APD-07 | Chicken scFv Phage Display Library Construction Kit

Fusion BioLabs Short Protocol | pAPD-ck-scFv

Version 2.0 | Modified November 8, 2026

Product Overview

The APD-07 Chicken scFv Phage Display Library Construction Kit provides amplification and assembly primers and the pAPD-ck-scFv phagemid for construction of chicken IgY-derived single-chain variable fragment (scFv) libraries. Chicken VH and VL repertoires are amplified from donor-derived cDNA, assembled in VH-(G4S)3 linker-VL format, and cloned by either validated restriction-enzyme strategy.

Key Features

Chicken scFv phage display library construction with alternative Method A (SacI/SpeI) and Method B (SfiI); common downstream purification, ligation, and transformation.

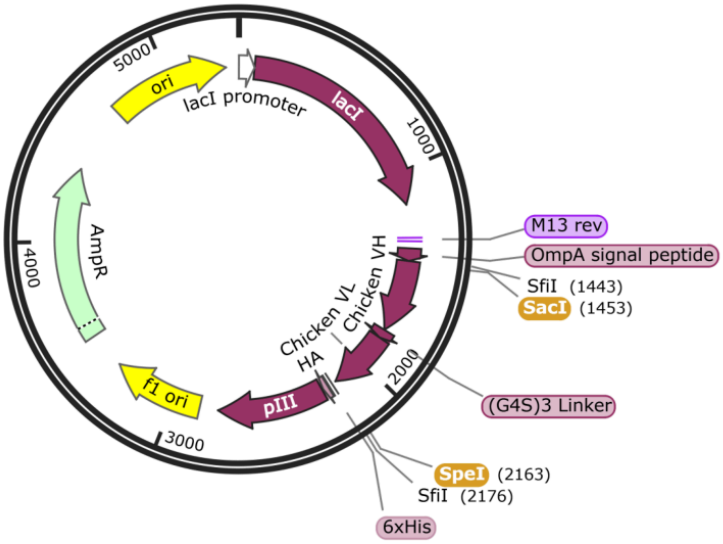
Specifications

Specification	Description
Catalog number	APD-07
Vector	pAPD-ck-scFv (5,349 bp)
Antibiotic resistance	Ampicillin (AmpR)
Expression	Inducible for downstream expression
Delivery	Transformation
Product type	Bacterial expression / phage display vector
Cloning method	Method A: SacI/SpeI; Method B: SfiI

Contents & Storage

Vial	Quantity	Description
1	100 µL, 10 µM	Chicken VH repertoire amplification primer mix (F+R)
2	100 µL, 10 µM	Chicken VL repertoire amplification primer mix (F+R)
3	100 µL, 10 µM	Chicken scFv assembly primer mix (F+R)
4	100 µL, 10 µM	M13 Reverse primer for scFv forward sequencing
5	100 µL, 10 µM	pIII Reverse primer for scFv reverse sequencing
6	10.0 µg	pAPD-ck-scFv plasmid in Tris-EDTA buffer

Store at -20°C; guaranteed stable for 12 months when properly stored.



Phagemid vector for chicken scFv library construction

5349 bp

Short Protocol

1. PCR Amplification of Chicken VH and VL Repertoires

Prepare ten 100 µL PCR reactions for each repertoire (1.0 mL total). Use Vial 1 for VH and Vial 2 for VL.

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 VH and VL separately; resolve on 2% agarose gel. Expected fragments: ~400 bp (VH), ~350 bp (VL). Excise, gel-purify and quantify. Expected yield ~3–5 µg per repertoire; repeat PCR if needed.

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

Combine chicken VH and VL repertoires using assembly primer mix (Vial 3) to generate VH-(G4S)3 linker-VL scFv. Prepare ten 100 µL reactions (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 3)	4 µL
Template combination	100 ng each VH and VL; 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 products; resolve on 2% agarose gel. Expected chicken scFv product ~760–800 bp. Gel-purify and quantify. Target ≥10–15 µg scFv repertoire DNA.

3. Restriction Digestion: Choose One Method

Prepare separate digestion reactions for 10 µg purified chicken scFv insert DNA and 10 µg pAPD-ck-scFv vector DNA. Each reaction uses the same 10× digestion buffer and a total volume of 200 µL.

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

3. Restriction Digestion (continued)

After digestion, add loading buffer and separate products on a 1% agarose gel. Excise the appropriate insert and vector backbone bands and purify using a commercial gel extraction kit. Original manual reports ~750–780 bp insert and ~4,600 bp vector backbone; verify against the selected restriction sites. Quantify recovered DNA; target ~1–4 µg purified DNA.

4. Library Ligation Using T4 DNA Ligase

Use the same downstream ligation for chicken scFv inserts generated by either digestion method.

Component	Chicken scFv library (200 µL reaction)
10× T4 DNA ligase buffer	20 µL
Digested pAPD-ck-scFv vector	1.9 µg
Digested chicken scFv repertoire	1.5 µg
T4 DNA ligase (20 units/µL)	5 µL
Nuclease-free water	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 ligation DNA and dissolve in 50 µL nuclease-free water.

5. Transformation and Phage Library Preparation

Combine 2 µL purified ligation with 25 µL TG1 electrocompetent cells (original 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. Original manual reports typical time constants of 3.5–4.5 ms and approximately 25 electroporations in total. Follow established competent-cell handling and recovery procedures.

Proceed with phage library preparation and panning.

Documentation Notes

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

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