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Fluorescence ELISA Based on CAT-regulated Fluorescence quenching of CdTe QDs for sensitive Detection of FB_1

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Optimization of the working conditions of coating antibody and competing antigen using a checkerboard method. the competitive inhibition rates: $(F_0\text{-}F)/F_0\times100\%$ was used to confirm the optimal parameters, where F_0 and F represented the fluorescence intensity of negative sample (FB_1-free) and an FB_1-spiked PBS solution (10 ng/mL), respectively. The results were shown in Table S1A-C

Table S1A Checkerboard method for optimization of the concentrations of anti-FB_1 mAb and FB_1-$CAT_{10:1}$ conjugate.

FB_1-$CAT_{10:1}$	anti-FB_1 mAbs (ug/mL)			
（ug/mL）	3.25	1.625	1.083	0.8125
40	14.35	22.20	20.99	69.40
20	36.92	52.64	52.86	68.16
10	67.80	76.66*	63.58	73.45

* The optimal concentrations of anti-FB_1 mAbs and FB_1-$CAT_{10:1}$ conjugate are 1.625 and 10 μg/mL, respectively.

Table S1B Checkerboard method for optimization of the concentrations of anti-FB1 mAb and FB1-CAT5:1 conjugate.

FB_1-$CAT_{5:1}$	anti-FB_1 mAbs (ug/mL)			
（ug/mL）	3.25	1.625	1.083	0.8125
40	8.25	42.43	63.93	63.72
20	16.89	59.16	72.98	77.60*
10	55.70	60.90	81.52	72.84

* The optimal concentrations of anti-FB_1 mAbs and FB_1-$CAT_{5:1}$ conjugate are 0.8125 and 20 μg/mL, respectively.

Table S1C Checkerboard method for optimization of the concentrations of anti-FB_1 mAb and FB_1-$CAT_{2:1}$ conjugate.

FB_1-$CAT_{2:1}$	anti-FB_1 mAbs (ug/mL)			
（ug/mL）	6.5	3.25	1.625	1.083
80	38.78	62.54	75.12	74.22*
40	61.55	75.91	74.81	68.80
20	67.20	75.87	62.65	59.45

* The optimal concentrations of anti-FB_1 mAbs and FB_1-$CAT_{2:1}$ conjugate are 1.083 and 80 μg/mL, respectively.

HRP-based conventional ELISA for FB_1 detection

96-well polystyrene microplate were modified with 20 μg/mL protein G (100 μL per well) in PBS (0.01M, pH 8.6) at 4 °C overnight. After washing the microplate three times with PBST (PBS, pH 7.4, 0.01 M, containing 0.05% Tween 20) and once with PBS, 100 μL of anti-FB_1 mAbs (1.30 μg/ml in PBS) were added into each well for 1 h incubation at 37℃. After washed three times with PBST and once with PBS, the plates were then blocked with blocking buffer (1 mg/mL of BSA in PBS) for 2 h at 37 °C. Subsequently, the plates were washed three times with PBST and once with PBS, a mixture containing 50μL of HRP-FB_1 (2.5 μg/mL in 0.01M PBS, pH 7.4) and 50μL of FB_1 standard solution (0 ~ 500 ng/mL in 0.01 M PBS containing 5% methanol, pH 7.5) was added into each well and incubated at 37℃ for 1 h, then the unbound content was removed by washing the microplate three times with PBST and twice with PBS. Then, 100 μL of TMB solution was added. After incubation for 15 min at 37℃, the reaction was terminated with 50 μL of 2 M H_2SO_4, and the absorbance of each well was measured at 450 nm using a microplate reader.

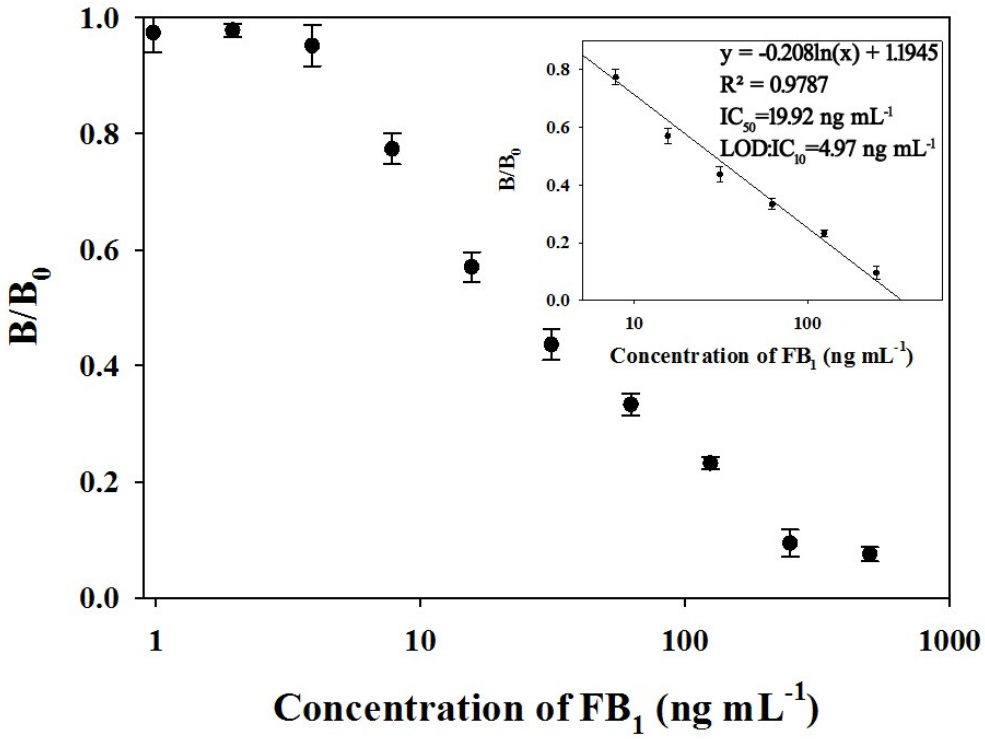

Figure S1. Standard curves for FB_1 detetction using HRP-based conventional ELISA. FB_1 standard solutions were obtained by diluting FB_1 stock solution using PBS. Vertical bars indicate the standard deviation (n=3). (B and B_0 represent the value of OD_{450nm} of the assay dealing with and without FB_1, respectively.)