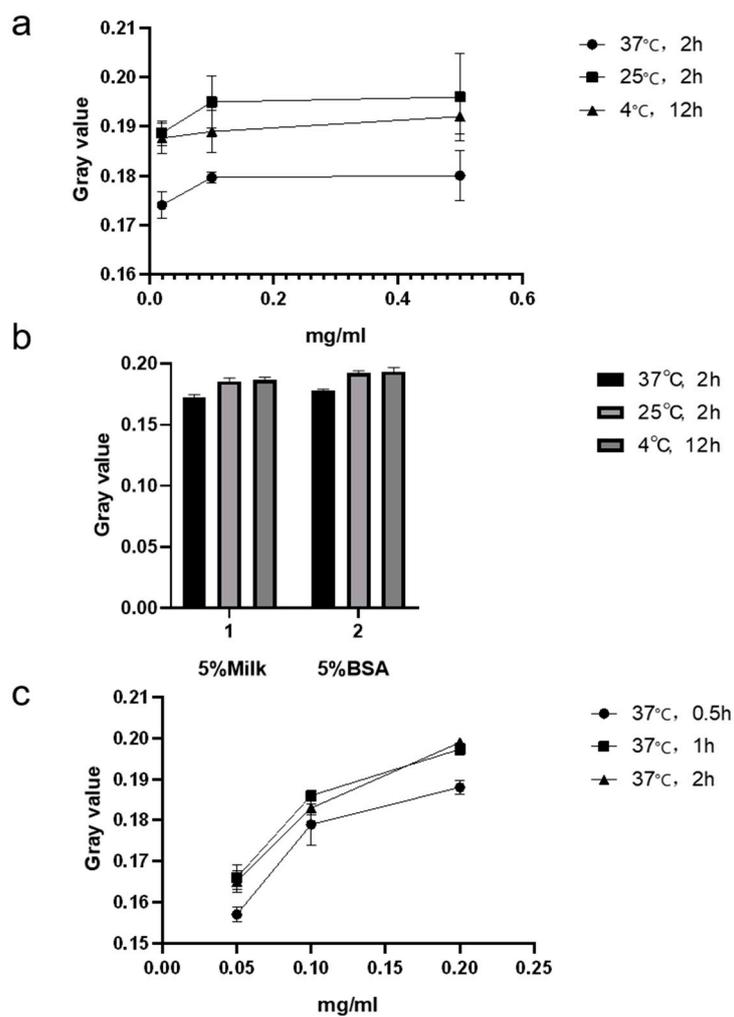
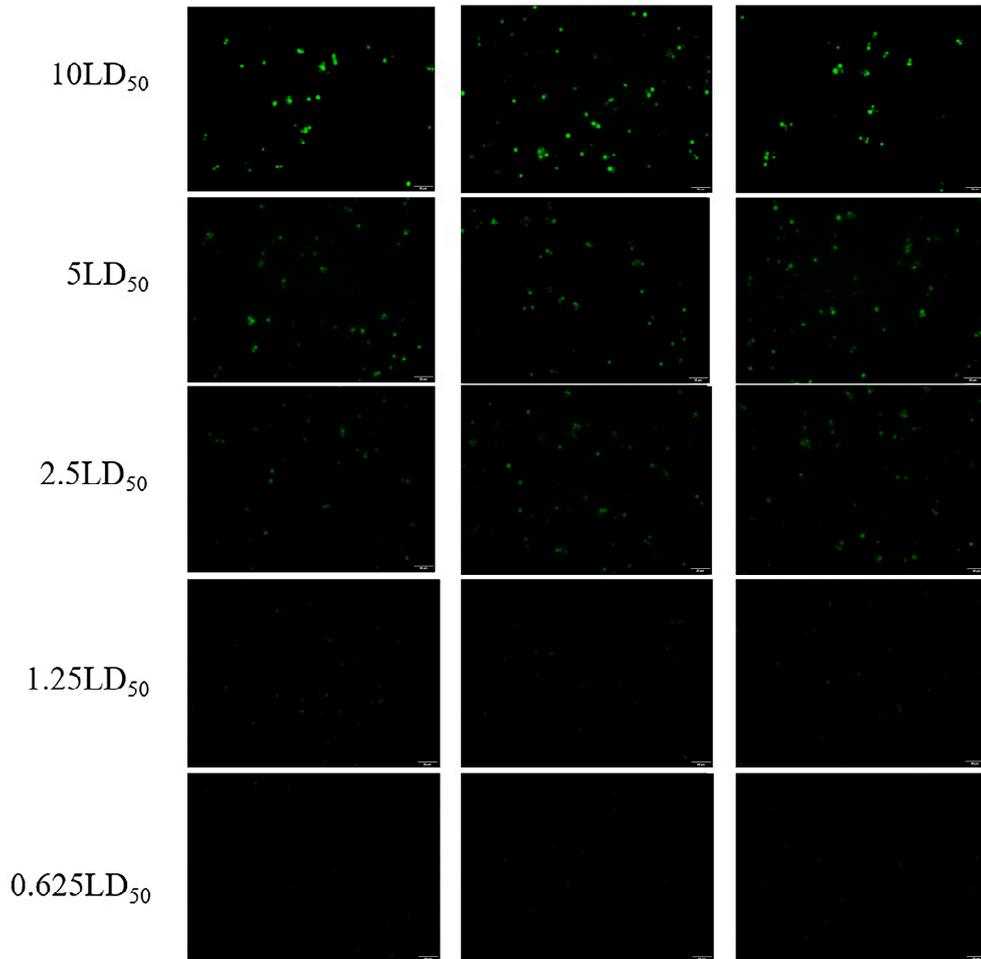


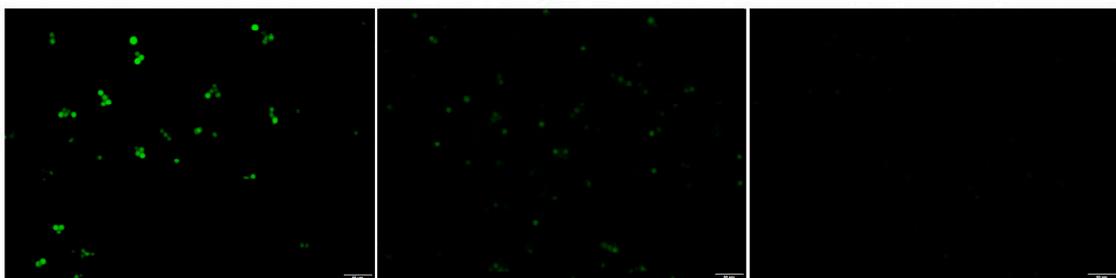
**Figure S1.** SDS-PAGE results of expression and purification of CPA recombinant proteins CPA<sub>C3</sub> and CPA<sub>N</sub>. (a) M: protein marker (GenStar M221), lane 1-2: purified recombinant CPA<sub>C3</sub> protein; (b) M: protein marker (Blue Plus) lane 3-4: purified recombinant CPA<sub>N</sub> protein.



**Figure S2.** Optimal conditions for microsphere fluorescence detection experiment. (a) The optimal coating concentration of CPA<sub>C3</sub> antibody to microspheres under the fixed capture concentration of 10 LD<sub>50</sub> CPA were determined by variable incubation time, temperature, and then photograph fluorescence imaging through observation of fluorescence microscope, and continue to get the specific value through the gray value analysis. (b–c) Similarly, the optimal blocking conditions of coating of fluorescence microscope, the dilution concentration (1:50, 1:100, 1:200 dilution of FITC-anti-CPA<sub>N</sub> antibody conjugate), incubation time of FITC-labeled CPA<sub>N</sub> antibody conjugate were also determined under a different blocking solution (5% skim milk-PBST-20, 5% BSA PBST-20 buffer) conditions. Triplicates were performed under different conditions.



**Figure S3.** Fluorescence pictures under different toxin concentrations. From top to bottom, the concentrations are 10LD<sub>50</sub>, 5LD<sub>50</sub>, 2.5LD<sub>50</sub>, 1.25LD<sub>50</sub>, respectively, 0.625LD<sub>50</sub>. Three biological replicates for each concentration. Scale bar = 50 μm.



**Figure S4.** Fluorescence pictures of different toxin concentrations in milk samples. The concentrations from left to right are  $10LD_{50}$ ,  $2.5LD_{50}$ , and  $0.625LD_{50}$ , respectively. Scale bar = 50  $\mu\text{m}$ .

**Table S1.** Determination of optimal antibody coating conditions (fix other conditions unchanged; choose toxin concentration of 10LD<sub>50</sub>, 3 biological replicates).

Temperature time	Average Gray Value		
	0.02mg/mL Capture antibody	0.1mg/mL Capture antibody	0.1mg/mL Capture antibody
37 °C, 2h	0.174	0.1795	0.18
25 °C, 2h	0.1885	0.195	0.196
4 °C 12h	0.1875	0.189	0.192

**Table S2.** Determination of optimal blocking conditions (fixing other conditions unchanged, choosing a toxin concentration of 10LD<sub>50</sub>, 3 biological replicates).

Temperature time	Average Gray Value	
	5%Skimmed milk powder	5%BSA
37 °C, 2 h	0.172	0.178
25 °C, 12 h	0.1855	0.192
4 °C, 12 h	0.187	0.1935

**Table S3.** The optimal incubation time and dilution of the fluorescence-labeled detection antibody (the concentration of detection antibody is 10 mg/mL and make 50, 100, 200-fold dilution; fixing other conditions unchanged, selecting the toxin concentration of 10LD<sub>50</sub>, 3 biological replicates).

Temperature time	Average Gray Value		
	1:50	1:100	1:200
37 °C, 30 min	0.188	0.179	0.157
37 °C, 1 h	0.197	0.186	0.166
37 °C, 2 h	0.199	0.183	0.165

**Table S4.** The gray value of the fluorescence images of different concentrations of alpha toxin.

LD <sub>50</sub>	Gray Value (TriPLICATE)			Mean	SD
10	0.202	0.193	0.191	0.202	0.193
5	0.097	0.098	0.091	0.097	0.098
2.5	0.054	0.053	0.053	0.054	0.053
1.25	0.029	0.024	0.028	0.029	0.024
0.625	0.016	0.012	0.015	0.016	0.012