Supporting information for:

Nanosilver-Mediated Enzyme-Free Electrochemical Immunosensor with Enhanced Stability for Aflatoxin B1 Detection in Food Safety

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Fig. S1a demonstrates that as the concentration of the coated antigen (AFB1-BSA) increases, the current intensity gradually goes up. As the concentration of the coated antigen continues to rise, the current intensity cannot increase further and has reached its maximum value. Therefore, a concentration of AFB_1-BSA at 0.56 μg/mL is selected. In **Fig. S1b**, the optimal concentration of AFB_1 monoclonal antibody was 0.49 μg/mL by comparing the current intensities of different AFB_1 monoclonal antibodies. **Fig. S1c** indicates the optimization of IgM concentration. As IgM concentration rises, current intensity increases, yet the growth rate decelerates at 30 μg/mL. Thus, 30 μg/mL is the optimal IgM concentration. **Fig. S1d** depicts the relationship between polarization time and current intensity at a voltage of 0.6 V. The polarization time exerts a significant influence on the experimental outcomes; as the polarization time of the solution increases, the current intensity first increases and then decreases. Moreover, excessive polarization time can lead to structural fatigue of the electrode, affecting subsequent experiments, thus the optimal polarization time is chosen as 15 s. **Fig. S1e** illustrates the relationship between pre-deposition time and current intensity of the electrode at a voltage of −1.6 V. As the pre-deposition time increases, the current intensity first increases and then decreases. Therefore, 7s is selected as the optimal pre-deposition time. **Fig. S1f** depicts the relationship between deposition time and current intensity of the electrode at a voltage of −1.2 V. As deposition time increases, the current intensity rises, indicating more Ag^+ in solution gain electrons and is reduced to metallic silver deposited on the electrode surface. However, as Ag^+ around the electrode decreases, due to mass transfer, distant Ag^+ moves slowly and can't reach the electrode in time.

After 60 s, although the current intensity still rises, the rate of increase slows. Therefore, 60 s is finally selected as the best deposition time. Nitric acid is added after the immunological process finishes. It doesn't interfere with the immune system, and its concentration is carefully optimized to avoid affecting subsequent electrochemical tests. **Fig. S1g** depicts the impact of nitric acid concentration on the experiment. Notably, the sample solution containing ammonium thiocyanate fails to detect the peak current of silver on the screen-printed electrode in the absence of nitric acid, highlighting the critical role of nitric acid in the process. As the concentration of nitric acid increases, both the oxidized Ag^+ concentration and current intensity increase. However, the solution turns to yellow and the current intensity begins to decline with the continuous addition of nitric acid. Therefore, the optimal concentration of nitric acid is 1 M. **Fig. S1h** shows that as the concentration of potassium nitrate increases, which enhances the conductivity of the solution, the current intensity gradually rises. However, beyond a certain concentration, the current intensity peaks and then starts to decline. Additionally, at excessively high concentrations of potassium nitrate, white crystals precipitate in the solution. Thus, the optimal concentration of potassium nitrate is set at 1.5 M. The effect of nitric acid oxidation time on NSP is depicted in **Fig. S1i**. The current intensity gradually increases with extended oxidation time, reaching its maximum after 60 minutes. Consequently, the optimal duration for nitric acid oxidation of NSP is determined to be 60 minutes. **Fig. S1j** details NH_4SCN concentration optimization. In the presence of excess NH_4SCN, Ag^+ reacts with SCN^- to form AgSCN precipitate. Subsequently, the AgSCN precipitate dissolves to produce a colorless solution of $NH_4[Ag(SCN)_2]$, thereby stabilizing the silver ions in the solution under room temperature and normal pressure. As the concentration of ammonium thiocyanate increases, the current intensity gradually increases. However, when the concentration of ammonium thiocyanate continues to rise, the rate of increase in current intensity diminishes, and white crystals precipitate from the solution. Therefore, the optimal concentration of ammonium thiocyanate is selected to be 2 M.

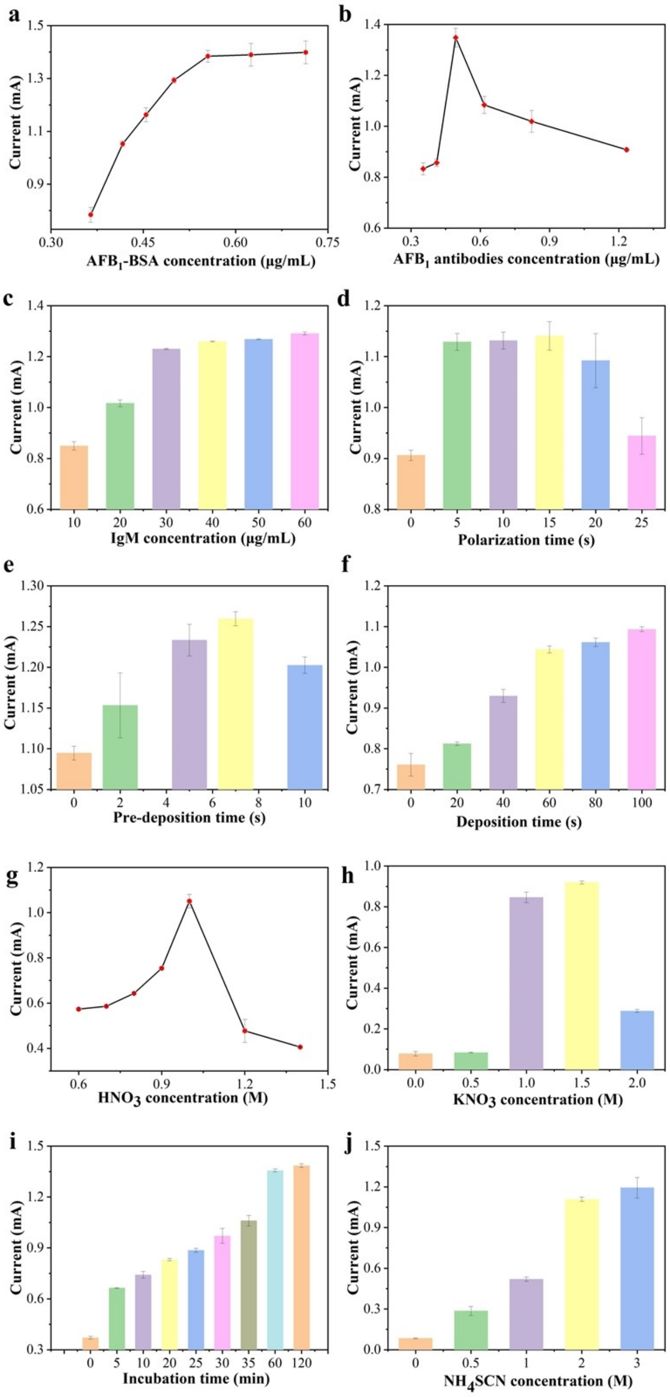

Fig. S1 Optimization of experimental conditions for the bio-enzyme-free electrochemical immunosensor: (a) Current intensity at varying AFB_1-BSA concentrations; (b) Current intensity at different AFB1 antibody concentrations; (c) Current intensity at various IgM concentrations; (d) Current intensity at different polarization times; (e) Current intensity with varying pre-deposition times; (f) Current intensity at different deposition times; (g) Current intensity at varying HNO_3 concentrations; (h) Current intensity at different KNO_3 concentrations; (i) Current intensity at varying HNO_3 incubation times; (j) Current intensity at different NH_4SCN concentrations.

Table S1 Spike recovery of AFB_1 from corn starch

Added (ng/mL)	**Detected (ng/mL)**	**RSD(%)**	**Recovery (%)**
0.1	0.109±0.004	3.81	109.42
1	1.037±0.040	3.82	103.73
10	10.29±0.035	0.34	102.90
100	100.984±1.375	1.36	100.98