APD-06 | Rabbit scFv Phage Display Library Construction Kit

Fusion BioLabs Short Protocol | pAPD-rbt-scFv

Product Overview

pAPD-rbt-scFv is a phagemid vector for constructing rabbit single-chain variable fragment (scFv) phage display libraries. Rabbit VH, Vκ, and Vλ variable-region repertoires are amplified from cDNA derived from peripheral blood lymphocytes or lymphoid tissues of immunized or non-immunized donors. VH and VL repertoires are assembled by overlap-extension PCR, digested using either validated cloning method, and ligated into the correspondingly digested pAPD-rbt-scFv vector.

Key Features

Efficient rabbit scFv display; validated alternative SacI/SpeI and SfiI cloning strategies; compatible downstream expression.

Specifications

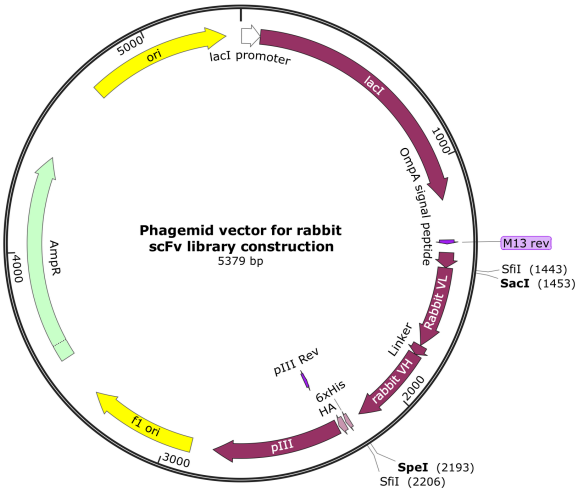
Specification	Description
Catalog number	APD-06
Antibiotic resistance	Ampicillin (AmpR)
Expression system	Inducible for downstream expression
Delivery type	Transformation
Product type	Bacterial expression / phage display vector
Cloning method	Restriction enzyme cloning: Method A (SacI/SpeI) or Method B (SfiI)

Contents & Storage

Vial	Quantity	Description
1	100 µL, 10 µM	Primer mix (F+R) for rabbit Vκ repertoire amplification
2	100 µL, 10 µM	Primer mix (F+R) for rabbit Vλ repertoire amplification
3	100 µL, 10 µM	Primer mix (F+R) for rabbit VH repertoire amplification
4	100 µL, 10 µM	Assembly primers (F+R) for scFv repertoire construction
5	10.0 µg	pAPD-rbt-scFv plasmid in Tris-EDTA buffer
6	100 µL, 10 µM	Forward sequencing primer for rabbit scFv insert (M13 Reverse)
7	100 µL, 10 µM	Reverse sequencing primer for rabbit scFv insert (pIII Reverse)

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

Vector for Library Construction



Short Protocol

1. PCR Amplification of Rabbit VH, Vκ, and Vλ Repertoires

Perform 10 reactions of 100 µL for each repertoire (1.0 mL total): Vκ uses Vial 1, Vλ uses Vial 2, and VH uses Vial 3.

Component	Amount per 100 µL reaction
10× PCR buffer, without Mg2+	10 µL
50 mM MgCl2	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

PCR 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. Run on 2% agarose gel; expected VH/Vκ/Vλ fragments are 380–420 bp. Excise and gel-purify the correct bands. Quantify DNA at 260 nm. Expected recovery: approximately 3–5 µg per repertoire; repeat and pool PCRs if needed.

2. Overlap-Extension PCR (OE-PCR) for Rabbit scFv Assembly

Assemble rabbit κ-scFv using VH + Vκ and rabbit λ-scFv using VH + Vλ. Use assembly primer mix (Vial 4). Prepare 10 reactions of 100 µL for each assembly (1.0 mL total).

Component	Amount per 100 µL reaction
10× PCR buffer, without Mg2+	10 µL
50 mM MgCl2	3 µL
10 mM dNTPs	2 µL
10 µM assembly primers (Vial 4)	4 µL
Template combination	100 ng of each VH and VL repertoire; volume varies
Taq DNA polymerase (10 units/µL)	0.4 µL
Nuclease-free water	To 100 µL

OE-PCR 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.

Pool reactions for each scFv repertoire separately. Run on 2% agarose gel; expected products are approximately 750–800 bp. Gel-purify and quantify. Target at least 10–15 µg scFv repertoire DNA; repeat and pool OE-PCRs if needed.

3. Restriction Digestion: Choose One Validated Method

Prepare **separate** reactions for 10 µg purified rabbit κ-scFv or λ-scFv insert DNA and 10 µg pAPD-rbt-scFv vector DNA. Both methods use the same validated universal 10× digestion buffer and 200 µL total reaction volume. Do not mix the two cloning methods.

Component (per reaction)	Method A: SacI/SpeI	Method B: SfiI
Insert DNA OR vector DNA	10 µg	10 µg
Universal digestion buffer (10×)	20 µL	20 µL
SacI (20 units/µL)	10 µL	—
SpeI (20 units/µL)	10 µL	—
SfiI (20 units/µL)	—	10 µL

Component (per reaction)	Method A: SacI/SpeI	Method B: SfiI
Nuclease-free water	To 200 µL	To 200 µL
Incubation temperature	37°C* 37°C	50°C
Incubation time	6 h or overnight* 6 h or overnight	6 h or overnight

After digestion, add loading buffer, separate products on a 1% agarose gel, excise the appropriate insert and vector backbone bands, and purify using a commercial gel extraction kit. The original protocol reports approximately 780 bp insert and 4,600 bp vector backbone; confirm fragment sizes for the selected method using the plasmid sequence/map. Quantify recovered DNA; the original target yield is 1–4 µg of each purified product.

4. Library Ligation Using T4 DNA Ligase

Prepare a separate ligation for the κ-scFv and λ-scFv libraries using the corresponding purified digested insert and vector.

Component	κ-scFv library	λ-scFv library
10× T4 DNA ligase buffer	20 µL	20 µL
Digested pAPD-rbt-scFv vector	1.9 µg	1.9 µg
Digested scFv 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

Thaw and resuspend ligase buffer at room temperature. Mix gently and briefly centrifuge. Incubate overnight at room temperature. Heat-inactivate at 65°C for 10 min. Purify the ligated DNA and dissolve in 50 µL nuclease-free water.

5. Transformation and Phage Library Preparation

As specified in the original manual, combine 2 µL purified ligation with 25 µL TG1 electrocompetent cells (specified efficiency >4 × 10^{10} cfu/µg plasmid DNA); transfer to a 0.1 cm electroporation cuvette and electroporate at 1.8 kV, 10 µF, and 600 ohms. The original manual reports typical time constants of 3.5–4.5 ms and approximately 25 electroporations in total. Follow validated competent-cell handling and recovery procedures.

Proceed with the established phage library preparation and panning workflow.

Documentation Notes

Method A uses SacI/SpeI digestion; Method B uses SfiI digestion. Both methods use the same DNA inputs, universal digestion buffer, and total reaction volume. Subsequent purification, ligation, transformation, and phage library preparation steps are shared.

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