APD-03 | Mouse Fab Phage Display Library Construction Kit

Fusion BioLabs Short Protocol | pAPD-m-Fab

Product Overview

The Mouse Fab Phage Display Library Construction Kit (APD-03) provides specialized PCR primer mixes and the pAPD-m-Fab phagemid vector for constructing mouse fragment antigen-binding (Fab) antibody libraries. The kit supports κ- and λ-light-chain repertoires and heavy-chain repertoires, with either light-chain-first or heavy-chain-first assembly.

Workflow Summary

Amplify V_H, V_κ, V_λ and corresponding constant-region fragments; assemble heavy- and light-chain repertoires; construct a light-chain or heavy-chain sub-library; then complete the mouse Fab library using the complementary repertoire.

Specifications

Specification	Value
Catalog number	APD-03
Vector	pAPD-m-Fab (6,074 bp)
Antibiotic resistance	Ampicillin (AmpR)
Expression	Inducible for downstream expression
Delivery	Transformation
Product type	Phage display / bacterial expression vector
Cloning method	SacI/XbaI for light-chain repertoires; XhoI/SpeI for heavy-chain repertoires

Kit Contents & Storage

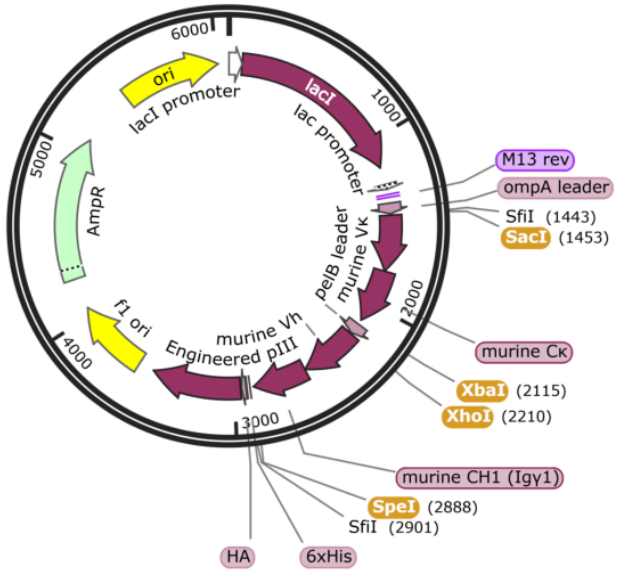
Vial	Quantity	Component
1	100 µL, 10 µM	Primer mix (F+R) for Vκ repertoire amplification
2	100 µL, 10 µM	Primer mix (F+R) for Vλ repertoire amplification
3	100 µL, 10 µM	Primer mix (F+R) for VH repertoire amplification
4	100 µL, 10 µM	Primer mix (F+R) for Cκ fragment amplification
5	100 µL, 10 µM	Primer mix (F+R) for Cλ fragment amplification
6	100 µL, 10 µM	Primer mix (F+R) for CH1 fragment amplification
7	100 µL, 10 µM	Assembly primers (F+R) for Vκ–Cκ repertoire construction
8	100 µL, 10 µM	Assembly primers (F+R) for Vλ–Cλ repertoire construction
9	100 µL, 10 µM	Assembly primers (F+R) for VH–CH1 repertoire construction
10	100 µL, 10 µM	M13 Reverse primer for forward sequencing
11	100 µL, 10 µM	pIII Reverse primer for reverse sequencing
12	10.0 µg in TE buffer	pAPD-m-Fab cloning vector

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

Vector for Library Construction

- 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

pAPD-m-Fab (6,074 bp). SacI/XbaI and XhoI/SpeI are the cloning enzyme pairs described in this protocol.

Short Protocol

1. PCR Amplification of Mouse Variable-Region Repertoires

Repertoire	Primer mix	Total PCR volume
Vκ	Vial 1	1.0 mL (10 × 100 µL)
Vλ	Vial 2	0.8 mL (8 × 100 µL)
VH	Vial 3	0.8 mL (8 × 100 µL)

Variable-Region PCR Reaction (100 µL)

Component	Volume
10× PCR buffer without Mg^{2+}	10 µL
50 mM $MgCl_2$	3 µL
10 mM dNTPs	2 µL
10 µM primer mix (F+R)	4 µL
cDNA template (500–8000 ng/µL)	1 µL
Taq DNA polymerase (10 units/µL)	0.4 µL
Nuclease-free water	79.6 µL

Thermal cycling: 94°C for 2 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 90 s; 72°C for 10 min; hold at 4°C.

Pool each repertoire separately. Resolve on 2% agarose gel, purify the 350–400 bp products, and quantify. Approximately 3–6 µg of each repertoire is anticipated; repeat PCR if necessary.

2. PCR Amplification of Mouse Constant-Region Fragments

Fragment	Primer mix	Total PCR volume
Cκ	Vial 4	0.6 mL (6 × 100 µL)
Cλ	Vial 5	0.6 mL (6 × 100 µL)
CH1	Vial 6	0.6 mL (6 × 100 µL)

Use pAPD-m-Fab (Vial 12) or a validated mouse Fab cDNA template as described in the source protocol. The source document contains an erroneous reference to “human CH1”; APD-03 is a mouse Fab kit.

Component	Volume per 100 µL
10× PCR buffer without Mg^{2+}	10 µL
50 mM $MgCl_2$	3 µL
10 mM dNTPs	2 µL
10 µM primer mix (F+R)	4 µL
Template (250 ng/µL)	0.2 µL
Taq DNA polymerase (10 units/µL)	0.4 µL
Nuclease-free water	To 100 µL

Thermal cycling: 94°C for 2 min; 25 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s; 72°C for 10 min; hold at 4°C. Gel-purify the approximately 350 bp CH1 and approximately 420 bp Cκ/Cλ fragments. Approximately 3–6 µg of each fragment is anticipated.

3. Overlap-Extension PCR Assembly

Assembly	Templates	Primer mix
Vκ–Cκ	Vκ + Cκ	Vial 7
Vλ–Cλ	Vλ + Cλ	Vial 8
VH–CH1	VH + CH1	Vial 9

Prepare ten 100 µL reactions for each assembly (1.0 mL total).

Component	Amount per 100 µL
10× PCR buffer without Mg^{2+}	10 µL
50 mM $MgCl_2$	3 µL
10 mM dNTPs	2 µL
10 µM assembly primers	4 µL
Two DNA templates	100 ng each; variable volume
Taq DNA polymerase (10 units/µL)	0.4 µL
Nuclease-free water	To 100 µL

Thermal cycling: 94°C for 2 min; 20 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 2 min; 72°C for 10 min; hold at 4°C. Gel-purify the approximately 750–800 bp assembled repertoires. Approximately 2–4 µg of each is anticipated; repeat assembly PCR if necessary.

4. Construction of Light-Chain or Heavy-Chain Sub-Libraries

4.1 Light-Chain Sub-Library — SacI/XbaI

Digest the Vκ–Cκ or Vλ–Cλ repertoire and the pAPD-m-Fab vector in separate 200 µL reactions.

Component	Light-chain repertoire	Vector
DNA	10 µg	5 µg
10× digestion buffer	20 µL	20 µL
SacI	100 units	100 units
XbaI	100 units	100 units
Nuclease-free water	To 200 µL	To 200 µL

Incubate at 37°C for 6 h or overnight. Gel-purify the approximately 650–700 bp light-chain fragment and approximately 5,400 bp vector backbone. The source protocol anticipates 1–4 µg purified product.

4.2 Heavy-Chain Sub-Library — XhoI/SpeI

Digest the VH–CH1 repertoire and pAPD-m-Fab vector in separate 200 µL reactions.

Component	Heavy-chain repertoire	Vector
DNA	10 µg	5 µg
10× digestion buffer	20 µL	20 µL

Component	Heavy-chain repertoire	Vector
XhoI	100 units	100 units
SpeI	100 units	100 units
Nuclease-free water	To 200 µL	To 200 µL

Incubate at 37°C for 6 h or overnight. Gel-purify the approximately 650–700 bp heavy-chain fragment and approximately 5,400 bp vector backbone. The source protocol anticipates 1–4 µg purified product.

4.3 Sub-Library Ligation and Transformation

Component	Amount
10× T4 DNA ligase buffer	20 µL
Digested vector backbone	1.9 µg
Matching digested repertoire	0.75 µg
T4 DNA ligase (20 units/µL)	5 µL
Nuclease-free water	To 200 µL

Gently mix, incubate overnight at room temperature, and heat-inactivate at 65°C for 10 min. Purify ligated DNA and dissolve in 50 µL nuclease-free water.

Transform 2 µL purified ligation into 25 µL TG1 electrocompetent cells (recommended efficiency >4 × 10^{10} cfu/µg). Electroporate in a 0.1 cm cuvette at 1.8 kV, 10 µF, and 600 Ω; typical time constant 3.5–4.5 ms. Plate 100 µL on 2YT with 50 µg/mL carbenicillin and incubate overnight at 37°C.

4.4 Large-Scale Sub-Library DNA Preparation

Scrape colonies into 100 mL 2YT containing 2% glucose and 50 µg/mL carbenicillin. Incubate for 60 min at 37°C; increase to 500 mL with 2YT/glucose and carbenicillin at 50 µg/mL, then incubate overnight at 37°C. Recover cells and isolate sub-library DNA.

5. Final Mouse Fab Library Construction

Two equivalent assembly routes are described: (A) add VH–CH1 to a light-chain sub-library using XhoI/SpeI, or (B) add Vκ–Cκ or Vλ–Cλ to a heavy-chain sub-library using SacI/XbaI.

5.1 Sub-Library Backbone Digestion

Component	Light-chain sub-library	Heavy-chain sub-library
DNA	10 µg	10 µg
10× digestion buffer	20 µL	20 µL
XhoI / SpeI	100 units each	—
SacI / XbaI	—	100 units each
Nuclease-free water	To 200 µL	To 200 µL

Incubate at 37°C for 6 h or overnight. Gel-purify the approximately 5,400 bp backbone and quantify. Approximately 1–2 µg purified backbone is anticipated.

5.2 Final Fab Library Ligation

Component	Route A: light-chain backbone	Route B: heavy-chain backbone
10× T4 DNA ligase buffer	20 µL	20 µL
Digested sub-library backbone	1.9 µg	1.9 µg
Complementary digested repertoire	0.75 µg VH–CH1	0.75 µg Vκ–Cκ or Vλ–Cλ
T4 DNA ligase (20 units/µL)	5 µL	5 µL
Nuclease-free water	To 200 µL	To 200 µL

Incubate overnight at room temperature, heat-inactivate at 65°C for 10 min, purify, and transform TG1 electrocompetent cells using the conditions in Section 4.3. The source protocol states an expected final mouse Fab library diversity of at least 10^{10}; actual diversity should be determined experimentally.

6. Phage Library Preparation and Panning

Proceed with the established phage library preparation and panning workflow.

Documentation Notes

This protocol documents SacI/XbaI and XhoI/SpeI cloning for APD-03. SfiI sites appear on the vector map but an SfiI cloning workflow is not specified here.

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