APD-04 | Human scFv Phage Display Library Construction Kit

Fusion BioLabs Short Protocol | pAPD-h-scFv

Product Overview

The Human scFv Phage Display Library Construction Kit (APD-04) provides primer sets and the pAPD-h-scFv phagemid vector for constructing human single-chain variable fragment (scFv) antibody libraries. Variable-region genes are amplified from cDNA derived from peripheral blood lymphocytes or lymphoid tissues of immunized or non-immunized donors.

Workflow

Amplify VH, Vκ and Vλ repertoires; assemble κ-scFv and λ-scFv repertoires; digest inserts and pAPD-h-scFv with SfiI; ligate and transform to establish human scFv libraries.

Specifications

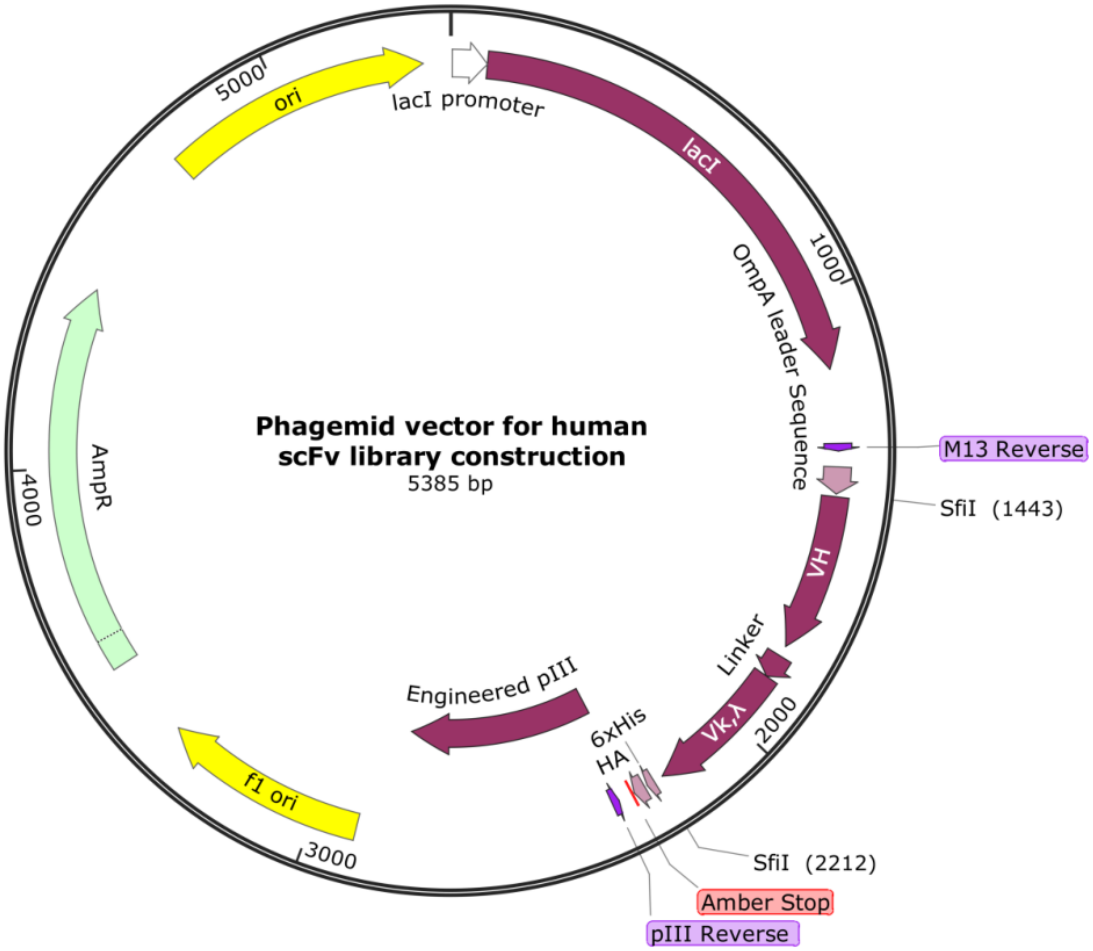
Specification	Value
Catalog number	APD-04
Vector	pAPD-h-scFv (5,385 bp)
Antibiotic resistance	Ampicillin (AmpR)
Expression	Inducible for downstream expression
Delivery	Transformation
Product type	Phage display / bacterial expression vector
Cloning method	SfiI (5′ and 3′ sites)

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	Assembly primers (F+R) for scFv repertoire construction
5	10.0 µg (20 µL at 500 ng/µL)	pAPD-h-scFv vector in Tris-EDTA buffer
6	100 µL, 10 µM	M13 Reverse primer for forward sequencing
7	100 µL, 10 µM	pIII Reverse primer for reverse sequencing

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 scFv library construction
5385 bp

Short Protocol

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

Prepare ten 100 µL reactions for each repertoire (1.0 mL total). Use Vial 1 for Vκ, Vial 2 for Vλ and Vial 3 for VH.

1. PCR Amplification of Human VH, Vκ and Vλ Repertoires (continued)

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

PCR Reaction Setup (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

Stage	Temperature and time
Initial denaturation	94°C, 2 min
30 cycles	94°C, 30 s; 56°C, 30 s; 72°C, 90 s
Final extension	72°C, 10 min
Hold	4°C

Pool each repertoire separately. Resolve products on a 2% agarose gel, excise the 350–400 bp bands and purify. Approximately 3–5 µg of each repertoire is anticipated; repeat PCR if necessary.

2. Overlap-Extension PCR for Human scFv Repertoires

Library	Templates	Primer mix	Total volume
κ-scFv	VH + Vκ	Vial 4	1.0 mL
λ-scFv	VH + Vλ	Vial 4	1.0 mL

Prepare ten 100 µL overlap-extension reactions for each library.

2. Overlap-Extension PCR (continued)

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 (Vial 4)	4 µL
VH and Vκ or Vλ 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; final extension at 72°C for 10 min; hold at 4°C.

Pool each scFv repertoire separately. Purify the approximately 750–800 bp bands from a 2% agarose gel. At least 10–15 µg of scFv repertoire DNA is anticipated; repeat overlap-extension PCR if necessary.

3. SfiI Restriction Digestion

Digest the scFv repertoire and the pAPD-h-scFv vector in separate reactions at 50°C for 6 hours or overnight.

Component	scFv repertoire	pAPD-h-scFv vector
DNA	10 µg	10 µg
10× digestion buffer	20 µL	20 µL
SfiI (20 units/µL)	10 µL	10 µL
Nuclease-free water	To 200 µL	To 200 µL

Separate products on a 1% agarose gel. Excise the approximately 750 bp scFv insert and approximately 4600 bp vector backbone. Purify and quantify each product. At least 1–4 µg of each purified product is anticipated.

4. Library Ligation and Transformation

Component	κ-scFv library	λ-scFv library
10× T4 DNA ligase buffer	20 µL	20 µL
Digested pAPD-h-scFv vector	1.9 µg	1.9 µg
Digested scFv repertoire	1.5 µg	1.5 µg
T4 DNA ligase (20 units/µL)	5.0 µL	5.0 µL
Nuclease-free water	To 200 µL	To 200 µL

Thaw and resuspend T4 DNA ligase buffer at room temperature. Mix the ligation reactions gently and briefly centrifuge. Incubate overnight at room temperature, then heat-inactivate at 65°C for 10 min. Purify the ligated samples and dissolve each in 50 µL nuclease-free water.

Transform 2 µL of purified ligation product into 25 µL TG1 electrocompetent cells (transformation efficiency greater than 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 Ω. A typical successful time constant is 3.5–4.5 ms.

5. Phage Library Preparation and Panning

Proceed with the established phage library preparation and panning workflow.

Documentation Notes

APD-04 uses SfiI restriction-enzyme cloning for human κ-scFv and λ-scFv library construction. The PCR, digestion, ligation and transformation workflows are described above.

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