Supporting Information

Ultrasensitive detection of deoxynivalenol by mass spectrometric immunoassay with cascade signal amplification

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Supplementary material

1. Experimental section

1.1 Chemicals and Materials

Tris-(hydroxymethyl) aminomethane (Tris), polyethylene glycol sorbitan monolaurate (Tween-20), Phi29 DNA polymerase, Phi29 DNA polymerase buffer, dNTP, streptavidin modified magnetic beads, ultrafiltration tube, and oligonucleotide purification kit were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China). All artificial antigens and antibodies were purchased from Zoonbio Biotechnology (Nanjing, Jiangsu, China). CircLigase, adenosine triphosphate (ATP) and CircLigase buffer were obtained from Biosearch Technologies (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore, Billerica, MA, USA) was used in all runs. All other reagents were of analytical grade.

The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd.(Shanghai, China) and were purified using high-performance liquid chromatography. Their sequences were expressed as follows:

Primer DNA: 5′-CHO-T_{40}-3′;

Fluorescence primer DNA: 5′-CHO-T_{40}-3′-6-FAM;

Circular template (5′-phosphate-A_{80}-3′);

The buffers were expressed as follows:

CircLigase buffer: 0.05 M 4-morpholinepropanesulfonic acid (MOPS) (pH 7.5), 0.01 M KCl, 5 mM $MgCl_2$, and 1 mM DTT;

Phi29 DNA polymerase buffer: 33 mM Tris-acetate (pH 7.9), 10 mM Mg-acetate, 66 mM K-acetate, 0.1% (v/v) Tween 20, and 1 mM DTT.

1.2 Apparatus

Transmission electron microscopy (TEM) images were collected on a JEM1400 electron microscope (JEOL, Japan). All mass spectra were acquired on Agilent 1290-6470 Ultra-High Performance Liquid Chromatography-Triple Quadrupole Mass Spectrometer, equipped with an electrospray ionization source (Agilent Technologies, Inc., USA). Fluorescence microscope images were taken using a Ci-L plus Upright Microscope (Nikon, Japan). The gel electrophoresis was preformed on the automatic gel-imaging system (Tanon, China).

1.3 Preparation of Antigen-Modified Magnetic Beads

Biotin was initially dissolved in dimethyl sulfoxide solvent. Then 13.2 μL 2mg/mL resulting biotin solution and 0.2 mg antigens were mixed in 300 μL 0.1M pH 7.4 phosphate-buffered saline (PBS) buffer. The reaction mixture was incubated at room temperature for 30 minutes, and the solution was purified and concentrated using an ultrafiltration tube (50 kDa) to remove unbound biotin and other residual reagents. Next, 500 μL of 10 mg/mL streptavidin-modified magnetic beads were mixed with 5 μL of 3 mg/mL biotinylated antigens solution in 0.1 M PBS buffer (pH 7.4). The mixture was incubated at 37 °C with gentle shaking for 30 minutes. After incubation, the magnetic beads were washed three times with 0.1 M PBS buffer at pH 7.4 containing 0.05% Tween-20 (PBST) to remove any unbound biotinylated antigens. Finally, the antigen-modified magnetic beads were resuspended in 1 mL of 0.1 M PBS buffer (pH 7.4) and stored at 4 °C for further use.

1.4 Preparation of Mass Tag-Modified Antibodies

A mixture of 10 μL of 1 mg/mL antibodies and 40 μL of 100 μM primer DNA was prepared in 1mL 0.05 M carbonate buffer solution (pH 11). The mixture was incubated at 4 °C for 12 hours. After the reaction, the solution was purified and concentrated at 10000 rpm to a final volume of 200 μL using an ultrafiltration tube

(50 kDa). The resulting mass tag-modified antibodies were stored at 4 °C for subsequent use.

1.5 Preparation of the Circular Template

The circular template for RCA was prepared according to the following protocol: An 80 μL ligation reaction mixture containing 10 μM linear template DNA, 2.5 mM $MnCl_2$, 10 U/μL CircLigase, 0.05 mM ATP, and 1× CircLigase buffer was prepared. The mixture was incubated at 60 °C for 3 hours to facilitate circularization, followed by heating at 80 °C for 10 minutes to inactivate the CircLigase enzyme. To remove any unligated linear DNA, 100 U of Exonuclease I and 100 U of Exonuclease III were added, and the reaction was incubated at 37 °C for 3 hours to hydrolyze the linear DNA into single nucleotides. The mixture was then heated at 80 °C for 20 minutes to inactivate the exonucleases. Finally, the circular template was purified and concentrated at 10000 rpm to a final volume of 50 μL using an oligonucleotide purification kit and stored at −20 °C for subsequent use.

1.6 Gel Electrophoresis

To validate the feasibility of the designed sensing strategy for DON detection, the reaction components were analyzed using 20% native polyacrylamide gel electrophoresis. In brief, samples containing different components were mixed with the loading buffer in a 5:1 ratio to form a 10 μL mixture. The mixture was then added to the loading well of a 20% PAGE gel prepared in a 0.5× Tris-Borate-EDTA (TBE) buffer. Electrophoresis was conducted using an electrophoresis instrument at 120 V for 60 min. Afterward, the gel was stained with 4S Green nucleic-acid colorant dye for 0.5 h and visualized using a Tanon fully automatic gel-imaging system.

1.7 Target Recognition, Amplification, and Detection

The experimental procedure was carefully designed to ensure analytical precision and reproducibility. Initially, 200 μL of the sample containing varying concentrations of DON, 10 μL of antigen-modified magnetic beads, and 10 μL of

mass-tag-modified antibody were added to a microcentrifuge tube and mixed thoroughly. The mixture was incubated at 37 °C for 30 minutes to allow for the formation of immunocomplexes. After incubation, the immunocomplexes were separated using a magnetic field, and the supernatant containing impurities were removed. The immunocomplexes were washed three times with PBST to ensure the removal of unbound components. Subsequently, 20 μL of RCA reaction mixture, containing 1 μL of circular template, 125 μM dNTPs, 4 U phi29 DNA polymerase, and 1× phi29 buffer were added to the immunocomplexes. The RCA reaction was carried out at 37 °C for 60 minutes, followed by enzyme inactivation at 80 °C for 5 minutes. The immunocomplexes were separated using a magnetic field and washed three times with PBST. Finally, to release the amplified DNA products, 100 U of Exonuclease I was added to the mixture was incubated in 1× Exonuclease I buffer at 37 °C for 30 minutes. The supernatant containing the released DNA was collected under a magnetic field and purified using an ultrafiltration tube (3 kDa) at 8000 rpm. The filtrate was then collected and analyzed using liquid chromatography–mass spectrometry (LC–MS).

1.8 LC–MS Conditions

The column used was ACQUITY UPLC BEH C18 (2.1 mm × 100 mm, with a particle size of 1.7 μm). The mobile phase comprised phase A (an aqueous solution containing 0.1% (v/v) formic acid and 5 mmol/L ammonium formate) and phase B (methanol). The gradient program consisted of 5% (v/v) of phase B from 0 to 2 minutes, followed by a linear increase from 5% (v/v) to 90% (v/v) of phase B from 2 to 6 minutes. The injection volume was 5 μL and the flow rate was 0.2 mL/minute. The column temperature was maintained at 40 °C. The ion source was Agilent Jet Stream Electrospray Ionization with scanning in positive ion mode and multiple reaction monitoring (MRM) used for the detection mode. The nebulizer pressure was set at 45 psi and the gas temperature was maintained at 300 °C with a gas flow rate of 10 L/minute. The temperature and flow rate of the sheath gas (N_2) were 350 °C and 11

L/minute, respectively. The capillary voltage was 3500 V and the nozzle voltage was 500 V.

2. Figure

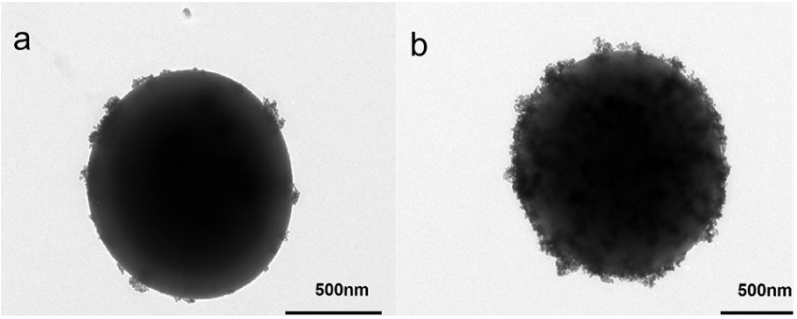

Figure S1. TEM image of (a) streptavidin-modified magnetic beads and (b) DNA-modified magnetic beads.

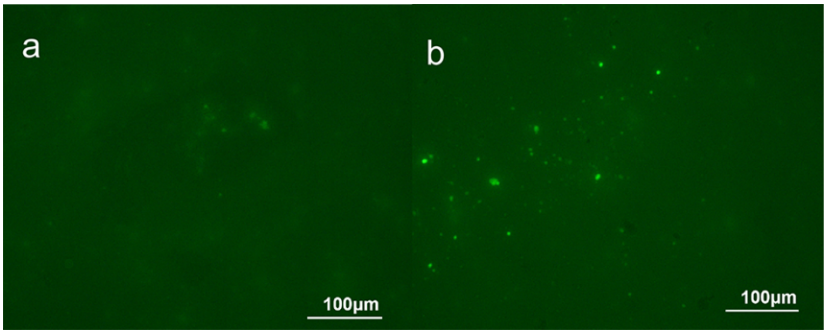

Figure S2. Fluorescence microscope images of magnetic beads (a) before and (b) after fluorescent DNA modification.

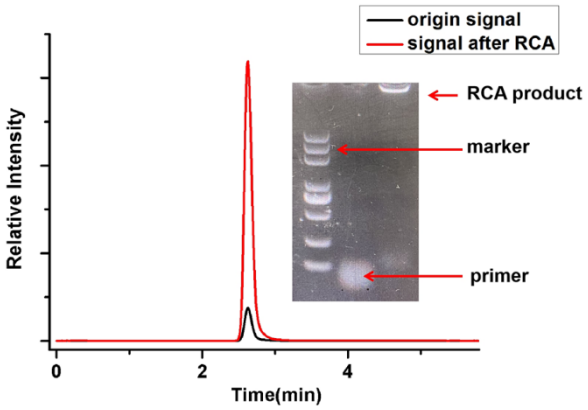

Figure S3. The variation of MS signals after RCA reaction.

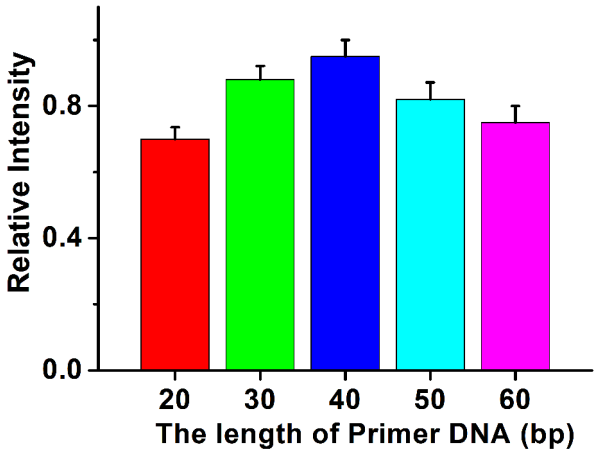

Figure S4. Comparison of the variation in length of Primer DNA, the error bars are the standard deviations of five parallel experiments.

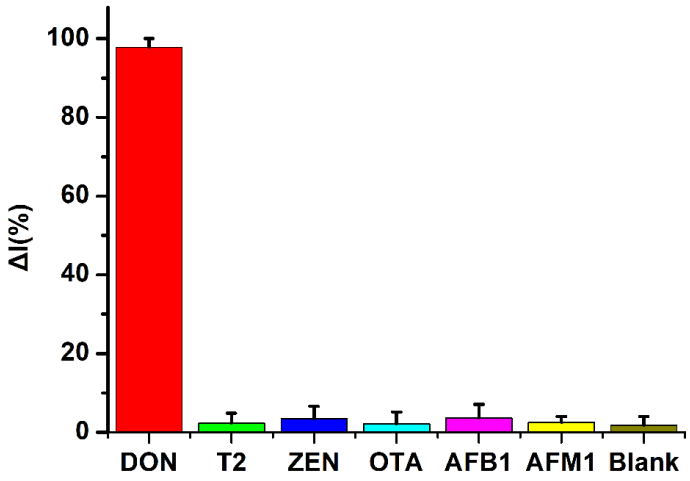

Figure S5. Comparison of the variation in signal intensity in the presence of different mycotoxins, the error bars are the standard deviations of five parallel experiments.

3. Table

Table S1. Recovery and precision of our method (n = 5).

Sample	Spiked Concentration (ng/mL)	Found Concentration (ng/mL)	Recovery Rate	CV (%)
wheat	1	1.14±0.08	108.2%	7.1%
	5	4.95±0.51	94.2%	10.3%
	10	11.05±1.31	110.8%	11.8%
corn	1	1.08±0.12	109.3%	11.1%
	5	5.24±0.52	107.5%	9.9%
	10	9.68±1.03	95.8%	10.6%

Table S2. Comparison of detection limits for different methods.

Methods	Linear Range (ng/mL)	LOD (ng/mL)
electrochemiluminescence immunosensor[1]	1-200	0.21
colourmetric immunosensor[2]	1-200	0.50
colourmetric immunosensor[3]	1-1000	0.435
electrochemical immunosensor[4]	0.01-10000	0.005
electrochemical immunosensor[5]	100-4500	63
fluorescent immunosensor	0.1-5000	0.004
lateral flow immunoassay[6]	0.24-8.51	0.24
liquid chromatography-mass spectrometry	0.375-750	0.10
This work	0.005-50	0.0024

References

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