Electronic Supporting Information for

A label-free fluorescence immunoassay system for the sensitive detection of the mycotoxin, ochratoxin A

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Materials and Methods

Reagents. Ochratoxin A (OTA) and ochratoxin B (OTB) were purchased from Sigma-Aldrich Chemical Co. (Yongin, South Korea) and Santa Cruz Biotechnology Inc. (CA, USA), respectively. Bovine serum albumin (BSA), mouse monoclonal antibody (Mouse-MAb), non-ionic surfactant tween 20, and MES salt were obtained from Sigma-Aldrich Chemical Co. Monoclonal anti-OTA antibody was purchased from Abcam Inc. (Cambridge, England) and desalted by using Zeba^{TM} Spin desalting columns (MWCO 7 kDa, Pierce Biotechnology, Inc.) before use. HPLC-grade methanol was obtained from Merck Chemical Corp. (Darmstadt, Germany). Red wine, white wine, and grape juice were purchased from Korean retail market (Daejeon, South Korea). All other chemicals and organic solvents used were of reagent grade or better. Standard solutions containing various concentrations of OTA in methanol were prepared for further assay.

Preparation of the fluorescence immunoassay system for OTA detection. For preparation of the OTA immunoassay system, 1 μL 1 mg mL^{-1} desalted anti-OTA solution was added to the fluorescence measurement cell along with 180 μL 25 mM MES buffer (pH 6.0) containing 0.3% tween 20. After addition of 20 μL of solutions containing various concentrations OTA in methanol, the cell was incubated at room temperature for 20 min followed by the fluorescent measurement.

Determination of specificity in the fluorescence immunoassay. In order to determine the specificity of the fluorescence immunoassay system, independent solutions were prepared containing OTA (20 and 100 ng mL^{-1}), and either 50 μg mL^{-1} BSA or 100 μg mL^{-1} of Mouse-MAb, and 10 μg mL^{-1} anti-OTA. In addition, solutions containing 10 μg mL^{-1} anti-OTA and 20 or 100 ng mL^{-1} OTB were subjected to the fluorescence immunoassay.

Sample preparation. To determine OTA in red wine, white wine, and grape juice, spiked with various concentrations of OTA, the samples were diluted ten times with 25 mM MES buffer (pH 6.0, containing 0.3% tween 20) to yield an OTA solution with a final methanol concentration of 10%. The solution obtained by mixing the spiked samples with anti-OTA (10 μg mL^{-1}) was incubated at room temperature for 20 min and subjected to a fluorescent measurement.

Instrumentation. The fluorescence intensities of all samples were recorded by using a LS 55 Fluorescence Spectrometer (PerkinElmer, Waltham, MA). Fluorescence spectra were recorded in the wavelength range of 400 - 600 nm with excitation at 390 nm with slit widths for excitation and emission of 10 nm.

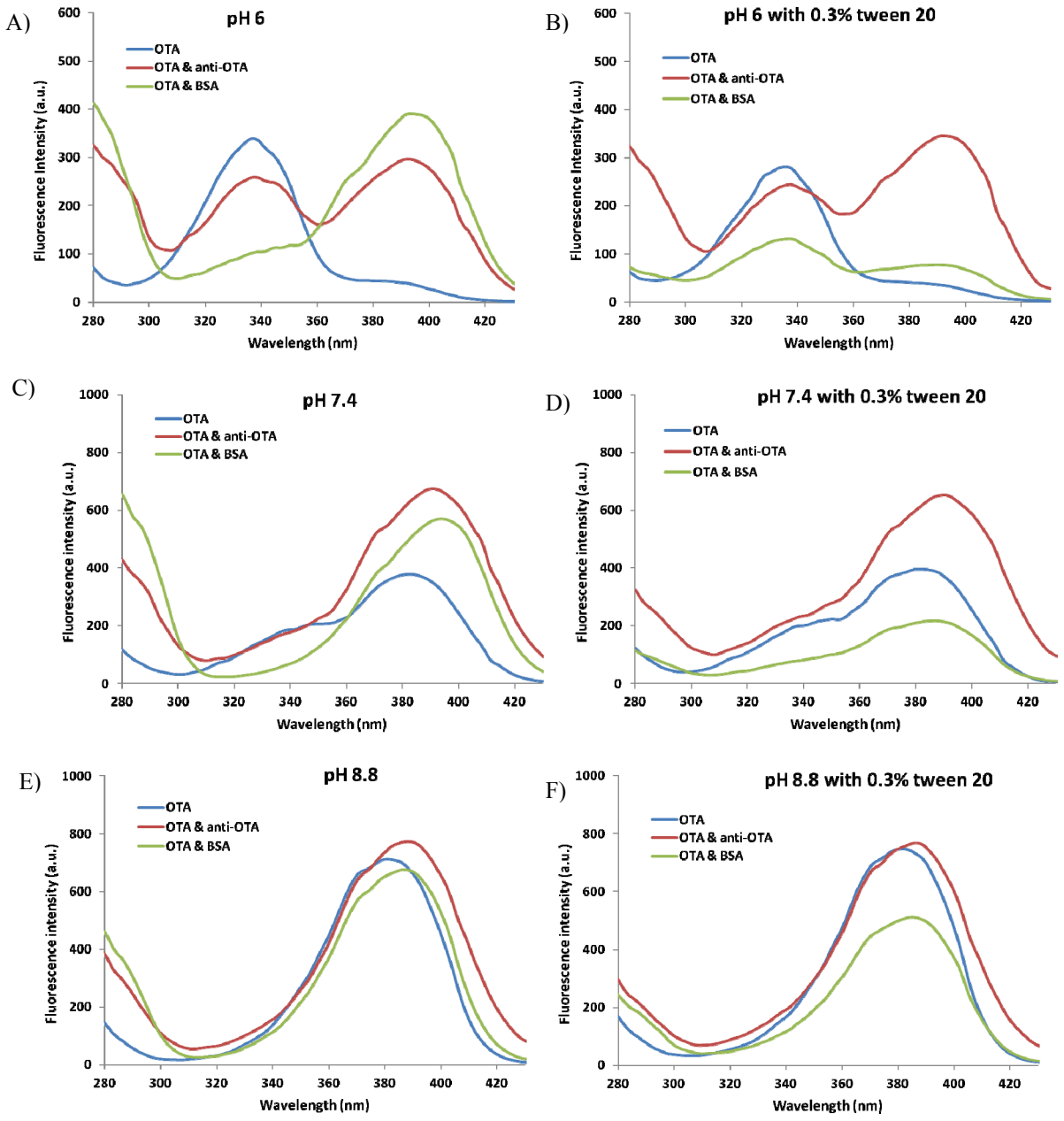


Fig. S1 Fluorescenceexcitation spectra of 500 ng mL^{-1} OTA, OTA coupled with anti-OTA (10 μg mL^{-1}) and BSA (50 μg mL^{-1}) in the 25 mM MES buffer (pH 6.0) (A), 25 mM MES buffer (pH 6.0) with 0.3% Tween 20 (B), 10 mM PB buffer (pH 7.4) (C), 10 mM PB buffer (pH 7.4) with 0.3% Tween 20 (D), 10 mM $NaHCO_3$ buffer (pH 8.8) (E), and 10 mM $NaHCO_3$ buffer (pH 8.8) 0.3% Tween 20 (F) (λ_{em} = 450 nm).

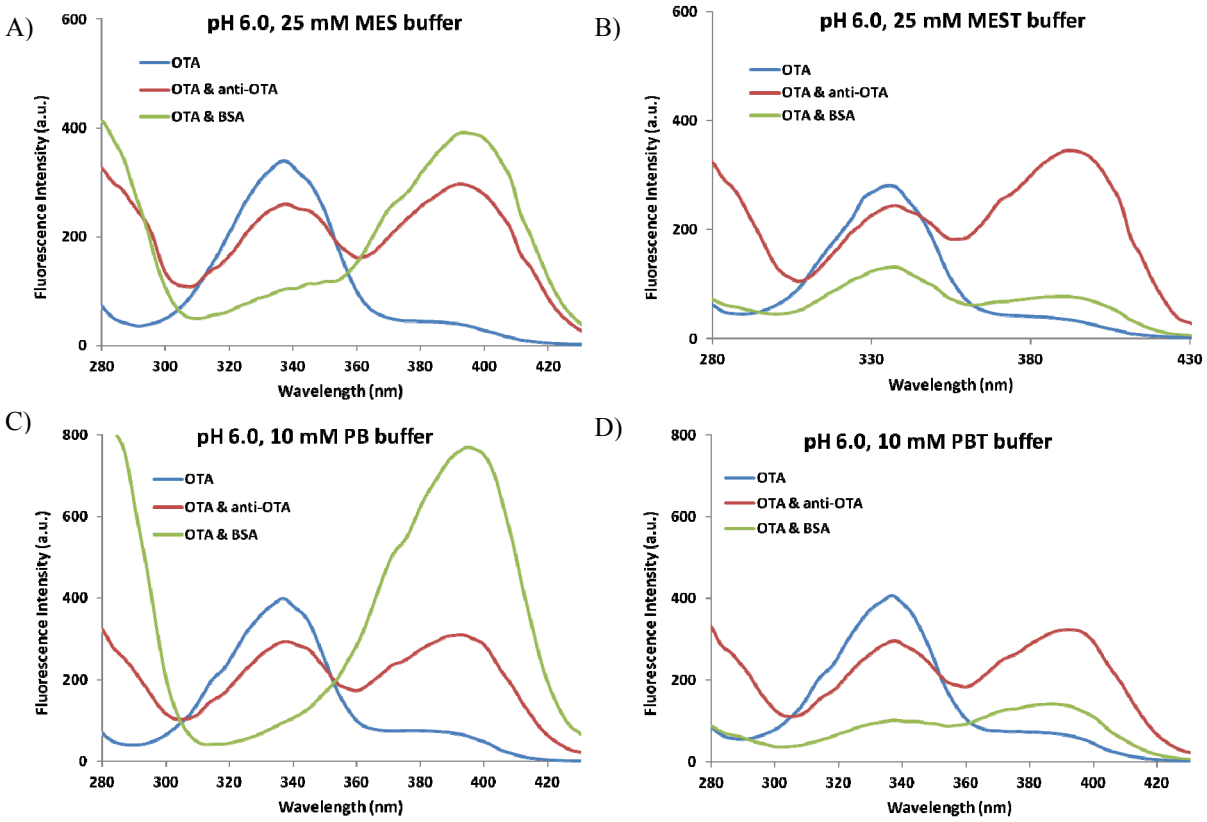


Fig. S2 Fluorescence excitation spectra of 500 ng mL^{-1} OTA, OTA coupled with anti-OTA (10 μg mL^{-1}) and BSA (50 μg mL^{-1}) in the 25 mM MES buffer (A), 25 mM MES buffer with 0.3% Tween 20 (MEST) (B), 10 mM PB buffer (C), 10 mM PB buffer with 0.3% Tween 20 (PBT) (D) under same pH condition 6.0. (λ_{em} = 450 nm).

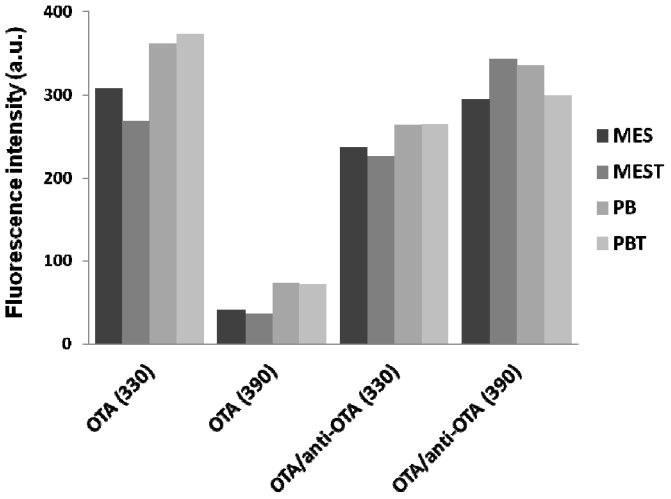
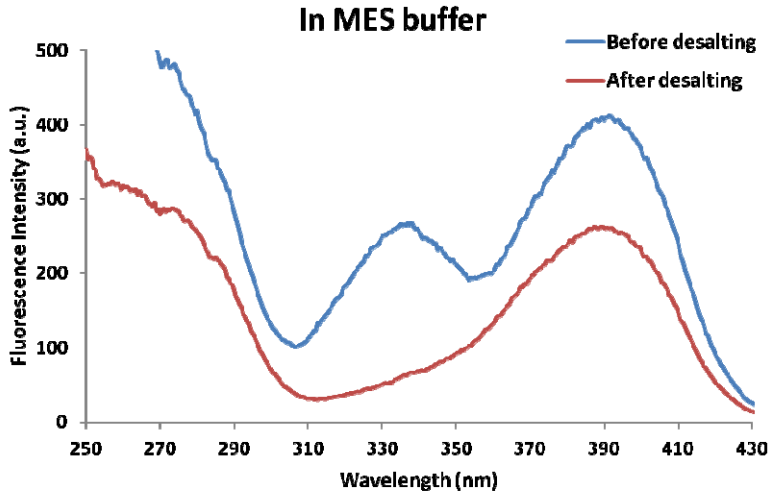


Fig. S3 Fluorescence intensities of OTA and OTA/anti-OTA complex in the pH 6.0 25 mM MES, 25 mM MEST, 10 mM PB, and 10mM PBT recorded at λ_{ex} of 340 and 390 nm under λ_{em} of 450 nm (OTA: 500 ng/mL; anti-OTA: 10 μg/mL; MEST: MES buffer with 0.3% tween 20; PBT: PB buffer with 0.3% tween 20).

A)



B)

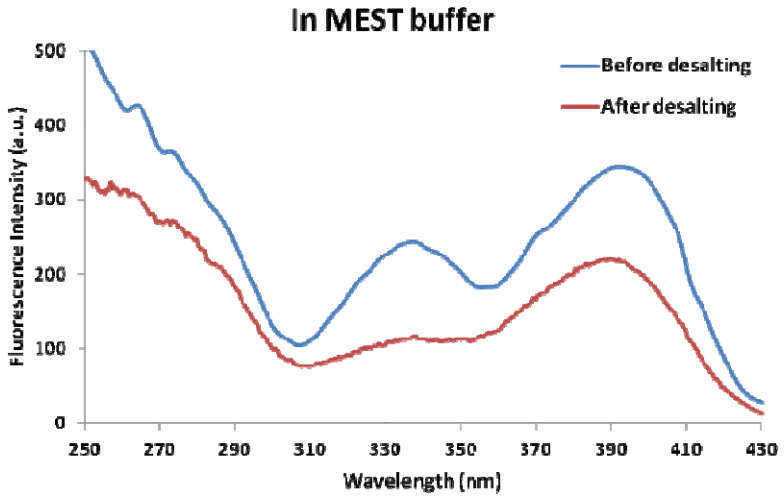
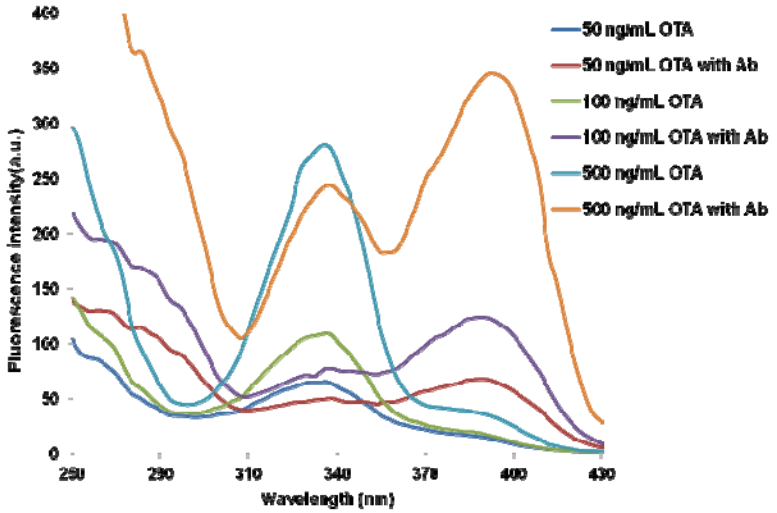


Fig. S4 Fluorescence excitation spectra of OTA/anti-OTA complex (OTA: 500 ng mL^{-1}; anti-OTA: 10 μg mL^{-1}) before and after desalting in pH 6.0 25 mM MES buffer (A) and MES buffer with 0.3% tween 20 (MEST) (B) (λ_{em}=450 nm).

A)



B)

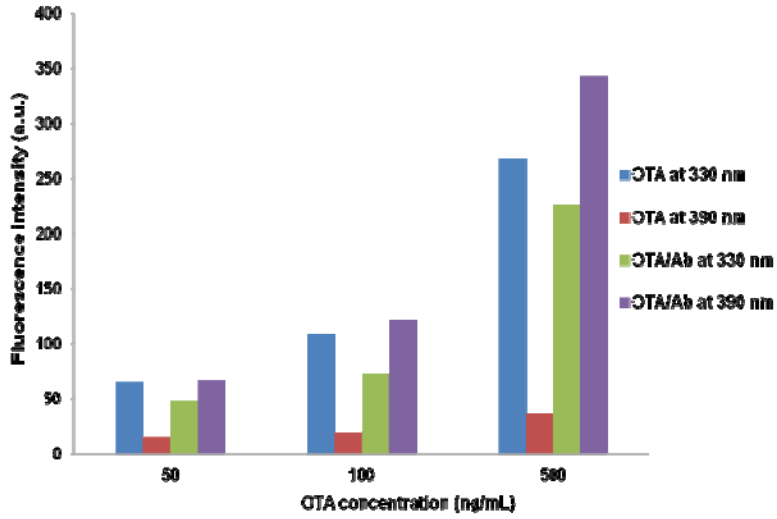


Fig. S5 A) Fluorescence excitation spectra of OTA and OTA/anti-OTA complex with various concentrations of OTA (50, 100, and 500 ng mL^{-1}) in pH 6.0 25 mM MES buffer with 0.3% tween 20 (λ_{em}=450 nm). B) Plot of fluorescence intensities at 330 nm and 390 nm from (A) as a function of concentration of OTA.

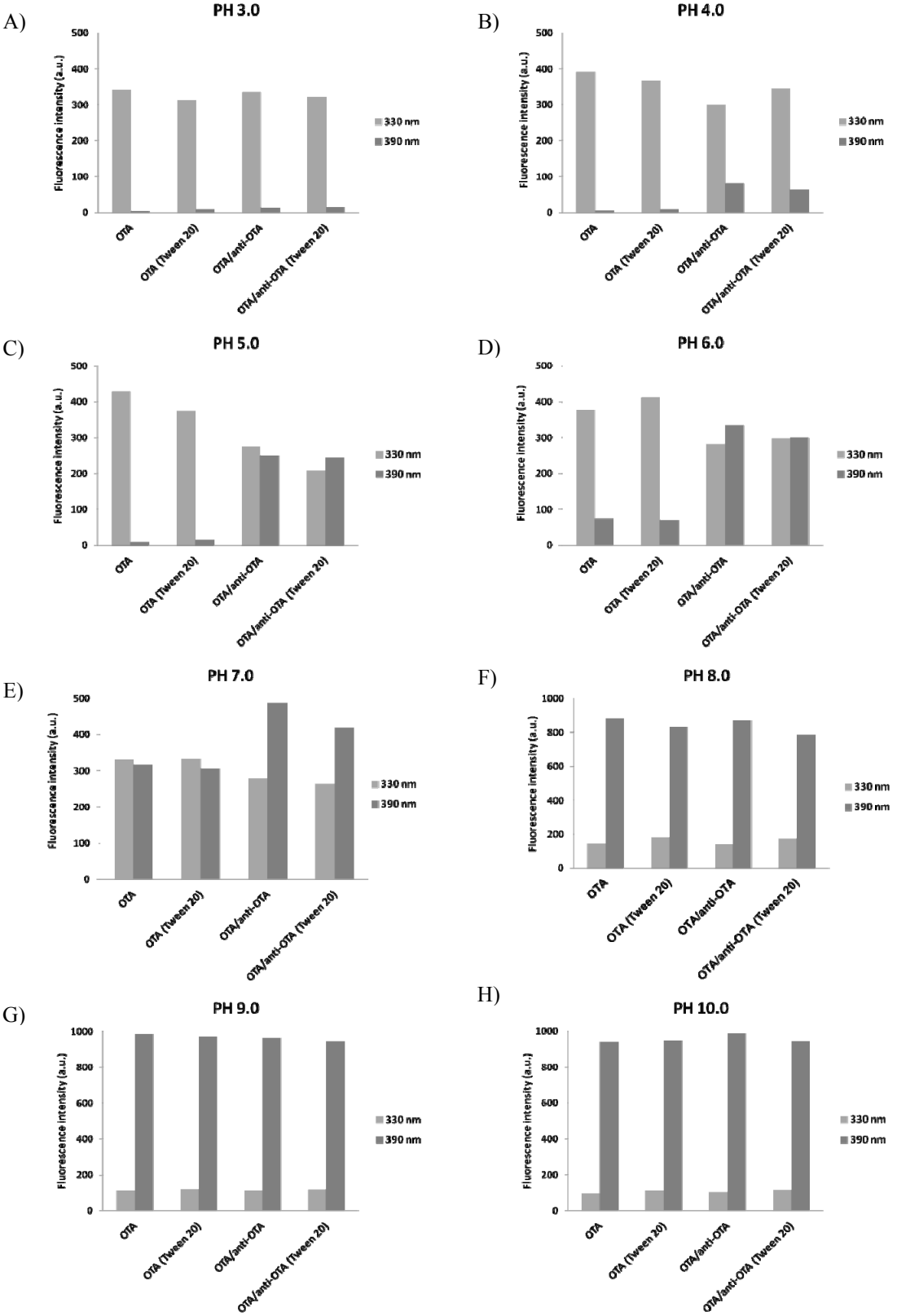


Fig. S6 Fluorescence intensities of OTA and OTA/anti-OTA complex in various pH condition with or without 0.3% tween 20 at excitation wavelength of 330 nm or 390 nm (λ_{em} = 450 nm). 500 ng mL^{-1} OTA and 10 µg mL^{-1} anti-OTA were used in this analysis. pH 3.0, 4.0, and 5.0: 10 mM acetate buffer; pH 6.0, 7.0, and 8.0: 10 mM PB buffer; pH 9.0 and 10.0: 10 mM $NaHCO_3$ buffer.

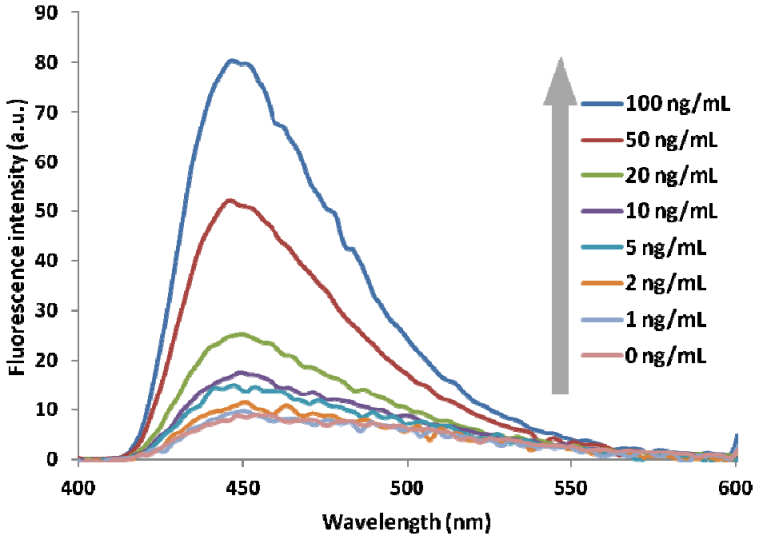


Fig. S7 Fluorescence emission spectra of solutions containing various concentrations of OTA under the conditions in which the OTA/anti-OTA complex is formed (λ_{ex}=390 nm; anti-OTA: 10 μg mL^{-1}).

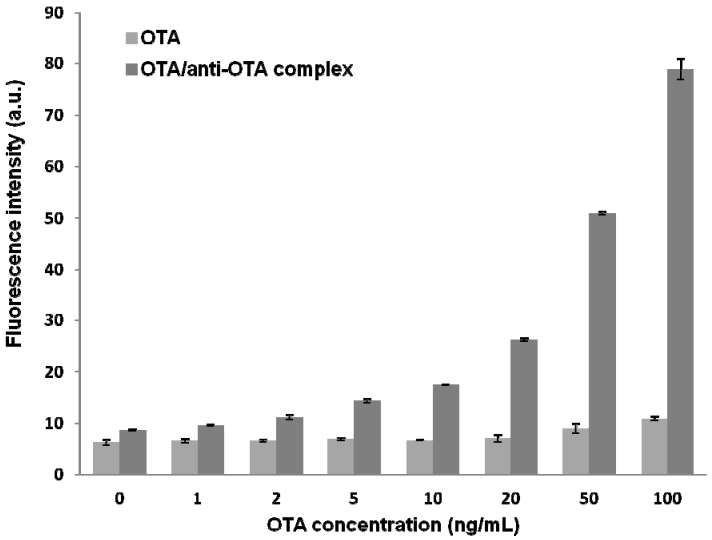


Fig. S8 Fluorescence intensities of the OTA/anti-OTA complex (dark gray; anti-OTA: 10 μg mL^{-1}) and free OTA (light gray) at 450 nm as a function of OTA concentrations (0, 1, 2, 5, 10, 20, 50, and 100 ng mL^{-1}). The fluorescence spectra were produced with excitation at 390 nm. The Error bars indicate standard deviations of data from experiments performed in triplicate.

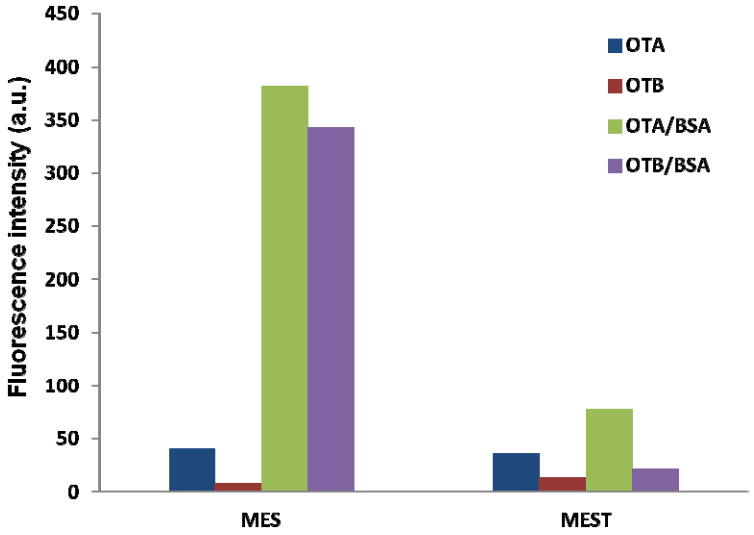
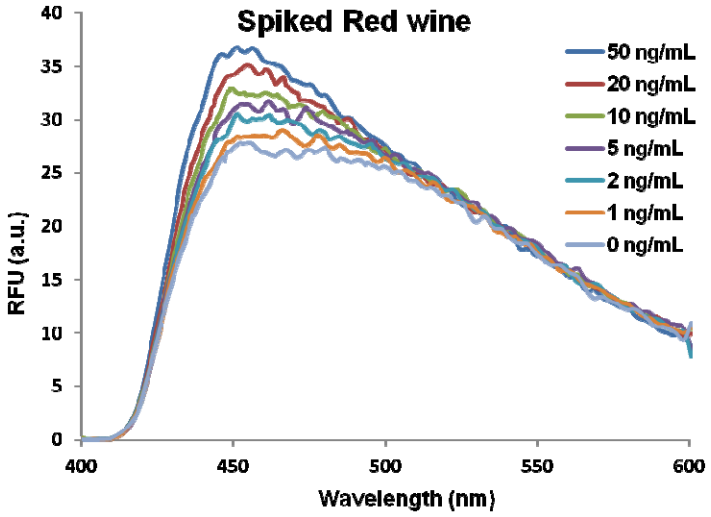


Fig. S9 Fluorescence intensities of OTA, OTB, OTA coupled with BSA (OTA/BSA) and OTB coupled with BSA (OTB/BSA) in the 25 mM MES buffer and MES buffer with 0.3% tween 20 (MEST). 500 ng mL^{-1} OTA and OTB, and 50 μg mL^{-1} BSA were employed in this assay (λ_{ex}=390 nm; λ_{em} = 450 nm).

A)



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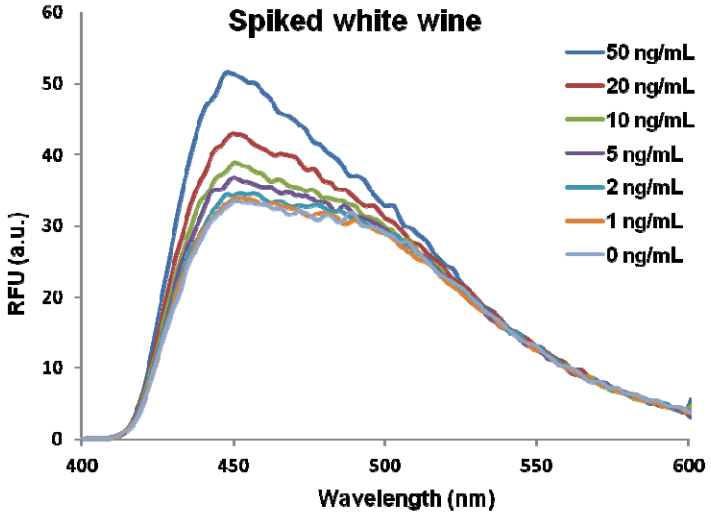
B)



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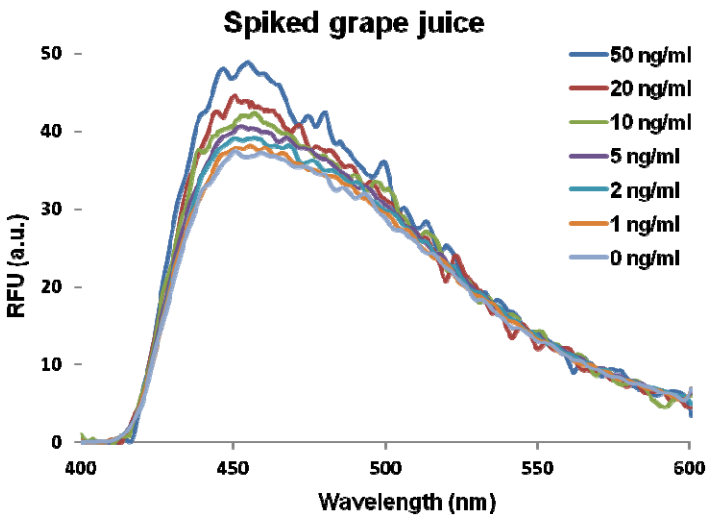
C)



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4 **Fig. S10** Fluorescence emission spectra of solutions containing various concentrations of OTA spiked
5 samples under the conditions in which the OTA/anti-OTA complex is formed (λ_{ex}=390 nm; anti-OTA:
6 10 μg mL^{-1}): A) Spiked red wine; B) Spiked white wine; C) Spiked grape juice.