Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Supplementary Figure 1

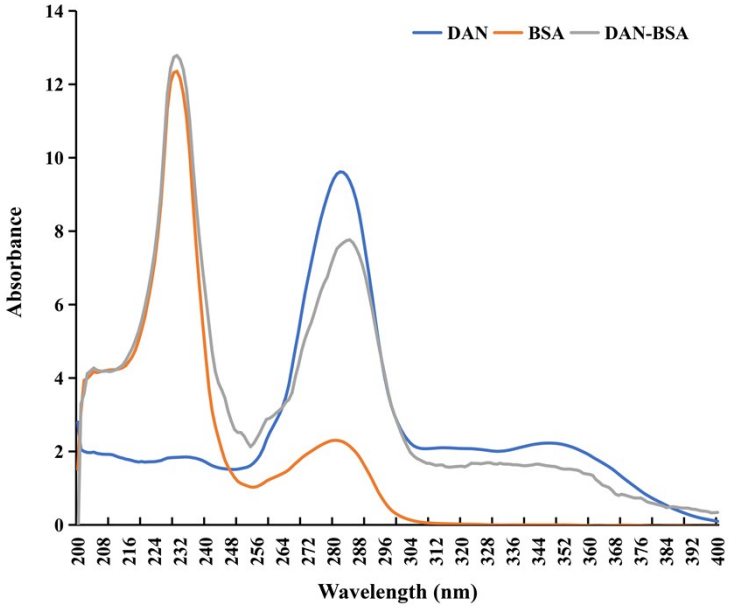

Fig. S1 Characterization of the couple of the DAN-BSA.

Supplementary Figure 2

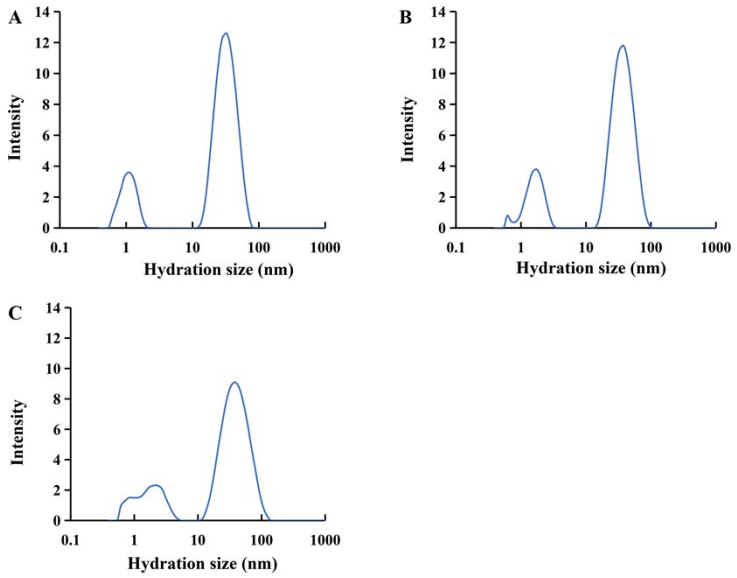

Fig. S2 Average hydrodynamic diameter of the prepared AgNPRs. (A) The prepared AgNPRs; (B-C) The AgNPRs etched induced by different concentrations of GOx: (B) 15 and (C) 30 ng/mL.

Supplementary Figure 3

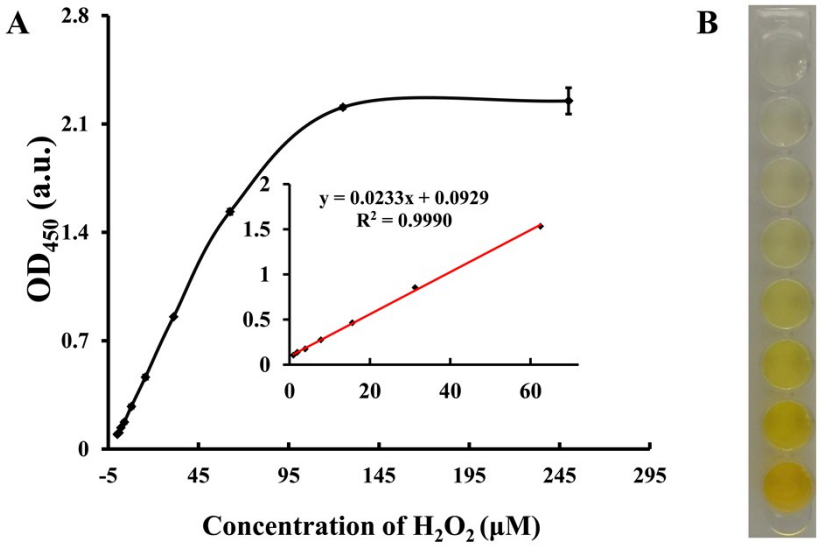

Fig. S3 (A) Calibration curve of TMB under H_2O_2 concentration ranging from 1.95 μM to 125 μM; (B) photographs of TMB under different H_2O_2 concentrations (0, 1.95,3.91, 7,81,15.63, 31.25,

62.5, 125 μM).

Supplementary Figure 4

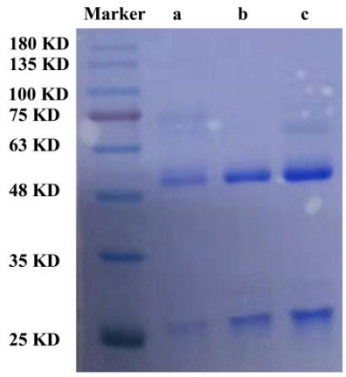

Fig. S4 Characterization of the mAb by SDS-PAGE. (a) 2.5 μg mAb, (b) 5 μg mAb, (c) 10 μg mAb.

Supplementary Figure 5

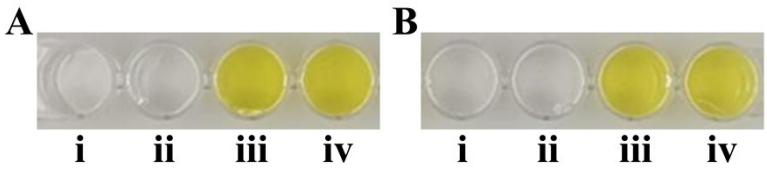

Fig. S5 Characterization of Biotin-mAb and Biotin-GOx. (A) The coupling result characterization of Biotin-mAb. i) ii) were added with 0.01 M PBS as the controls, iii) iv) were added with coupled Biotin-mAb; (B) The coupling result characterization of Biotin-GOx. i) ii) were added with 0.01 M PBS as the controls, iii) iv) were added with coupled Biotin-GOx.

Supplementary Figure 6

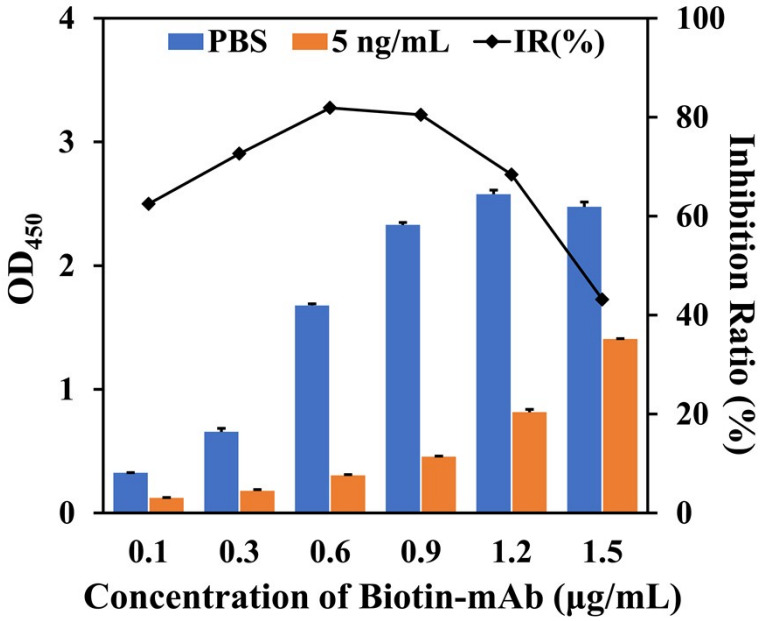

Fig. S6 The concentration optimization of Biotin-mAb in ELISA based on TMB as signal transducer. The error bars represent the standard deviation of three measurements.

Supplementary Figure 7

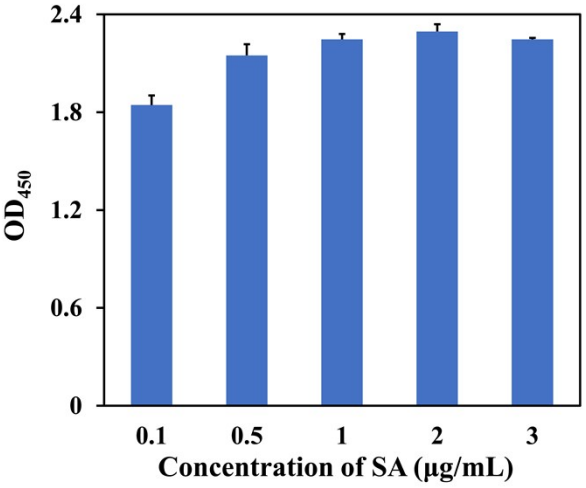

Fig. S7 The concentration optimization of SA in ELISA based on TMB as signal transducer. The

error bars represent the standard deviation of three measurements.

Supplementary Figure 8

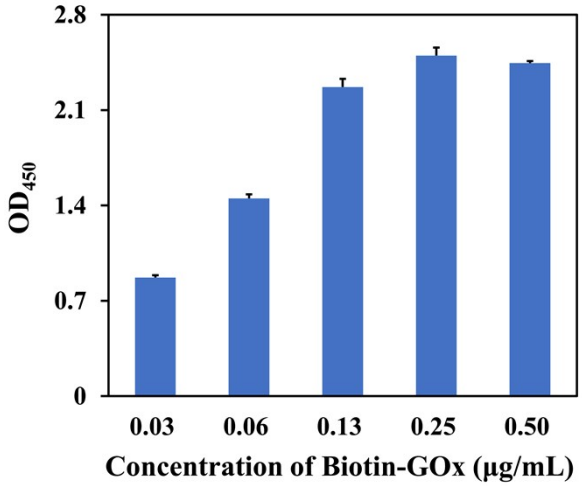

Fig. S8 The concentration optimization of GOx-Biotin in ELISA based on TMB as signal transducer. The error bars represent the standard deviation of three measurements.

Supplementary Figure 9

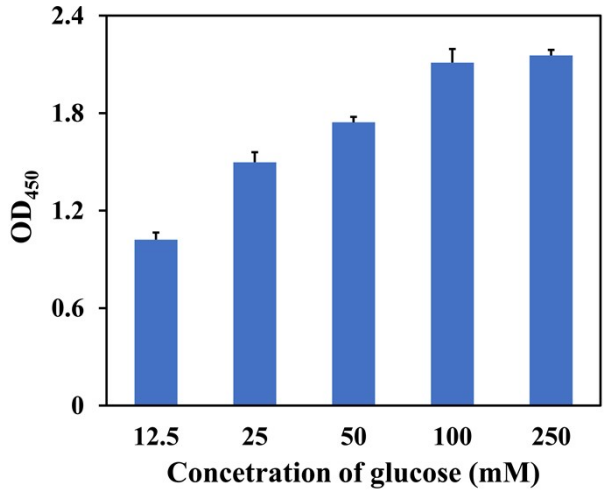

Fig. S9 The concentration optimization of glucose in ELISA based on TMB as signal transducer. The error bars represent the standard deviation of three measurements.

LC-MS/MS analysis

The reliability of the proposed AgNPR etching pELISA method was confirmed using LC-MS/MS. The detailed operating procedures were performed according to GB/T 22985-2008. 2 g milk sample was weighed to the nearest 0.01 g and placed in a 50 mL plastic centrifuge tube. The standard of DAN was added to the 50 mL plastic centrifuge tube. Ten milliliters of acetonitrile were added, and the mixture was shaken for 1 min in a vortex shaker. After centrifugation at 5000 rpm for 5 min, the supernatant was filtered into a chicken heart bottle. Five milliliters of a phosphate buffer solution and 10 mL of acetonitrile were added to the residue, the steps described above were repeated, and the supernatant was combined and rotary-evaporated at 50 ℃ until all the acetonitrile was distilled off. Then, 5 mL of a

phosphate buffer solution was added and mixed with the precipitate. The treated solution was transferred to a reservoir, and the chicken heart bottle was washed with 5 mL of phosphate buffer. The wash solution was added to the reservoir and passed through the HLB solid phase extraction cartridge at a flow rate of approximately 1 mL/min. After the sample solution was completely discharged, it was sequentially rinsed with 4 mL of water and 4 mL of 25% an aqueous methanol solution, drained, and eluted with 4 mL of 5% ammonia in methanol in a 10 mL graduated centrifuge tube. The eluate was incubated at 50 ℃. When the eluent was reduced to approximately 0.2 mL under a nitrogen flow, the concentration process was terminated. The volume of eluent was adjusted to 1 mL with a methanol-formic acid solution. After centrifugation at 5000 r/min for 5 minutes, the supernatant was passed through a 0.2 μm filter for the LC-MS/MS analysis. Chromatographic separation was achieved with an Agilent Zorbax Eclipse XDBC18 column using solvent A (ammonium acetate, 10 mM) and solvent B (methanol) as the mobile phase. Initial gradient conditions were set to 80% solvent A, reduced with a linear gradient to 40% from 0 min to 5 min, then further decreased to 5% from 5 min to 9 min. At 9 min, the gradient was programmed to the initial conditions to re-equilibrate the column for 2 min. The flow rate was 0.2 mL/min, and the injection volume was 15 μL in the full injection mode. Ionization was achieved using electrospray ionization in positive ion mode. High-purity argon gas was used as the collision gas. The monitored ion pairs for DAN were m/z 358.14/340.00 (quantitative ions) and 358.14/313.80 (qualitative ions).

Table S1

Comparison of recovery and precision between the proposed pELISA and LC–MS/MS for quantitative DAN determination.

DAN added (ng/mL)	Proposed pELISA (n = 3)			LC–MS/MS (n = 3)		
	DAN recovered (ng/mL)	Recovery (%)	CV (%)	DAN recovered (ng/mL)	Recovery (%)	CV (%)
30	36.38	121.26	0.6	26.22	87.4	5.96
60	62.07	103.45	0.99	43.32	72.2	8.34
90	102.6	114	1.21	73.44	81.6	8.8