

Electronic Supplementary Material (ESI) for RSC Advances.

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Supporting Information

Development of a horseradish peroxidase-nanobody fusion protein for visual detection of ochratoxin A by dot immunoassay

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Table S1 The primers for constructing the recombinant plasmids pET25b-Nb28-HRP and pET25b-HRP-Nb28

Name	The sequence of primers	Protein	Recombinant plasmid
HN-HF	GGAATTCC <u>CATATG</u> CAGTTAACGCCGACTTTCTA CGATAAC	HRP	PET25b-HRP-Nb28
HN-HR	CCGCCAGAGCCACCTCCGCCTGAACCGCCTCC TCCTGAGTTCGAGTTTACGACTCGGC		
HN-NF	G TTCAGGCGGAGGTGGCTCTGGCGGTGGCGG ATCCATGGCCATGGCCCAGTTGC	Nb28	
HN-NR	ATAAGAAT <u>GCGGCCG</u> CTTGTGGTTTTGGTGTC TTGGGTTC		
NH-NF	GGAATTCC <u>CATATG</u> ATGGCCATGGCCCAGTTGC	Nb28	PET25b-Nb28-HRP
NH-NR	CCGCCAGAGCCACCTCCGCCTGAACCGCCTCC TCCTTGTGGTTTTGGTGTCTTGGGTTC		
NH-HF	G TTCAGGCGGAGGTGGCTCTGGCGGTGGCGG ATCCCAGTTAACGCCGACTTTCTACGAT	HRP	
NH-HR	ATAAGAAT <u>GCGGCCG</u> CTGAGTTCGAGTTTACG ACTCG		

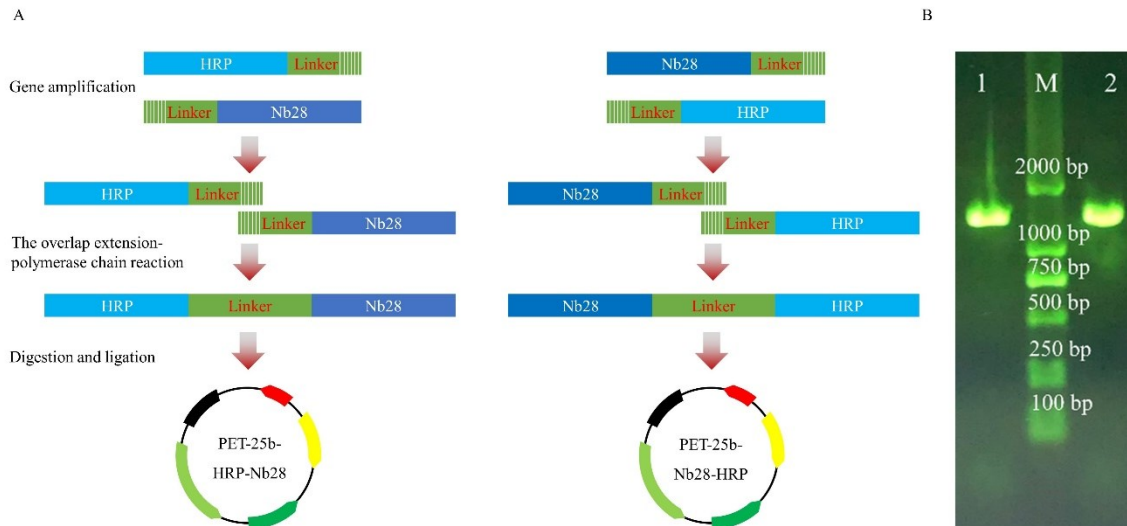


Fig.S1 Construction of the recombinant plasmids pET25b-HRP-Nb28 and pET25b-Nb28-HRP (A) and DNA agarose analysis of the gene fragments of HRP-Nb28 and Nb28-HRP (B). Lane 1: HRP-Nb28 fragment; Lane 2: Nb28-HRP fragment; M: D2000 DNA Ladder.

Table S2 Amino acid sequences of the fragments of HRP-Nb28 and Nb28-HRP

DNA fragment	Amino acid sequence (5'-3')
Nb28-HRP	MAMAQLQLVESGGGLVQAGGSLRLSCAASGSTVGVNAMDMGWYRQ APGKQRELVAAIINGGGSTNLADSVKGRFTISRDKAKRTLYLQMNSLK PEDTAVYYCYVRSGLVYWGQGTQVTVSSEPKTPKQGGGGGGGG GSGGGGSQLTPTFYDNPCPNVSNIVRDTIVNELRSDPRIAASILRLHFHD CFVNGCDASILLDNTTSFRTEKDAFGNANSARGFPVIDRMKAAVESAC PRTVSCADLLTIAAQSVTLAGGPSWRVPLGRRDSLQAFDLANANLP APFFTLPLKDSFRNVGLNRSSDLVALSGGHTFGKNQCRFIMDRLYNFS NTGLPDPTLNTTYLQTLRGLCPLNGNLSALVDFDLRTPTIFDNKYVNL EEQKGLIQSDQELFSSPNATDTIPLVRSFANSTQTFNFAFVEAMDRMGN ITPLTGTGGQIRLNCRVVNSNS
HRP-Nb28	QLTPTFYDNPCPNVSNIVRDTIVNELRSDPRIAASILRLHFHDCFVNGCD ASILLDNTTSFRTEKDAFGNANSARGFPVIDRMKAAVESACPRTVSCA DLLTIAAQSVTLAGGPSWRVPLGRRDSLQAFDLANANLPAPFFTLPL QLKDSFRNVGLNRSSDLVALSGGHTFGKNQCRFIMDRLYNFSNTGLPD PTLNTTYLQTLRGLCPLNGNLSALVDFDLRTPTIFDNKYVNL EEQKGL IQSDQELFSSPNATDTIPLVRSFANSTQTFNFAFVEAMDRMGNITPLTGT QGQIRLNCRVVNSNSGGGGGGGGGGGGSMAMAQLQLVESGGGLV QAGGSLRLSCAASGSTVGVNAMDMGWYRQAPGKQRELVAAIINGGG STNLADSVKGRFTISRDKAKRTLYLQMNSLKPEDTAVYYCYVRSGLV LVYWGQGTQVTVSSEPKTPKQ

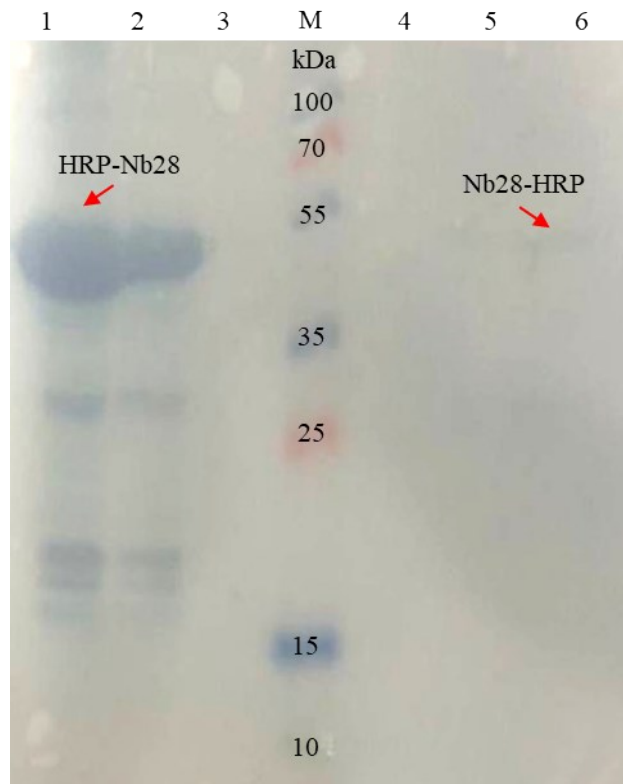


Fig.S2 Western blot analysis of the auto-induction expression of fusion proteins HRP-Nb28 (left) and Nb28-HRP (right). Lane 1 and 6: The precipitated protein of the induced *E.coli* cell after sonication; Lane 2 and 5: Total protein of the induced *E.coli* cell; lane 3 and 4: The supernatant protein of the induced *E.coli* cell after sonication; lane M: Prestained protein ladder. The red arrows point to the target proteins.

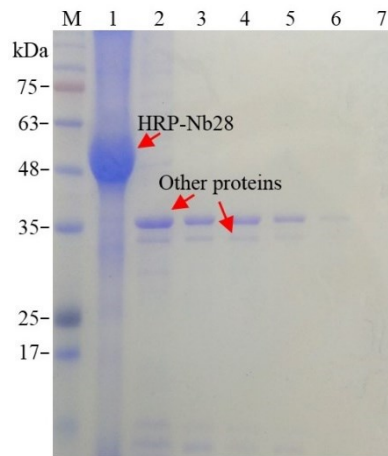


Fig. S3 SDS-PAGE analysis of the purification of HRP-Nb28 inclusion body. Lane M: Prestained protein ladder; Lane 1: The purified inclusion body; Lane 2-4: Supernatants collected after washing the inclusion body with wash buffer I; Lane 5-7: Supernatants collected after washing the inclusion body with wash buffer II.

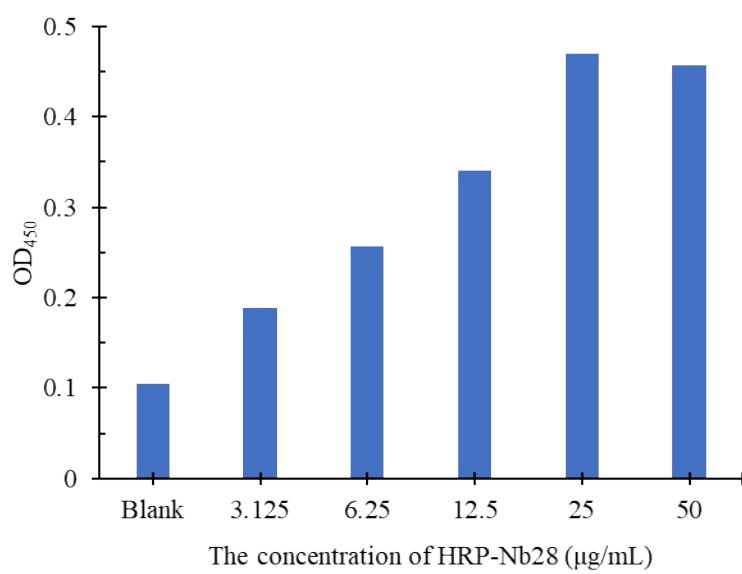


Fig. S4 The performance analysis of HRP-Nb28 fusion protein

Table S3 Comparison of the proposed method with other reported OTA detection methods

No.	Methods	Cut-off value	Detection time	References
1	Dot ELISA	10 ng/mL	10 min	This work
2	Test strip	16 ng/mL	10 min	1
3	Test strip	5 ng/mL	5-10 min	2
4	Dot ELISA	0.625 ng/mL	21 min	3
5	Dot ELISA	0.3125 ng/mL	< 6 min	4

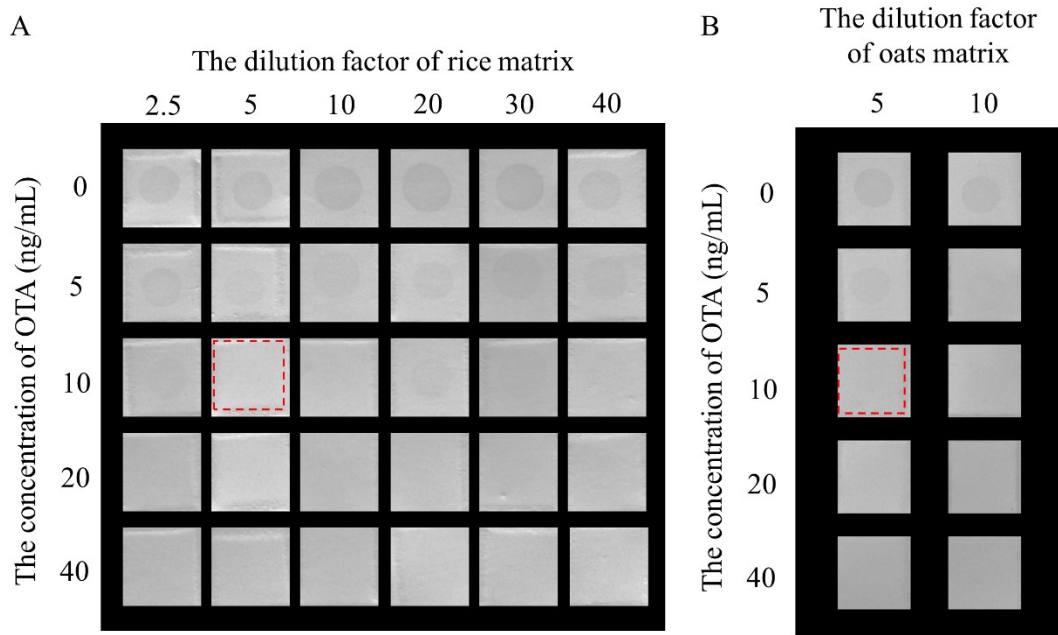


Fig.S5 Evaluation of the matrix effect of rice (A) and oats (B) samples at different dilutions on the performance of HN-DIA.

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