APD-08 | Camelid VHH Phage Display Library Construction Kit

pAPD-VHH | Catalog No. APD-08 | Version 2.0 | Modified November 8, 2026

Product Overview

The pAPD-VHH phagemid and supplied primer set support construction of camelid VHH antibody phage display libraries. VHH repertoires are amplified from cDNA prepared from peripheral blood lymphocytes or lymphoid tissue of immunized or non-immunized donors, digested using NcoI/NotI, and ligated into the pAPD-VHH vector.

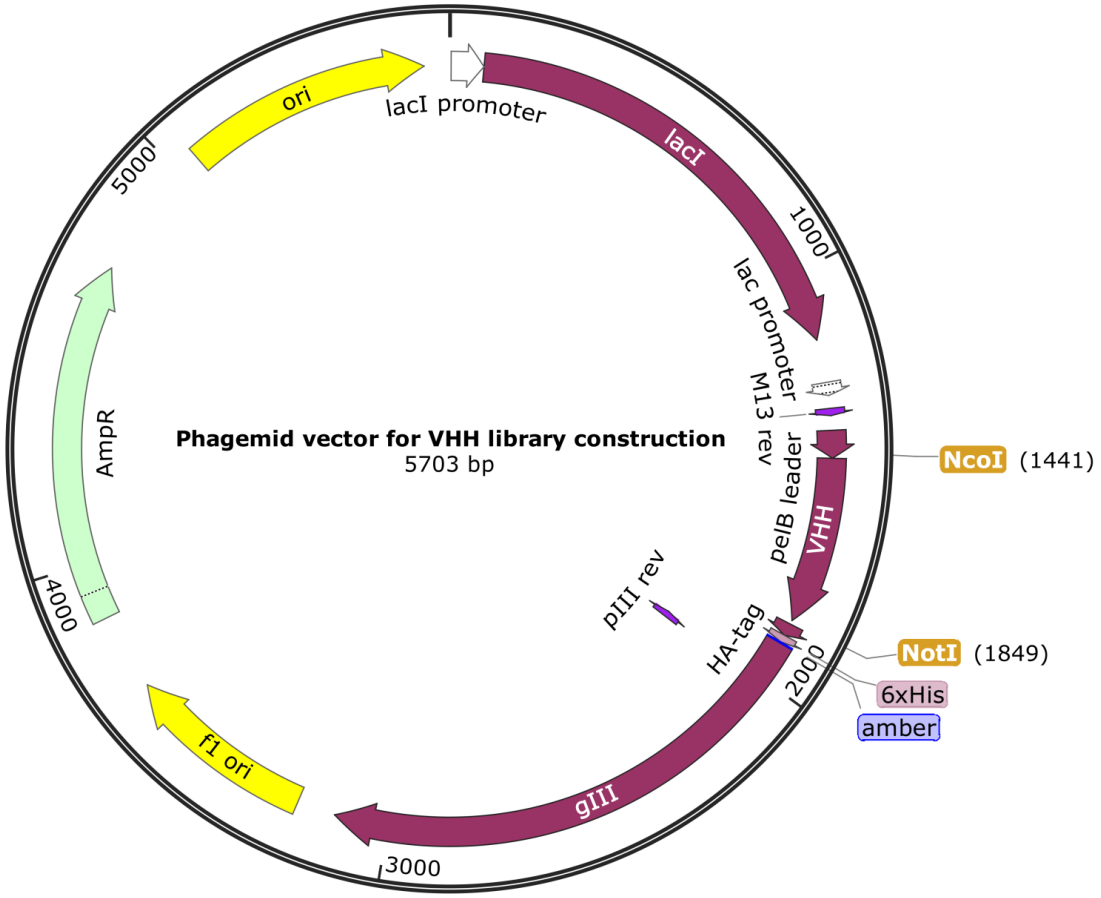
Kit Contents

Vial	Quantity	Component
1	100 µL, 10 µM	VHH repertoire amplification primer mix: 4 forward and 2 reverse oligos (normalized concentration)
2	100 µL, 10 µM	M13 Reverse primer for VHH forward sequencing
3	100 µL, 10 µM	pIII Reverse primer for VHH reverse sequencing
4	10.0 µg	pAPD-VHH phagemid vector in Tris-EDTA buffer

Cloning Method

This protocol documents one cloning method only: NcoI/NotI. No SfiI method is included.

pAPD-VHH Vector Map



Phagemid vector for VHH library construction

5703 bp

Short Protocol

1. PCR Amplification of Camelid VHH Repertoires

Prepare ten 100 µL PCR reactions using Vial 1 (1.0 mL total).

1. PCR Amplification of Camelid VHH Repertoires (continued)

Component	Per 100 µL PCR
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), Vial 1	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 stage	Temperature / duration
Initial denaturation	94°C, 2 min
30 cycles: denaturation	94°C, 30 sec
30 cycles: annealing	55°C, 45 sec
30 cycles: extension	72°C, 90 sec
Final extension	72°C, 10 min
Hold	4°C

Combine reactions in a 2.0 mL nuclease-free tube. Add loading buffer and separate products on a 1% agarose gel. Expect VHH repertoire fragments of approximately 400–450 bp. Excise the target bands, purify and quantify. It anticipates approximately 10–15 µg of VHH repertoire DNA; repeat PCR if needed.

2. NcoI/NotI Restriction Digestion

Digest purified VHH repertoires and pAPD-VHH vector in separate reactions at 37°C for 6 hours or overnight.

Component	VHH repertoire	pAPD-VHH vector
DNA	10 µg	10 µg
10× digestion buffer	20 µL	20 µL
NcoI (20 units/µL)	10 µL	10 µL
NotI (20 units/µL)	10 µL	10 µL
Nuclease-free water	To 200 µL	To 200 µL

Run digestion products on a 1% agarose gel. Expected sizes: VHH inserts approximately 400–430 bp and digested vector backbone approximately 5,300 bp. Excise and purify the appropriate bands. It anticipates at least 1–4 µg of each purified product.

3. Library Ligation Using T4 DNA Ligase

Component	200 µL reaction
10× T4 DNA ligase buffer	20 µL
Digested pAPD-VHH vector	2.5 µg
Digested VHH repertoires	1.5 µg
T4 DNA ligase (20 units/µL)	5.0 µL
Nuclease-free water	To 200 µL

Thaw and resuspend the 10× ligase buffer at room temperature. Mix gently, briefly centrifuge, and incubate overnight at room temperature. Heat-inactivate at 65°C for 10 minutes. Purify the ligation products and dissolve in 50 µL nuclease-free water.

4. Transformation and Library Construction

Transform 2 µL purified ligation product into 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 Ω. It specifies a typical time constant of 3.5–4.5 ms and approximately 25 individual electroporations.

5. Phage Library Preparation and Panning

Proceed with the established phage library preparation and panning workflow.

Documentation Notes

APD-08 uses NcoI/NotI cloning only. The Data Sheet and Short Protocol use consistent vector identification, four-vial kit contents and restriction sites.

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