

1 Electronic Supplementary Material (ESI) for Analytical Methods.

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Supplementary Information

5 **Phage-mediated double-nanobody sandwich immunoassay for detecting alpha**

6 **fetal protein in human serum**

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27 **Table S1** The screening of paired antibodies

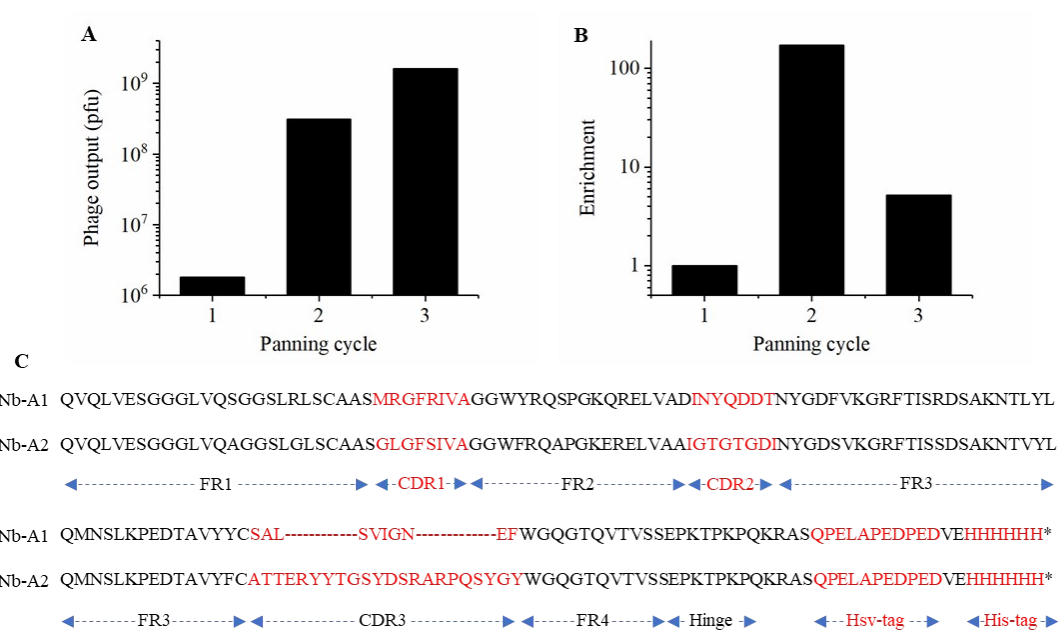
Detection antibody Caputre antibody	AFP	Phage-A1	Phage-A2
	concentration		
Nb-A1	50 ng/mL	0.079	2.858
	0 ng/mL	0.09	0.194
Nb-A2	50 ng/mL	0.15	0.187
	0 ng/mL	0.07	0.196

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29 **Table S2** Optimization of the concentrations of capture antibody and detection
30 antibody by checkerboard titration

Phage-A2 (pfu/mL)	Nb-A1 (μg/mL)					
	62.72	31.36	15.68	7.84	3.92	1.96
2×10^{13}	0.562	0.347	0.228	0.221	0.182	0.180
1×10^{13}	0.556	0.295	0.226	0.194	0.168	0.168
5×10^{12}	0.505	0.299	0.211	0.201	0.167	0.162
2.5×10^{12}	0.491	0.294	0.208	0.182	0.156	0.145
1.25×10^{12}	0.448	0.295	0.201	0.182	0.152	0.143
6.25×10^{11}	0.432	0.268	0.179	0.180	0.143	0.140
3.125×10^{11}	0.462	0.244	0.175	0.168	0.137	0.132
1.5625×10^{11}	0.422	0.263	0.158	0.163	0.127	0.110

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34 **Figure S1** Selection of anti-AFP nanobodies phages and amino acid sequences

35 analysis of positive phage clones. (A) The enrichment of phage in three rounds

36 biopanning; (B) The output phage particles in three rounds biopanning; (C) Amino

37 acid sequences of selected anti-AFP nanobodies containing Hsv-tag and His-tag.

38 **Table S3** Comparison of the proposed method with other reported AFP detection
 39 methods

No.	Methods	LOD	References
1	Phage-mediated ELISA	0.237 ng/mL	This work
2	Metal-linked immunoassay	0.1 ng/mL	1
3	Plasmonic ELISA	0.23 ng/mL	2
4	ELISA	0.48 ng/mL	3
5	AuNP-based ELISA	2 ng/mL	4

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