuge the contents.
- Run the sample on a 1% agarose gel and an appropriate molecular weight marker.



*Expect results: ~1600 bp for digested Fab repertoires (either k-Fab repertoires or λ-Fab repertoires) products, and ~4600 bp for digested pAPD-h-Fab vector backbone.*

- Cut out the correct-size bands, and purify the DNA using any commercial gel DNA extraction kit.
- Quantitate yields using a NanoDrop Spectrophotometer at 260 nm

*Expect results: At least 1-4 µg of each purified Fab repertoire or digested pAPD-h-Fab vector backbone.*

6. Library ligation using T4 DNA ligase

Components	200 µl reaction for k-Fab library	200 µl reaction for λ-Fab library
T4 DNA Ligase Buffer (10X)*	20 µl	20 µl
Digested pAPD-h-Fab	1.9 µg	1.9 µg
Digested Fab repertoires	1.5 µg	1.5 µg
T4 DNA Ligase (20 units/µl)	5.0 µl	5.0 µl
Water, nuclease-free	to 200 µl	to 200 µl

*\*The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.*

- Gently mix the reaction by pipetting up and down and microfuge briefly.
- Incubate overnight at room temperature.
- Heat inactivate at 65°C for 10 minutes.
- Chill on ice and heart transform 1-5 µl of the reaction into 50 µl competent cells.
- Purify the ligated samples and dissolve in 50 µl nuclease-free water.
- Transform 2 µl of purified ligation samples into 25 µl tube with TG1 electrocompetent cells (efficiency should be  $>4 \times 10^{10}$  cfu/µg of plasmid DNA) for 2 min.
- Transfer the mix to electroporation cuvette (0.1 cm gap) and electroporate at 1.8 kV, 10 µF and 600 Ohms. Typical time constants are 3.5 to 4.5 msec for successful electroporation.

7. Proceed with the phage library preparation and panning.