# APD-02 | Human Fab Phage Display Library Construction Kit

Fusion BioLabs Short Protocol | pAPD-h-Fab

## Product Overview

The Human Fab Phage Display Library Construction Kit (APD-02) provides PCR primer mixes and the pAPD-h-Fab phagemid vector for constructing human fragment antigen-binding (Fab) antibody libraries. Human $V_H$, Vκ, and Vλ repertoires are amplified from cDNA; CH1, Cκ, and Cλ fragments are amplified using the supplied vector as template. Overlap-extension PCR generates human κ-Fab and λ-Fab repertoires.

## Workflow Summary

Amplify human variable-region repertoires and vector-derived constant-region fragments; assemble heavy- and light-chain fragments; complete the Fab repertoires; select SfiI (Method A) or SacI/SpeI (Method B) digestion; ligate and transform.

## Specifications

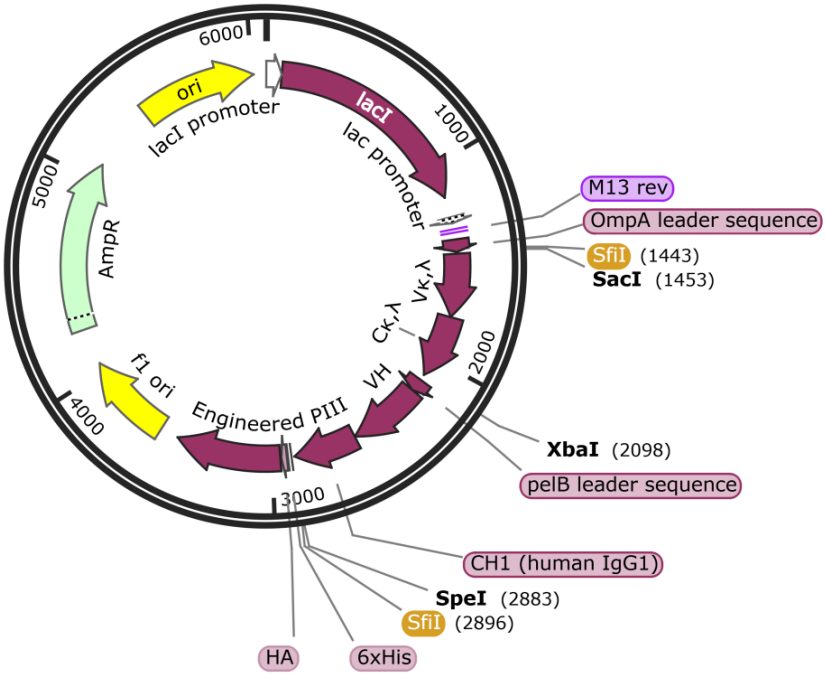
| Specification | Value |
|---|---|
| Catalog number | APD-02 |
| Vector | pAPD-h-Fab (6,069 bp) |
| Antibiotic resistance | Ampicillin (AmpR) |
| Expression | Inducible for downstream expression |
| Delivery | Transformation |
| Product type | Phage display / bacterial expression vector |
| Cloning method | Method A: SfiI; Method B: SacI/SpeI (confirmed alternative) |

## Kit Contents & Storage

| Vial | Quantity | Component |
|---|---|---|
| 1 | 100 µL, 10 µM | $V_H$ repertoire amplification primer mix |
| 2 | 100 µL, 10 µM | Vκ repertoire amplification primer mix |
| 3 | 100 µL, 10 µM | Vλ repertoire amplification primer mix |
| 4 | 100 µL, 10 µM | CH1 fragment amplification primer mix |
| 5 | 100 µL, 10 µM | Cκ fragment amplification primer mix |
| 6 | 100 µL, 10 µM | Cλ fragment amplification primer mix |
| 7 | 100 µL, 10 µM | $V_H$–CH1 assembly primers |
| 8 | 100 µL, 10 µM | Vκ–Cκ assembly primers |
| 9 | 100 µL, 10 µM | Vλ–Cλ assembly primers |
| 10 | 100 µL, 10 µM | Fab repertoire assembly primers |
| 11 | 100 µL, 10 µM | M13 Reverse primer for forward sequencing |
| 12 | 100 µL, 10 µM | pIII Reverse primer for reverse sequencing |
| 13 | 10.0 µg in TE buffer | pAPD-h-Fab cloning vector and constant-region PCR template |

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

## Vector for Library Construction




**Phagemid vector for human Fab library construction**
6069 bp

pAPD-h-Fab (6,069 bp). Method A uses SfiI (1443 and 2896); Method B uses SacI (1453) and SpeI (2883).

## Short Protocol

## 1. PCR Amplification of Human Variable-Region Repertoires

| Repertoire | Primer mix | Total PCR volume |
|---|---|---|
| $V_H$ | Vial 1 | 1.0 mL (10 × 100 µL) |
| Vκ | Vial 2 | 0.8 mL (8 × 100 µL) |
| Vλ | 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. Gel-purify the approximately 350–400 bp products. Anticipated recovery is 3–6 µg per repertoire.

## 2. PCR Amplification of Human Constant-Region Fragments

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

Use pAPD-h-Fab vector (Vial 13) as the template. Prepare a 100 µL reaction with 10 µL 10× PCR buffer, 3 µL 50 mM $MgCl_2$, 2 µL 10 mM dNTPs, 4 µL primer mix, 0.2 µL vector template (250 ng/µL), 0.4 µL Taq polymerase (10 units/µL), and 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. Gel-purify approximately 350 bp CH1 and approximately 420 bp Cκ/Cλ fragments.

## 3. Overlap-Extension PCR Assembly

| Assembly | Templates | Primer mix |
|---|---|---|
| Linker–$V_H$–CH1 | $V_H$ + CH1 | Vial 7 |
| Vκ–Cκ–linker | Vκ + Cκ | Vial 8 |
| Vλ–Cλ–linker | Vλ + Cλ | 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. Gel-purify approximately 750–800 bp assembled repertoires; anticipated yield is 2–4 µg.

## 4. Final Human Fab Repertoire Assembly

Combine Vκ–Cκ–linker or Vλ–Cλ–linker with linker–$V_H$–CH1 using Vial 10 assembly primers. Use the 100 µL overlap-PCR formulation in Section 3 with 25 cycles and a 3-minute extension at 72°C. Purify approximately 1,500 bp Fab products; anticipated yield is 10–20 µg per repertoire.

## 5. Restriction Digestion: Select One Cloning Method

Digest the assembled Fab repertoire and pAPD-h-Fab vector in separate reactions. Select one cloning method and use compatible insert ends.

| Component (per reaction) | Method A: SfiI | Method B: SacI/SpeI |
|---|---|---|
| Insert DNA OR vector DNA | 10 µg | 10 µg |
| 10× universal digestion buffer | 20 µL | 20 µL |
| SfiI (20 units/µL) | 10 µL | — |
| SacI (20 units/µL) | — | 10 µL |
| SpeI (20 units/µL) | — | 10 µL |
| Nuclease-free water | To 200 µL | To 200 µL |
| Incubation temperature | 50°C | 37°C |
| Incubation time | 6 h or overnight | 6 h or overnight |

After digestion, resolve the products by 1% agarose gel electrophoresis. Gel-purify the Fab insert and vector backbone, and quantify the purified DNA. Proceed to the shared ligation and transformation workflow in Section 6.

Method B: SacI/SpeI is a Fusion BioLabs-confirmed alternative. Use the appropriate SacI/SpeI-compatible insert-end design and verified enzyme/buffer compatibility.

## 6. Fab Library Ligation and Transformation

| Component | κ-Fab library | λ-Fab library |
|---|---|---|
| 10× T4 DNA ligase buffer | 20 µL | 20 µL |
| Digested pAPD-h-Fab vector | 1.9 µg | 1.9 µg |
| Digested Fab repertoire | 1.5 µg | 1.5 µg |
| T4 DNA ligase (20 units/µL) | 5 µL | 5 µL |
| Nuclease-free water | To 200 µL | To 200 µL |

## 6. Fab Library Ligation and Transformation (continued)

Gently mix and incubate the ligation overnight at room temperature. Heat-inactivate at 65°C for 10 min, then purify ligated DNA. Transform TG1 electrocompetent cells using the established workflow.

Electroporation settings documented for APD-02: 0.1 cm cuvette, 1.8 kV, 10 µF, 600 Ω; typical time constant 3.5–4.5 ms. Confirm instrument and competent-cell compatibility before use.

## 7. Phage Library Preparation and Panning

Proceed with the established phage library preparation, rescue, and panning workflow.

## Documentation Notes

Both cloning strategies have been confirmed by Fusion BioLabs. Method A uses SfiI digestion; Method B uses SacI/SpeI digestion. The amplification, Fab assembly, ligation, and transformation sections are shared.

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