Supplementary Information

Time-resolved fluorescence immunochromatographic assay developed using two idiotypic nanobodies for rapid, quantitative, and simultaneous detection of aflatoxin and zearalenone in maize and its products

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25 **Contents:**

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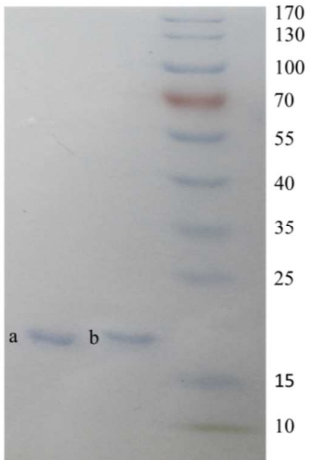
33 **Purification of the anti-idiotypic nanobodies for AFB_1 and ZEN**



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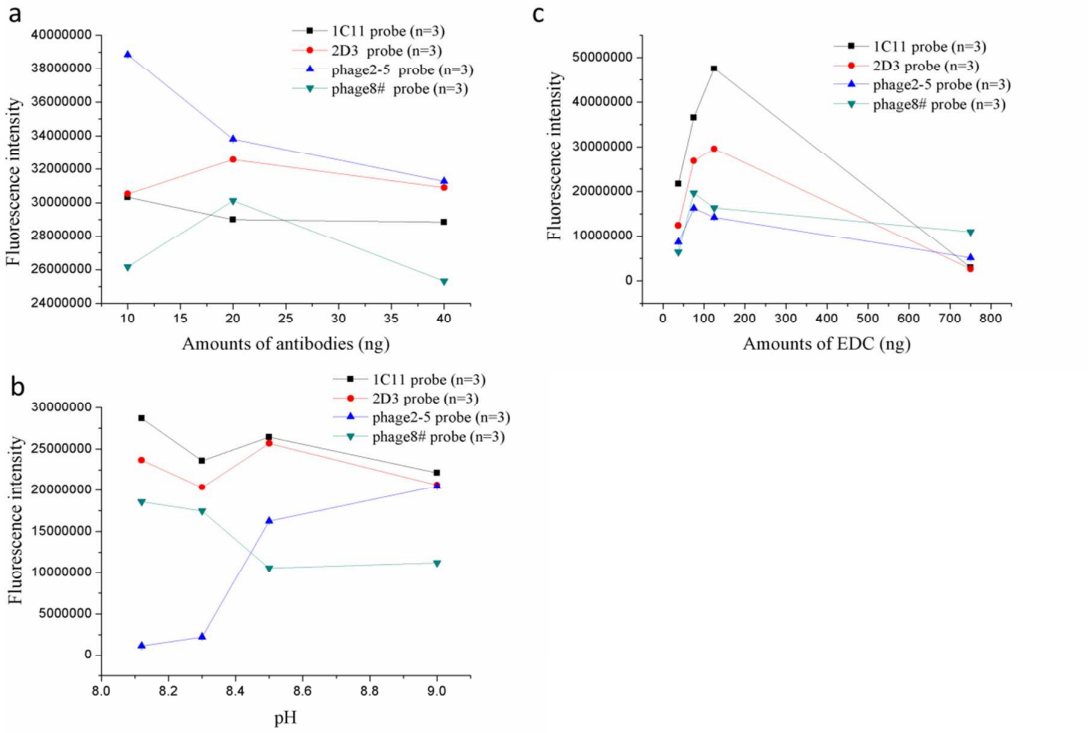
35
36 **Figure S1. Extraction and purification of nanobody**
37 **(a, phage 8#; b, phage 2-5).**
38
39 **Preparation of Eu/Tb(III)-nanosphere fluorescence probe**



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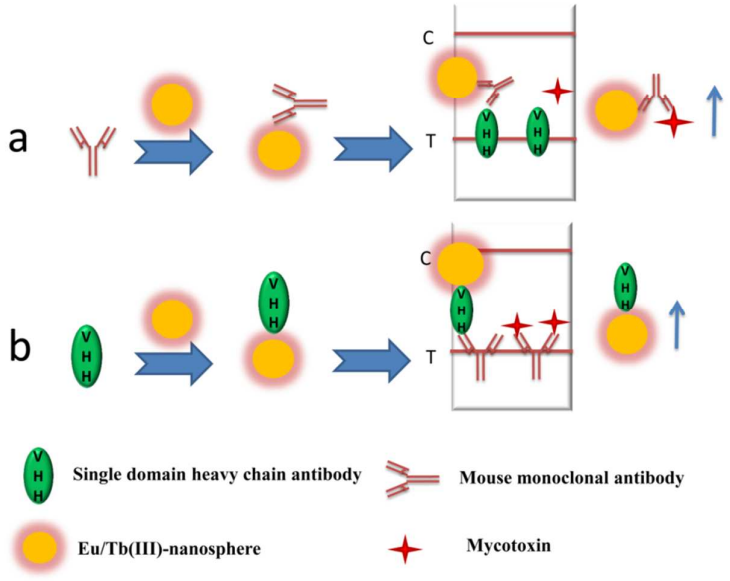
41 **Figure S2. (a) Effect of amounts of antibodies, (b) pH of BB buffer, (c) and amounts of EDC on**
42 **fluorescence intensity.**
43

44 **Preparation of two patterns of competitive TRFICA**

45 The test strip for the AINnd-TRFICA pattern had a T line coated with the phage 2-5 or
46 phage 8# and a C line coated with the rabbit anti-mouse IgG. The test strip for the mAb-TRFICA
47 pattern had a T line coated with the 1C11 or 2D3 and a C line coated with the rabbit anti-apical
48 IgG. All the immunoreagents were spurted onto the nitrocellulose membrane (HF07502S25,
49 Millipore, Bedford, MA, USA) at the rate of 0.4 – 0.8 μg/cm. The nitrocellulose membrane was
50 dried for 2 h at 37°C. The sample pad was treated with blocking buffer (2.9% Na_2HPO_4 + 0.3%
51 NaH_2PO_4 + 1% Tween 20 + 1% PVPK 30 + 0.25% EDTA + 0.5% BSA + 0.02% NaN_3) and
52 allowed to stand at 37°C for 6 h. Then, sample pad, NC membrane, and absorbent pad were pasted
53 onto a plastic scale board, overlapping with each other by 1 mm. Then, the assembly was cut into
54 4 × 60 mm strips with CM 4000 Guillotine Cutter.



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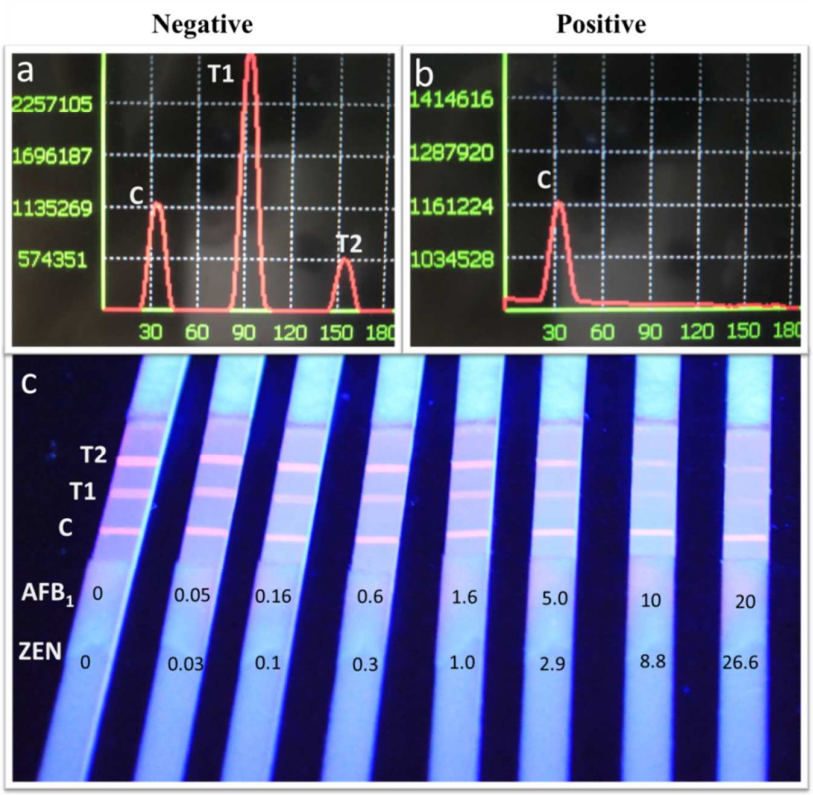
56 **Scheme S1. Schematic diagram of (a) AINnd-TRFICA for mycotoxin, (b) mAb-TRFICA for**
57 **mycotoxin.**

58 **AIdnb-TRFICA for dual mycotoxins**



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60 **Figure S3. (a) negative. (b) positive. (c) Responses of the strips to AFB_1 and ZEN with different**

61 **concentrations. The concentrations of AFB_1 and ZEN (ng mL^{-1}) were increased from left to right.**

62 The appropriate amounts of antigen and antibody were optimized in an immunoassay to

63 obtain a higher sensitivity. Therefore, the amounts of antigen on T and C lines and dilution of

64 antibody probes were optimized (Table S1).

65 **Table S1. T/C values under various conditions.**

	Antigen on T line (ng mL^{-1})	C line (ng mL^{-1})	Probe Dilute factor	T/C
	0.25	0.05-0.25	100-300	-
Phage2-5	0.50	0.05-0.25	100-300	0.124-0.548
	1.00	0.05-0.25	100-300	0.152-1.412
	0.25	0.05-0.25	200-400	-
Phage8#	0.50	0.05-0.25	200-400	0.392-1.284
	1.00	0.05-0.25	200-400	0.285-2.065

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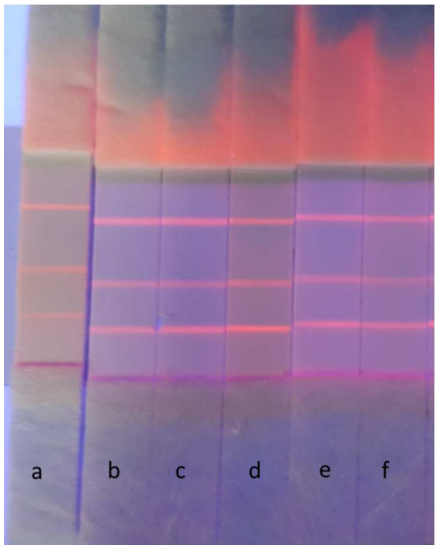

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68 **Figure S4. Effects of different analysis buffer on the release of nanosphere-probe (a, 1% PVPK**
69 **30 + 1% Sucrose + ddH_2O; b, 1% PVPK 30 + 1% BSA + ddH_2O; c, 1% PVPK 30 + 2% Sucrose + 1%**
70 **Tween 20 + ddH_2O; d, 2% Sucrose + 1%Tween 20 + ddH_2O; e, 2% Sucrose + 1% PVPK 30 +**
71 **ddH_2O).**

72

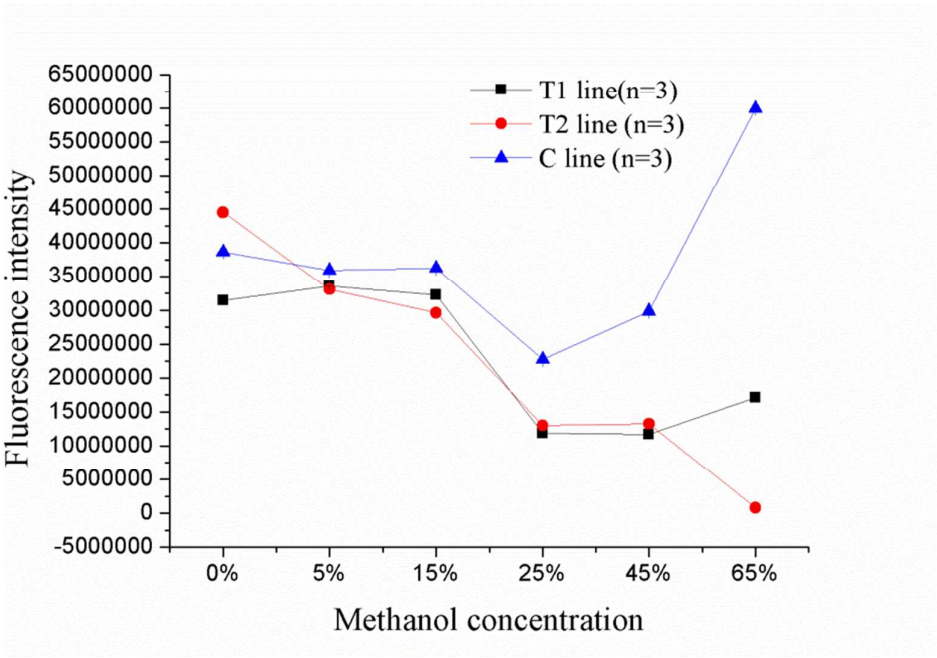

Figure S5. Effect of concentration of methanol on T/C value.

75 **Validation of the developed AIdnb-TRFICA for dual mycotoxin**

76 **Table S2. Recoveries of AFB_1 and ZEN in spiked samples by AIdnb-TRFICA**

Mycotoxins	samples	Spiked concentration (ng)	Mean recovery (%)	CV (%)[a]
AFB_1	Yellow maize	10	106.6	7.6
		20	90.3	5.4
		50	83.3	6.8
	White maize	10	94.2	8.1
		20	80.5	7.4
		50	73.9	7.7
	Maize flour	10	81.4	9.1
		20	72.6	8.2
		50	83.8	9.6
ZEN	Yellow maize	20	91.0	7.7
		100	85.3	6.6
		500	81.9	7.1
	White maize	20	80.2	10.3
		100	78.5	9.8
		500	77.7	8.3
	Maize flour	20	87.2	10.6
		100	82.1	9.6
		500	75.6	5.8

77 [a] Coefficient of variation

78 **LC-MS/ MS analysis used in validation experiments**

79 The ground sample (5 g) was mixed with 10 mL of acetonitrile/water (80:20 v/v) and
80 shaken for 2 h. The clear extract was filtered using a 0.22-μm membrane filter before analysis by
81 LC-MS/MS.

82 The LC-MS system was an Accela HPLC connected to a triple quadrupole MS analyzer
83 with an electrospray ionization (ESI) interface (Thermo Fisher Scientific, USA). A 10-μL aliquot
84 was injected into a C18 column (Hypersil Gold, 100 mm × 2.1 mm i.d., 3 μm, Thermo Fisher
85 Scientific) at 30°C column temperature. The gradient was composed of solvents (A, v/v) 50%
86 methanol-acetonitrile and (B, v/v) 0.1% formic acid in water at a flow rate of 200 μL/min. The
87 gradient elution was performed as follows: 0 min, 15% A; 8.5 min, 50% A; 10 min, 50% A; 11.5
88 min, 70% A; 13.5 min, 70% A; 15 min, 100% A; 15.01 min, 15% A; 20 min,15% A.

89 The MS detection was equipped with an ESI source in negative and positive selected
90 reaction monitoring (SRM) modes[1]. MS was measured with the following conditions: for the
91 positive scan mode, spray voltage, 4000 V; sheath gas pressure, 20 psi; capillary temperature,
92 330°C; however, for the negative scan mode, aux gas pressure, 8 psi;. spray voltage, 3000 V and
93 capillary temperature, 270°C. The parent and two daughter ions were 313/241.1, 285 for AFB_1 and
94 317.1/130.8, 174.9 for ZEN.

Reference

1. Wu, R.; Zhang, W.; Wang, X.. Food Chem 2016, 204, 334-342.