

Colorimetric ELISA with acid – base indicator for sensitive detection of ochratoxin A in corn samples

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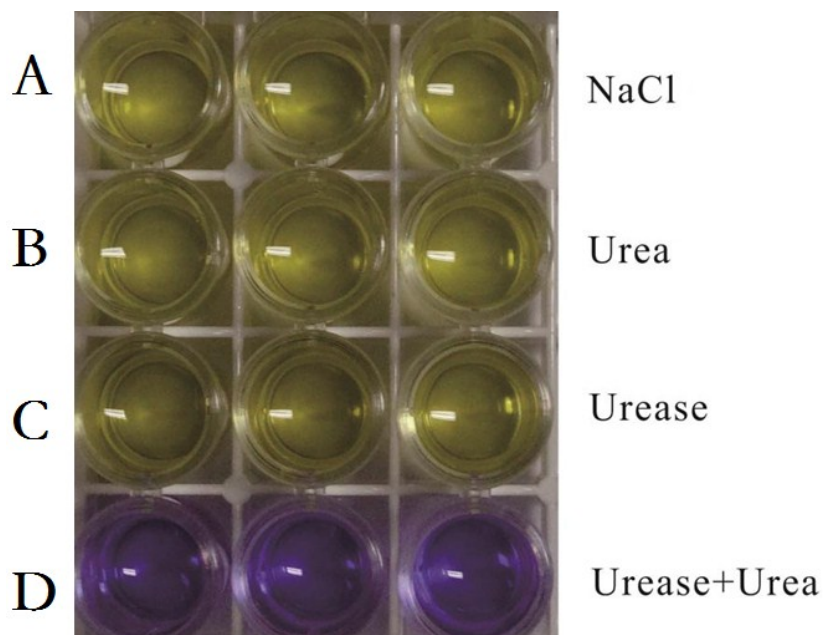


Figure S1. The effects of urea and urease@OTA on BCP colored change. A: 10 μ L of BCP dye (8 mg/mL) dissolved in 0.128 mol/L NaCl solution (pH 5.96); B: 10 μ L of BCP dye (8 mg/mL) dissolved in 0.128 mol/L NaCl solution (pH 5.96) containing 0.1 mol/L urea; C: 10 μ L of BCP dye (8 mg/mL) dissolved in 0.128 mol/L NaCl solution (pH 5.96) containing 100 μ g/mL urease@OTA;. D: 10 μ L of BCP dye (8 mg/mL) dissolved in 0.128 mol/L NaCl solution (pH 5.96) containing 0.1 mol/L urea and 100 nmol/L urease@OTA .

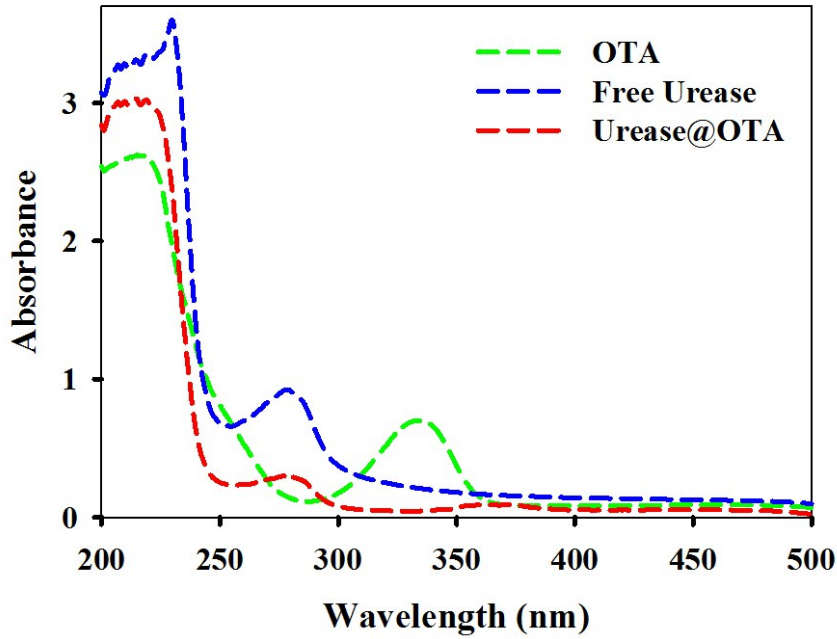


Figure S2. UV-vis absorption spectra of OTA (green line), urease (blue line), and urease@OTA conjugates (red line). Compared with that of urease, the UV-vis spectrum of Urease@OTA conjugates displayed two characteristic peaks at 280 and 363 nm, respectively. The concentrations of OTA, urease, and urease@OTA were 10, 760, and 300 $\mu\text{g/mL}$, respectively. According to derivation of LambertBeer law, the following formula can be used to calculate the conjugation ratio:

$$C_a/C_b = (AC_{am} \cdot KB_{bm} - AC_{bm} \cdot KB_{am}) / (AC_{bm} \cdot KA_{am} - AC_{am} \cdot KA_{bm}),$$

Where (C_a/C_b) is the optical density of the conjugate at the maximum absorption wavelengths of A and B. KA_{am} and KB_{bm} are the molar extinction coefficients of A and B at their maximum absorption wavelength. KA_{bm} represents the molar extinction coefficient of A at the maximum absorption wavelength of B. KB_{am} stands for the molar extinction coefficient of B at the maximum absorption wavelength of A. The molar extinction coefficient is calculated by the following formula: where $KY_{xm} =$

$AY_{xm}/(\rho_x/M_x)$, Y stands for A or B; x stands for a (OTA) or b (urease); ρ_x is the concentration of x; M_x is the molar mass of x; and AY_{xm} is the optical density of Y at the maximum absorption wavelength of x. The primary data are displayed below, and the optical densities can be obtained from the UV-vis spectrum.

$\rho_a = 10 \text{ } \mu\text{g/mL}$, $\rho_b = 760 \text{ } \mu\text{g/mL}$, $M_a = 403.8$, $M_b = 480000$, $AA_{am} = 0.6999$, $AA_{bm} = 0.1516$, $AB_{am} = 0.2093$, $AB_{bm} = 0.9239$, $AC_{am} = 0.0453$, and $AC_{bm} = 0.3016$. Thus, the C_a/C_b value is 5.73.

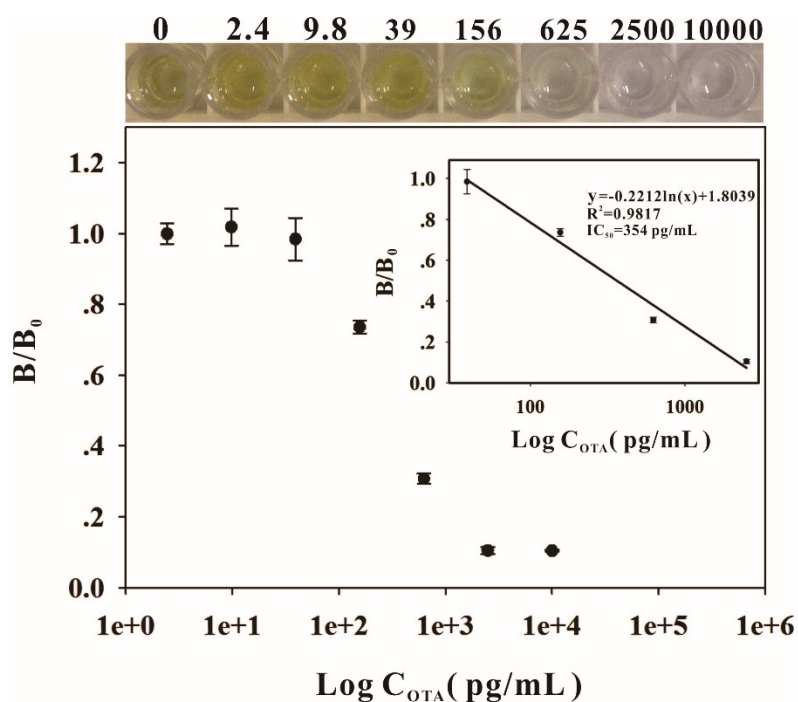


Figure S3. Quantitative immunoassay of OTA using HRP-based conventional ELISA in 0.02 M PB buffer with pH 6.5 and contains 5% methanol. The inset shows the calibration curve with OTA concentration ranging from 39 pg/mL to 2500 pg/mL ($R^2 = 0.9817$). At the top, the color of TMB acts as the qualitative analysis by naked eye. The cutoff limit of the HRP-based conventional ELISA is about 625 pg/mL. The error bars represent the standard deviation of three measurements.

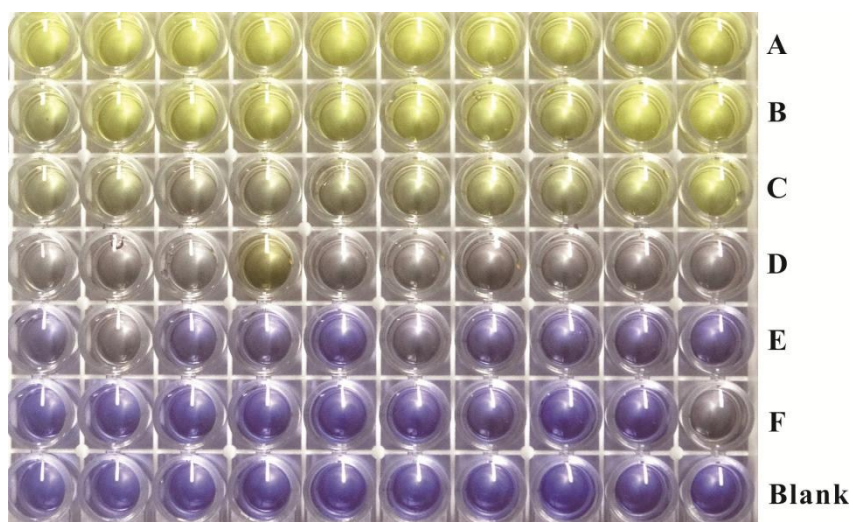


Figure S4. Reliability of the proposed method for the OTA qualitative detection with naked eye was evaluated by analyzing OTA spiked samples in corn extract. A: OTA (160 pg/mL), B: OTA (100 pg/mL), C: OTA (60 pg/mL), D: OTA (50 pg/mL), E: DON (20 ng/mL), F: OTA (10 pg/mL), and blank (0 pg/mL). Blank test was performed by adding 0.128 mol/L NaCl solution (pH 5.96) containing 10% methanol. All samples were analyzed by the proposed method for 10 times.

Table S1. Concentrations of anti-OTA mAb and urease@OTA were optimized using the checkerboard titration method.

Dilution of urease@OTA	Dilution of anti-OTA mAb			
	2000	4000	8000	16000
10	7.7447	7.4097	7.1351	6.7550
20	6.2164	5.6613	5.4074	4.9652
40	6.0173	5.3246	4.1733	3.8903
80	5.2251	3.1667	2.5652	2.0118
160	2.8657	2.4234	1.7457	1.4016*
320	2.2161	1.6704	1.4645	0.9708
640	0.9358	0.8219	0.5060	0.2782

Notes: The initial concentrations of mouse anti-OTA mAbs and urease@OTA were 0.31 mg/mL and 1.0 mg/mL, respectively. The OD_{588/434} value is represents the signal output. The red mark denotes optimal conditions.