Fusion BioLabs

Antibody Phage Display Library Construction Kit

pAPD-m-Fab: Mouse Fab phage display library construction kit

Catalog#: APD-03

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-m-Fab is the phagemid vector for construction of a fragment antigen-binding (Fab) library for **mouse** 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 Variable repertoires of V_H, V_k, V_λ and constant regions of C_{H1}, C_κ and C_λ.
- Combine V_H repertoires and C_{H1} fragment to create V_H-C_{H1} repertoires, V_k repertoires and C_κ fragment to create V_k-C_k repertoires, V_λ repertoires and C_λ fragment to create V_λ-C_λ repertoires, using a simple two-fragment PCR assembly procedure respectively.
- Restriction enzyme digestion **pAPD-m-Fab** vector and V_k-C_k repertoires or V_λ-C_λ repertoires with SacI/XbaI, or **pAPD-m-Fab** vector and V_H-C_{H1} repertoires with XhoI/SpeI.
- Ligation of digested and purified fragment into corresponding restriction enzymes digested and purified **pAPD-m-Fab** vector to make either **Light chain sub-library** or **heavy chain sub-library**.
- Restriction enzyme digestion **light chain sub-library** and V_H-C_{H1} repertoires with XhoI/SpeI or **heavy chain sub-library** and $V_{k,\lambda}$-$C_{k,\lambda}$ repertoires with SacI/XbaI, to make mouse 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

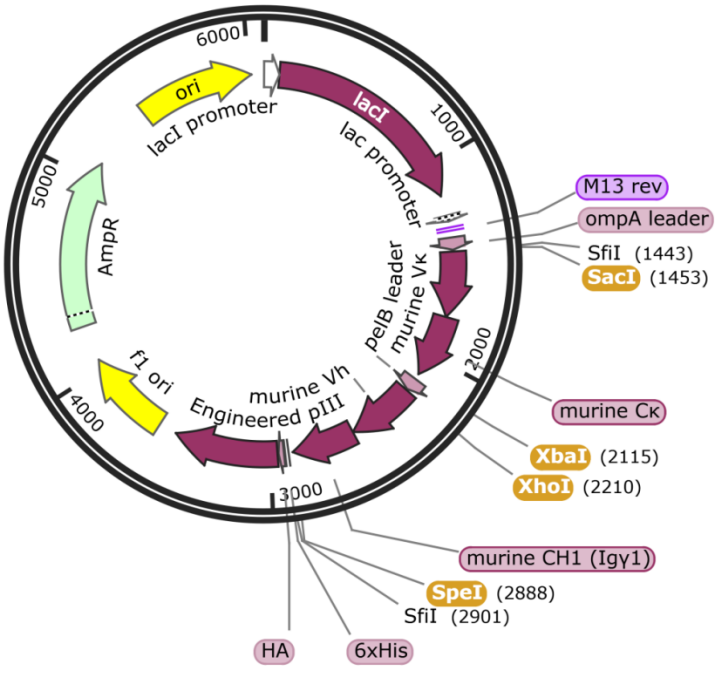
Antibiotic Resistance	Ampicillin (Amp^R)
Constitutive or Inducible System	Inducible for downstream expression
Delivery Type	Transformation
Product Type	Bacterial Expression Vector
Cloning Method	Restriction Enzymes for (5'-SacI and 3'-XbaI for $V_{k,\lambda}$-$C_{k,\lambda}$ repertoires; 5'-XhoI and 3'-SpeI for V_H-C_{H1} repertoire)

Contents & Storage

Primer Set		
Vial 1	100 µl, 10 µM	Primer mix (F+R) for V_k repertoire amplification
Vial 2	100 µl, 10 µM	Primer mix (F+R) for V_λ repertoire amplification
Vial 3	100 µl, 10 µM	Primer mix (F+R) for V_H repertoire amplification
Vial 4	100 µl, 10 µM	Primer mix (F+R) for C_k fragment amplification
Vial 5	100 µl, 10 µM	Primer mix (F+R) for C_λ fragment amplification
Vial 6	100 µl, 10 µM	Primer mix (F+R) for C_{H1} fragment amplification
Vial 7	100 µl, 10 µM	Assembly Primer mix (F+R) for V_k-C_k repertoire construction
Vial 8	100 µl, 10 µM	Assembly Primer mix (F+R) for V_λ-C_λ repertoire construction
Vial 9	100 µl, 10 µM	Assembly Primer mix (F+R) for V_H-C_{CH1} repertoire construction
Vial 10	100 µl, 10 µM	M13 rev Forward sequencing primer
Vial 11	100 µl, 10 µM	pIII rev Reverse sequencing primer
pAPD-m-Fab cloning vector for phage display mouse Fab library construction		
Vial 12	10.0 µg 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 mouse Fab library construction
6074 bp

Protocols

1. PCR amplification of the V_H repertoires, V_k repertoires and V_λ repertoires using following condition.

   - Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
V_k repertoires	Vial 1	1.0 ml (100 µl /rxn x 10 rxns)
V_λ repertoires	Vial 2	0.8 ml (100 µl /rxn x 8 rxns)
V_H repertoires	Vial 3	0.8 ml (100 µl /rxn x 8 rxns)

   - PCR setup and PCR protocol

Component	Amount	PCR Protocol		
10 x PCR buffer, -Mg^{2+}	10 µl	Initial denaturation		94°C for 2 min
50 mM $MgCl^2$	3 µl	30 PCR cycles	Denature	94°C for 30 sec
10 mM dNTPs	2 µl		Anneal	56°C for 30 sec
10 µM Primer mix (F+R)	4 µl		Extend	72°C for 90 sec
cDNA template (500-8000 ng/µl)	1 µl	Final Extension		72°C for 10 min
Taq DNA Polymerase (10 units/µl)	0.4 µl	Hold		4°C, indefinitely
Water, nuclease-free	79.6 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: 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 µg 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_λ fragments using cloned mouse Fab vectors (Vial 12) as templates via following condition.

   - Primer mix set and total PCR reaction volume

PCR amplification	Primer mix set	Volume
C_k fragment	Vial 4	0.6 ml (100 µl /rxn x 6 rxns)
C_λ fragment	Vial 5	0.6 ml (100 µl /rxn x 6 rxns)
C_{H1} 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^{2+}	10 µl	Initial denaturation		94°C for 2 min
50 mM $MgCl^2$	3 µl	25 PCR cycles	Denature	94°C for 30 sec
10 mM dNTPs	2 µl		Anneal	56°C for 30 sec
10 µM Primer mix (F+R)	4 µl		Extend	72°C for 60 sec
PCR template (**Vial 11 or cDNA**, 250 ng/µl)	0.2 µl	Final Extension		72°C for 10 min
Taq DNA Polymerase (10 units/µl)	0.4 µl	Hold		4°C, indefinitely
Water, nuclease-free	79.6 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: 350 bp for C_{H1} fragment, 420 bp for C_k fragment and C_λ 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. Assembly PCR to generate V_k-C_k repertoires, V_λ-C_λ repertoires, and V_H-C_{H1} repertoires according to the below template combination and assembly primers.

- Assembly primer set and total PCR reaction volume

Overlapping extension PCR	Template combination	Assembly primers	Volume
V_k-C_k repertoires	V_k repertoires & C_k fragment	Vial 7	1.0 ml (10 rxns)
V_λ-C_λ repertoires	V_λ repertoires & C_λ fragment	Vial 8	1.0 ml (10 rxns)
VH-C_{H1} repertoires	V_H repertoires & C_{H1} fragment	Vial 9	1.0 ml (10 rxns)

- PCR setup and PCR protocol

Component	Amount	PCR Protocol		
10 x PCR buffer, -Mg^{2+}	10 µl	Initial denaturation		94°C for 2 min
50 mM $MgCl_2$	3 µl	20 PCR cycles	Denature	94°C for 30 sec
10 mM dNTPs	2 µl		Anneal	56°C for 30 sec
10 µM Assembly primers	4 µl		Extend	72°C for 2 min
Template combination (**100 ng each**)	x µl	Final Extension		72°C for 10 min
Taq DNA Polymerase (10 units/µl)	0.4 µl	Hold		4°C, indefinitely
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 V_k-C_k repertoires, V_λ-C_λ repertoires and VH-C_{H1} 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. Making **Light chain sub-library** or **Heavy chain sub-library**

4.1 Making **Light chain sub-library**

4.1.1 *Repertoires and vector digestion and purification*

Components	*V_k-C_k repertoires* or *V_λ-C_λ repertoires*	pAPD-m-Fab vectors
DNA	10 µg	5 µg
10X digestion buffer	20 µl	20 µl
SacI	100 units	100 units
XbaI	100 units	100 units
Water, nuclease-free	to 200 µl	to 200 µl

- Restriction digestion at 37°C for overnight or 6 h.
- 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: 650~700 bp for digested repertoires (either V_k-C_k repertoires or V_λ-C_λ repertoires) products, and ~5400 bp for digested pAPD-m-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 repertoire or digested pAPD-m-Fab vector backbone.

4.1.2 *Light chain sub-library* ligation using T4 DNA ligase

Components	Amount
T4 DNA Ligase Buffer (10X)*	20 µl
Digested pAPD-m-Fab (SacI/XbaI)	1.9 µg
Digested *V_k-C_k* or *V_λ-C_λ repertoires (SacI/XbaI)*	0.75 µg
T4 DNA Ligase (20 units/µl)	5.0 µl
Water, nuclease-free	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 x 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.
- Plate out 100 µL from each transformation onto 2YT with 50 µg/ml Carbenicillin (2YT-Carb) plates and incubate overnight at 37°C.

4.1.3 Large-Scale Preparation of *Light chain sub-library* DNA

- Scrape cells off all plates into 100 ml 2YT with 2% Glucose and 50 µg/ml Carbenicillin (2YT-Glu-Carb) and incubate a further 60 min at 37°C.
- Increase volume to 500 mL with 2TY–GLU, increase carbenicillin to 50 µg/mL, and incubate overnight at 37°C.
- Recover cells by centrifugation and prepare DNA by your reliable method.

4.2 Making **Heavy chain sub-library**

4.2.1 Repertoires and vector digestion and purification

Components	*V_H-C_{H1} repertoires*	pAPD-m-Fab vectors
DNA	10 µg	5 µg
10X digestion buffer	20 µl	20 µl
SacI	100 units	100 units
XbaI	100 units	100 units
Water, nuclease-free	to 200 µl	to 200 µl

- Restriction digestion at 37°C for overnight or 6 h.
- 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: 650~700 bp for digested repertoires products, and ~5400 bp for digested pAPD-m-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 purified repertoire or digested pAPD-m-Fab vector backbone.

4.2.2 *Heavy chain sub-library* ligation using T4 DNA ligase

Components	Amount
T4 DNA Ligase Buffer (10X)*	20 µl
Digested pAPD-m-Fab (XhoI/SpeI)	1.9 µg
Digested *V_H-C_{H1} repertoires (XhoI/SpeI)*	0.75 µg
T4 DNA Ligase (20 units/µl)	5.0 µl
Water, nuclease-free	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 x 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.
- Plate out 100 µL from each transformation onto 2YT with 50 µg/ml Carbenicillin (2YT-Carb) plates and incubate overnight at 37°C.

4.2.3 Large-Scale Preparation of *Heavy chain sub-library* DNA

- Scrape cells off all plates into 100 ml 2YT with 2% Glucose and 50 µg/ml Carbenicillin (2YT-Glu-Carb) and incubate a further 60 min at 37°C.
- Increase volume to 500 mL with 2TY–GLU, increase carbenicillin to 50 µg/mL, and incubate overnight at 37°C.
- Recover cells by centrifugation and prepare DNA by your reliable method.

5. Mouse Fab Library construction from either *Light chain sub-library or Heavy chain sub-library*

5.2.1 *Light chain sub-library and Heavy chain sub-library* digestion and purification

Components	*Light chain sub-library*	*Heavy chain sub-library*
DNA	10 µg	10 µg
10X digestion buffer	20 µl	20 µl
XhoI	100 units	/
SpeI	100 units	/
SacI	/	100 units
XbaI	/	100 units
Water, nuclease-free	to 200 µl	to 200 µl

- Restriction digestion at 37°C overnight or 6 h.
- 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: 5400 bp for digested Light chain sub-library or Heavy chain sub-library 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 1-2 µg of Light chain sub-library or Heavy chain sub-library backbone.

5.2.2 *Final mouse Fab library* ligation using T4 DNA ligase

Components	Light chain sub-library	Heavy chain sub-library

T4 DNA Ligase Buffer (10X)*	20 µl	20 µl
Digested *Light chain sub-library (XhoI/SpeI)*	1.9 µg	/
Digested *V_H-C_{H1} repertoires (XhoI/SpeI)*	0.75 µg	/
Digested *Heavy chain sub-library (SacI/XbaI)*	/	1.9 µg
Digested *V_k-C_k* or *V_λ-C_λ repertoires (SacI/XbaI)*	/	0.75 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 x 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.
- Plate out 100 µL from each transformation onto 2YT with 50 µg/ml Carbenicillin (2YT-Carb) plates and incubate overnight at 37°C.
- The expected final mouse Fab library diversity should be at least 10^{10}.

6. Proceed with the phage library preparation and panning.