Supporting Information

CdSe/ZnS quantum dot-encoded maleic anhydride-grafted PLA microspheres prepared through membrane emulsification for multiplexed immunoassays of tumor markers

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Reagents and chemicals

Cadmium oxide (CdO, 99.5%), zinc acetate (99.9%), selenium powder (Se, 99.9%), sulfur powder (S, 99.99%), oleic acid (OA, 90%), Thimerosal, 1-octadecene (ODE, 90%), trioctylphosphine (TOP, 97%) ,Tellurium (Te, 99.9%), sodium borohydride ($NaBH_4$, ≥96%), cadmium chloride ($CdCl_2$•$2.5H_2O$, 99.0%), sodium hydroxide (NaOH, ≥96%), sodium dihydrogen phosphate dihydrate (NaH_2PO_4•$2H_2O$, ≥99%) and disodium hydrogen phosphate dodecahydrate (Na_2HPO_4•$12H_2O$, ≥99%) were provided from Sinopharm Chemical Reagent Co., Ltd. Phosphate buffer saline (PBS, 0.1 M, pH =7.4), phosphate buffer saline tween-20 (PBST, 0.05% tween-20 in the PBS) were self-prepared. Double distilled water was used throughout the experiment. All reagents were purified without further purification.

Recipes of buffers for immunoassays

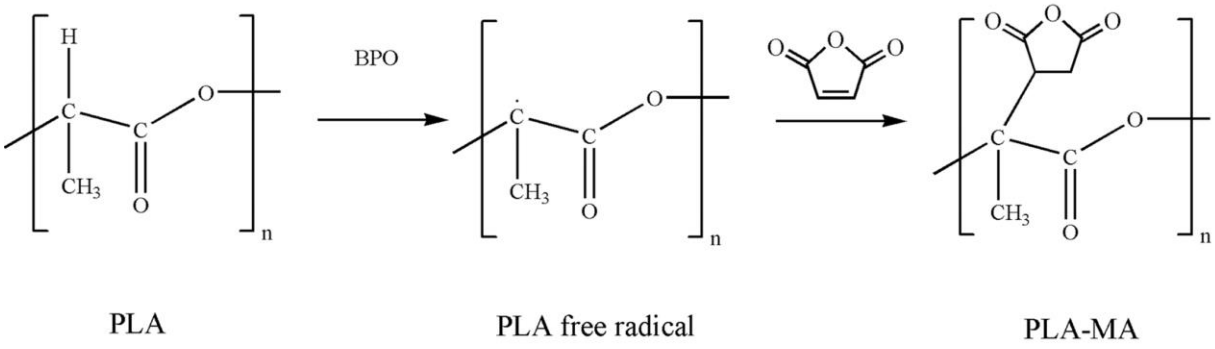
Table S1. Buffers formulations for immunoassays

Name of buffer	Component	PH	Storage
PBS	0.7260g Na_2HPO_4•$12H_2O$ 1.6146g NaCl 0.0480g KH_2PO_4 0.0403g KCl 200mL H_2O	7.4	Filter Sterilize and store at 4 ˚C for all Buffers
Washing Buffer	20mL PBS 10μL Tween-20	7.4	
Activation Buffer	0.6000g NaH_2PO_4 0.4mL 0.2g/mL NaOH 50mL H_2O	6.2	
Storage Buffer	20mL PBS 4μL Tween-20 0.0200g BSA 0.0040g Thimerosal	7.4	
Assay Buffer	20mL PBS 0.0200g BSA	7.4	

0.0040g Thimerosal

Preparation of polylactic acid-maleic anhydride (PLA-MA)

PLA-MA was synthesized by referring to the previous literature with the more stable. Firstly, 5g PLA (MW=110000), 0.5g MA, 0.01g BPO were all mixed in in 50mL dichloromethane, and the solution was kept in a vacuum drying oven at room temperature for 24 h, so that the reaction mixture is fully integrated together. Secondly, the white solid mixture obtained by drying was reacted in an oven at 110 ℃ for 18 h. After the reaction was completed, light yellow solid mixture was obtained. Next, the product was collected, purified by chloroform lysate and ether precipitation several times, centrifuged at 10000 rpm for 3 minutes, and repeated 3 times. Finally, the solid mixture was vacuum dried at 40 ℃ for 10 h. The resulting product was designated PLA-MA and stored at room temperature for later use.



Scheme S1. Schematic diagram of the preparation of polylactic acid-maleic anhydride

Synthesis of CdSe/ZnS QDs

To synthesize green-emitting QDs (520 nm), 0.4 mmol CdO and 4.0 mmol $Zn(CH_3COOH)_2$ were placed in a 100 mL four-neck flask, and 9 mL OA was added to the flask. After adding 10 mL of ODE, the mixture was heated to 180 ℃ to react until clear. Then, the temperature was lowered to 150 ℃ and reacted for 20 minutes to remove water vapor. The mixture was heated to 300 ℃, 4mL of TOP solution containing 0.4 mmol Se and 4.0 mmol S was quickly injected into the reaction flask, and then the temperature was set to 280 ℃ for reaction. Take a small part every 30 s to observe under the UV lamp until it becomes obvious green and stop the reaction.

Dissolve and purify with chloroform and acetone several times, and finally store in chloroform for later use. For the synthesis of yellow light-emitting QDs (580 nm), except that the amounts of Se and S are changed to 0.3 mmol and 4.1 mmol, respectively, the rest of the process is the same as the synthesis of green light-emitting quantum dots.

Synthesis of water-soluble CdTe QDs

The water-phase CdTe QDs protected by MPA were synthesized by hydrothermal method. 67 μL MPA and 91.3 mg CdTe·2.5H_2O were mixed in 40 mL of water, and the pH of the solution was adjusted to 11.0 with 1 M NaOH solution with stirring. At this time, in a nitrogen atmosphere, NaHTe is obtained by the reduction reaction of Te powder and $NaBH_4$, and 1 mL of NaHTe solution (0.04 M) is transferred with a syringe, and the above-mentioned cadmium source solution is injected. Then the reaction mixture was heated to reflux (100 ℃) under normal pressure, and a condenser was attached. By controlling the heating time, CdTe crystal nuclei with different crystal nucleus diameters are obtained, and a small amount of reaction solution is continuously taken out and placed under ultraviolet light for observation. After the fluorescence emission of the desired particle size is obtained, the reaction is stopped, the reaction solution is separated with ethanol, and centrifuged at 10,000 rpm for 10 minutes, repeated 3 times. Naturally dry, keep at 4 ℃ and avoid light.

Immunodetection of tumor markers

In this paper, four tumor markers of CA125, CA199, CA724, CEA were selected for immunoassay experiments. The experimental program is shown in Fig 3. First, take a certain amount of PLA-MA microsphere suspension, add 20 microliters of solutions containing 70 mg/mL EDC and 70 mg/mL S-NHS respectively, and react at room temperature for 30 minutes in the dark to activate the microspheres. The activated microspheres were fully dispersed and suspended in PBS buffer, and appropriate amount of corresponding capture antibody was added to incubate at room

temperature and avoid light for 3 h to coat antibody X-Ab1 (X=CA125, CA199, CA724, CEA). Then, 1wt% BSA was added to the antibody-coated microspheres and reacted at room temperature for 30 minutes to block the unreacted active ester groups on the surface of the microspheres. Wash and centrifuge several times after each operation. Secondly, a certain amount of secondary antibody (X-Ab2), 10 μL EDC solution (10 mg/mL), 500 μL CdTe QDs (660 nm, 2 mg/mL) were mixed uniformly, and the reaction was shaken for 3h in the dark. Then, 1wt% BSA was added to the reaction with shaking at room temperature to block the unreacted active sites on the surface of the CdTe QDs, labeled X-Ab2-CdTe QDs. Then, 150 μL of buffer solutions containing different concentrations of tumor marker antigen (X-Ag) were separately mixed with PLA-MA microspheres with specific antibodies and incubated for 1 h at room temperature in the dark. Wash and centrifuge several times to remove unreacted antigen. Immediately, a certain amount of X-Ab2-CdTe QDs was added to the above system and reacted at room temperature and protected from light for 1 h to construct a sandwich immune structure, and then washed and centrifuged several times. Finally, the PLA-MA quantum dot microspheres with a sandwich immune structure on the surface were reconstituted into 200 μL suspension, and transferred to a flow cytometer for flow cytometry analysis.

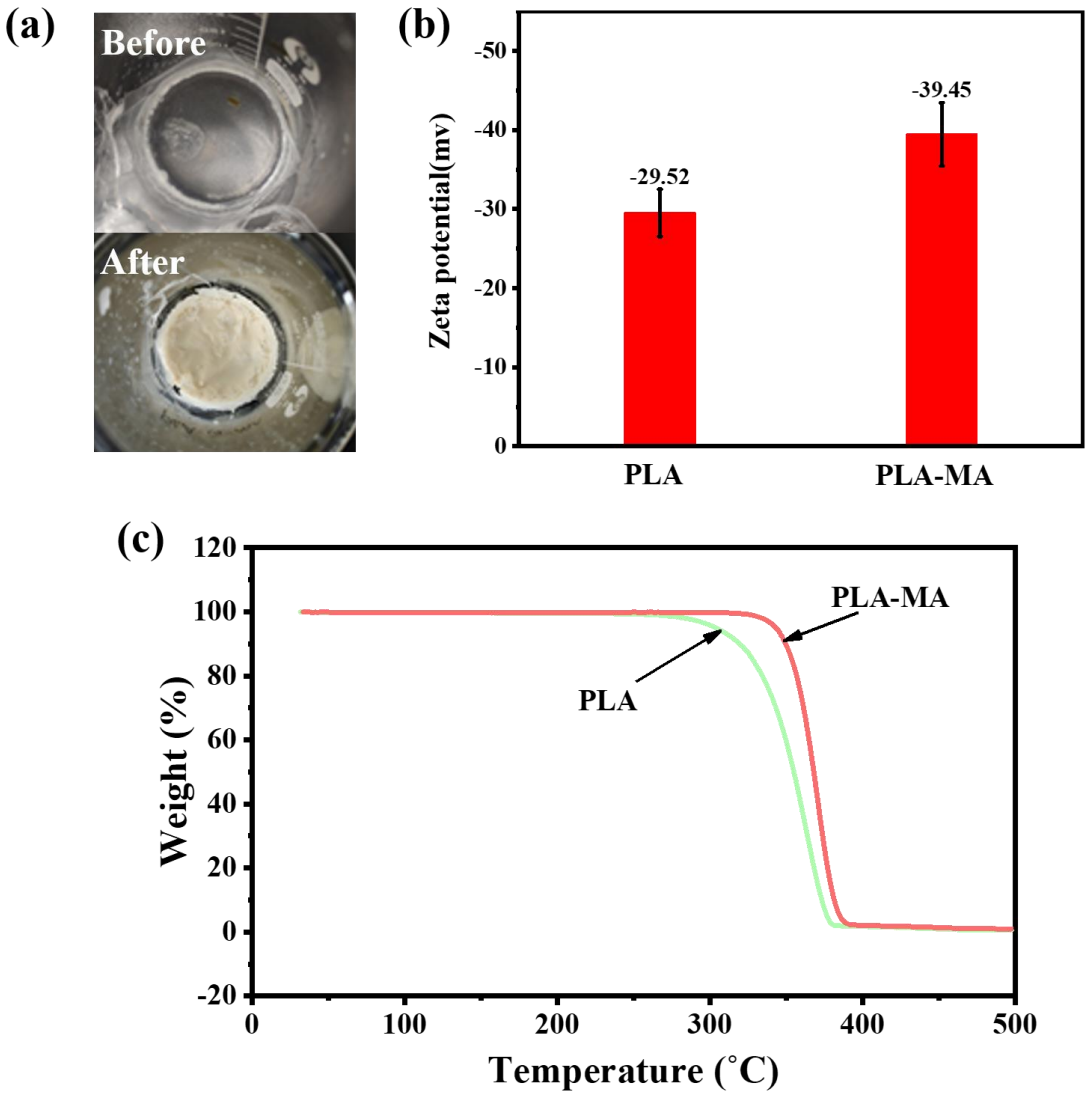


Fig. S1 (a) The morphology of PLA-MA before and after the reaction; (b) the zeta potential of PLA and PLA-MA; (c) the thermogravimetric analysis of PLA-MA.

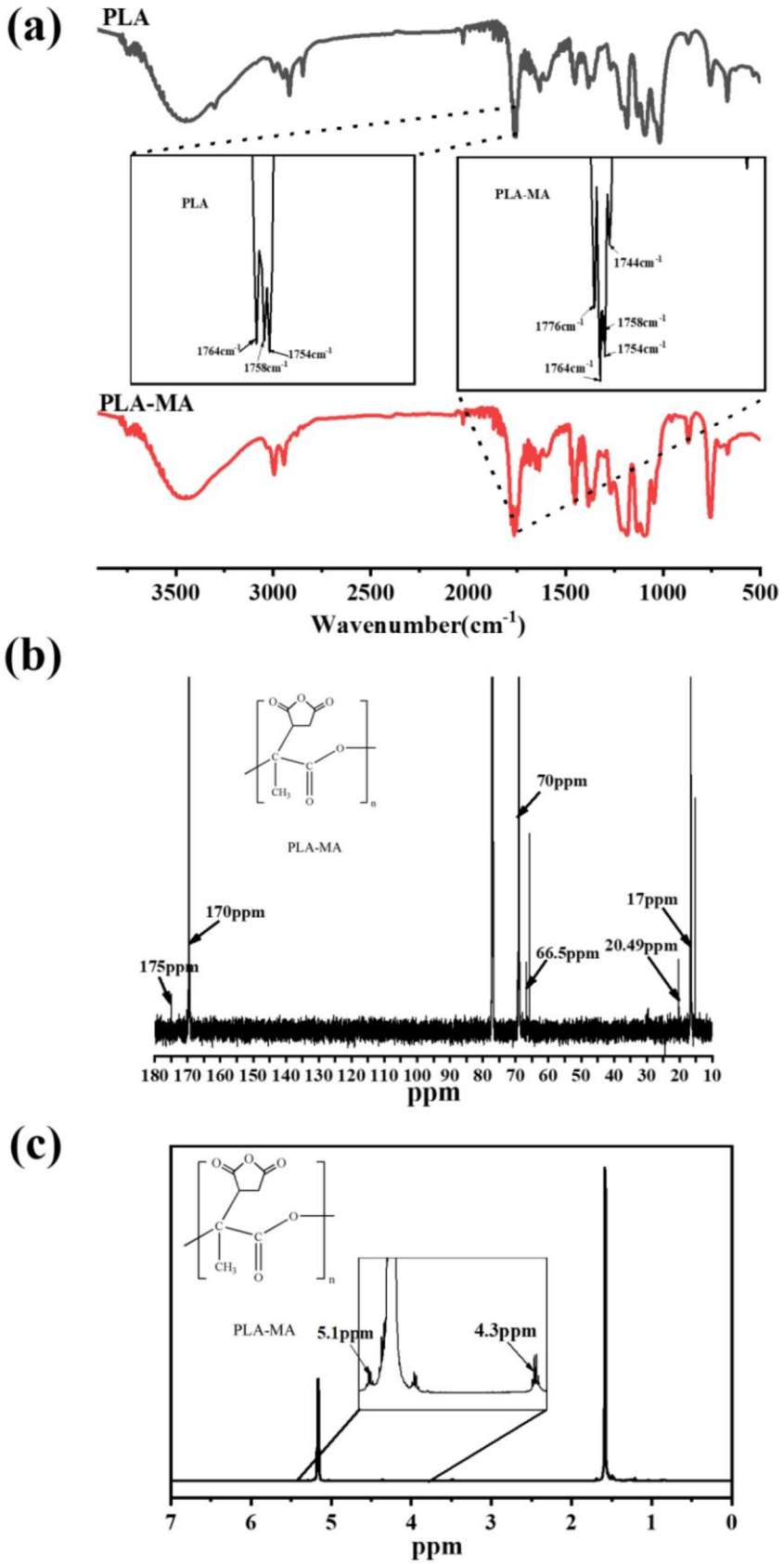


Fig. S2 (a) Fourier transform infrared spectra of PLA and PLA-MA; (b) ^{13}C-NMR spectrum of PLA-MA; (c) ^{1}H-NMR spectrum of PLA-MA

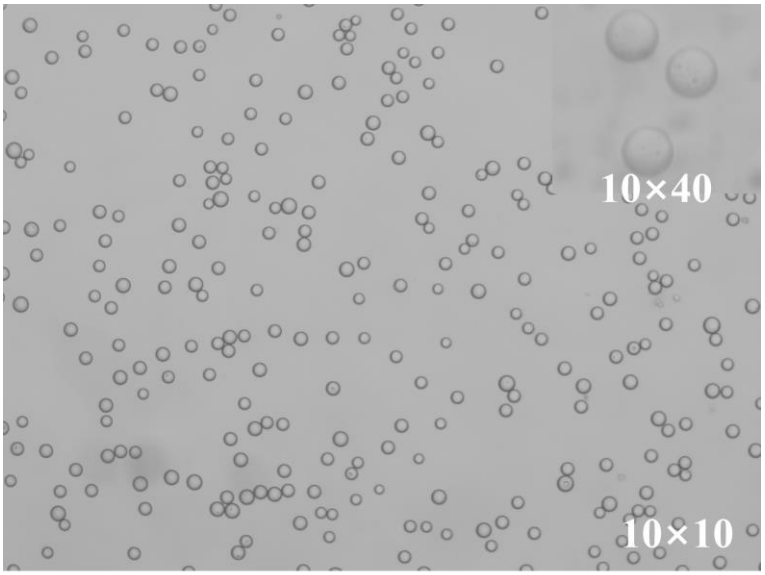


Fig. S3 Optical microscope image of PLA-MA microspheres

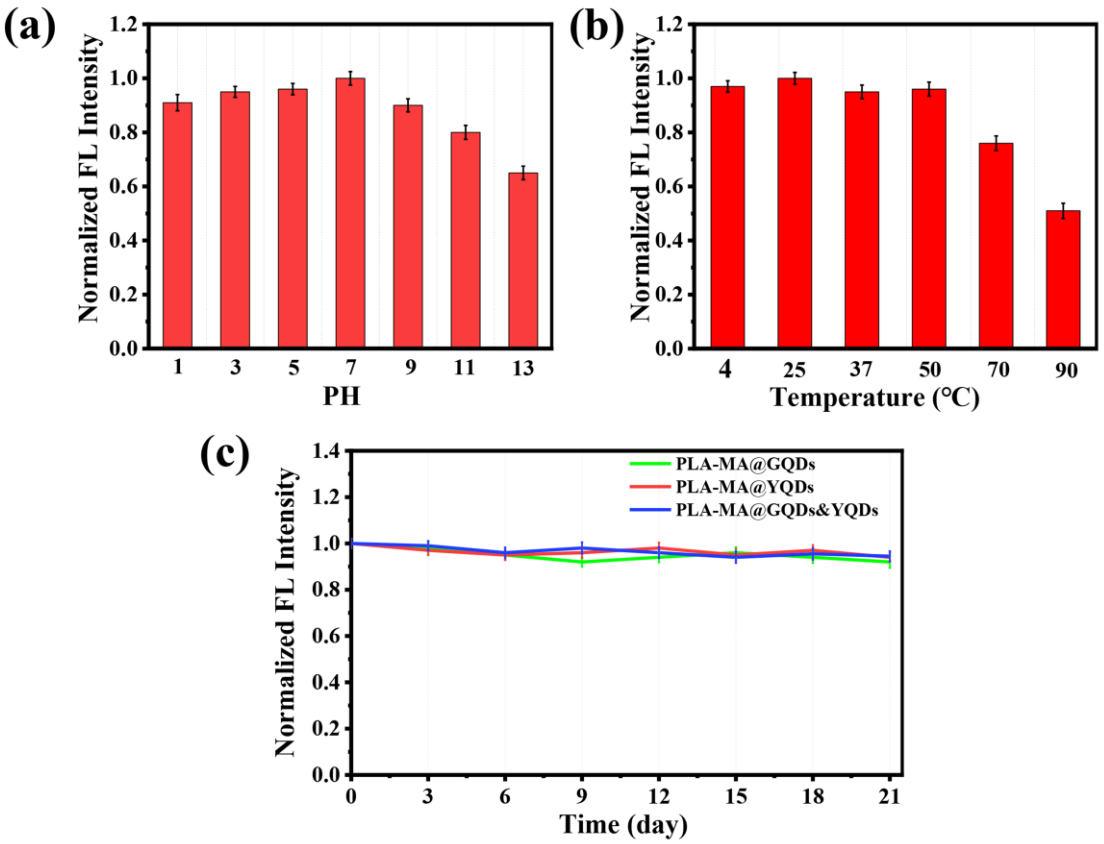


Fig. S4 Fluorescence stability of PLA-MA Fluorescent Microspheres. (a) Temperature dependent stability: PLA-MA fluorescent microspheres were suspended in PBS (pH=7.4) and stored at different temperatures including 4 ℃, 25 ℃, 37 ℃, 50 ℃, 70 ℃ and 90 ℃ for 24 h. (b) pH-dependent stability: PLA-MA Fluorescent Microspheres were suspended in PBS with different pH values (1, 3, 5, 7, 9, 11, and 13, respectively) and stored at room temperature for 24 h. (c) Time dependent stability: PLA-MA fluorescent microspheres were kept at room temperature for 21 days.

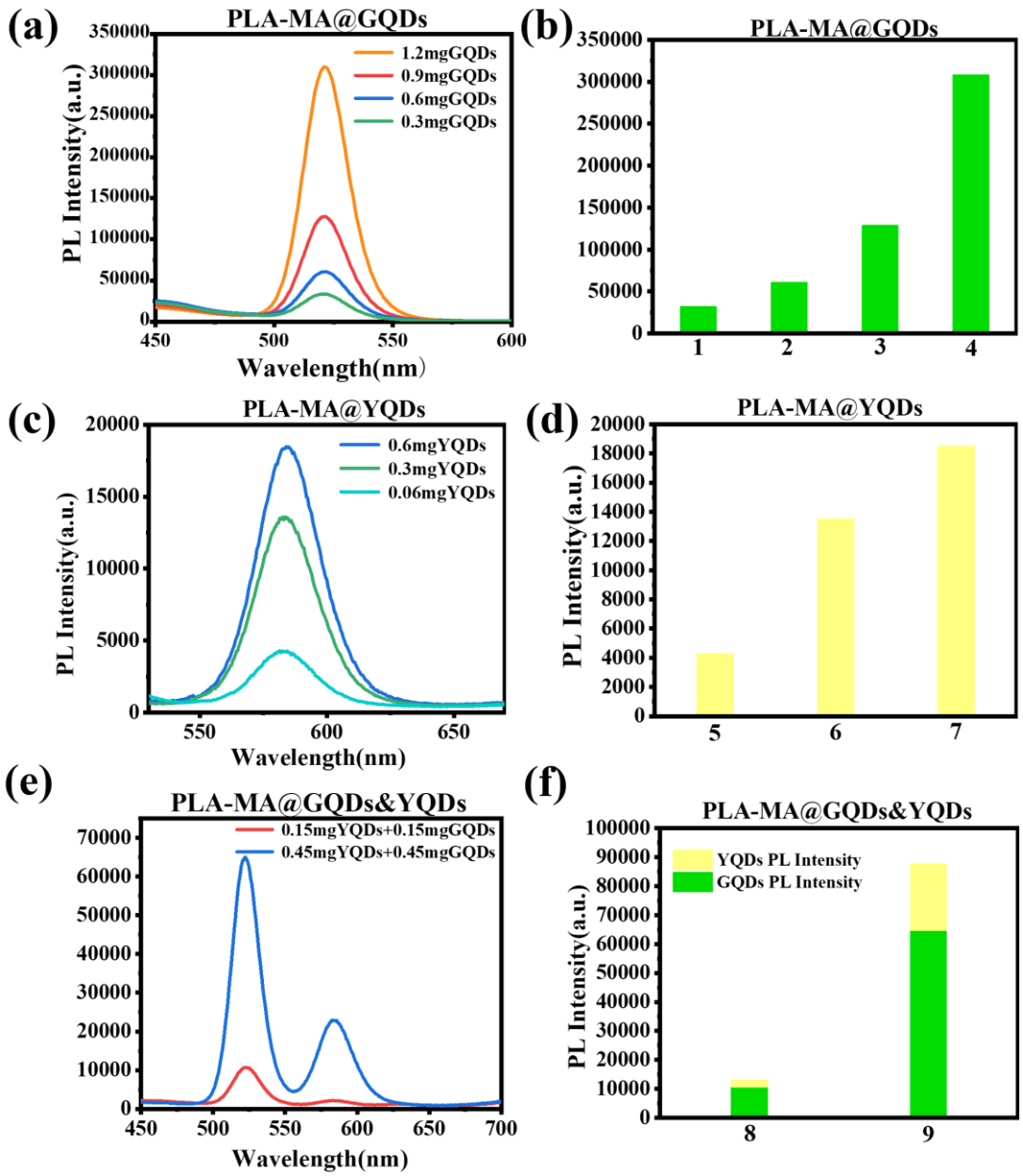


Fig. S5 Fluorescence spectra of PLA-MA fluorescent microspheres. (a) The fluorescence spectrum of PLA-MA@GQDs; (b) The maximum fluorescence intensity of PLA-MA@YQDs; (c) The fluorescence spectrum of PLA-MA@YQDs; (d) The maximum fluorescence intensity of PLA-MA@YQDs; (e) Fluorescence spectra of PLA-MA@GQDs&YQDs; (f) maximum fluorescence intensity of PLA-MA@GQDs&YQDs.

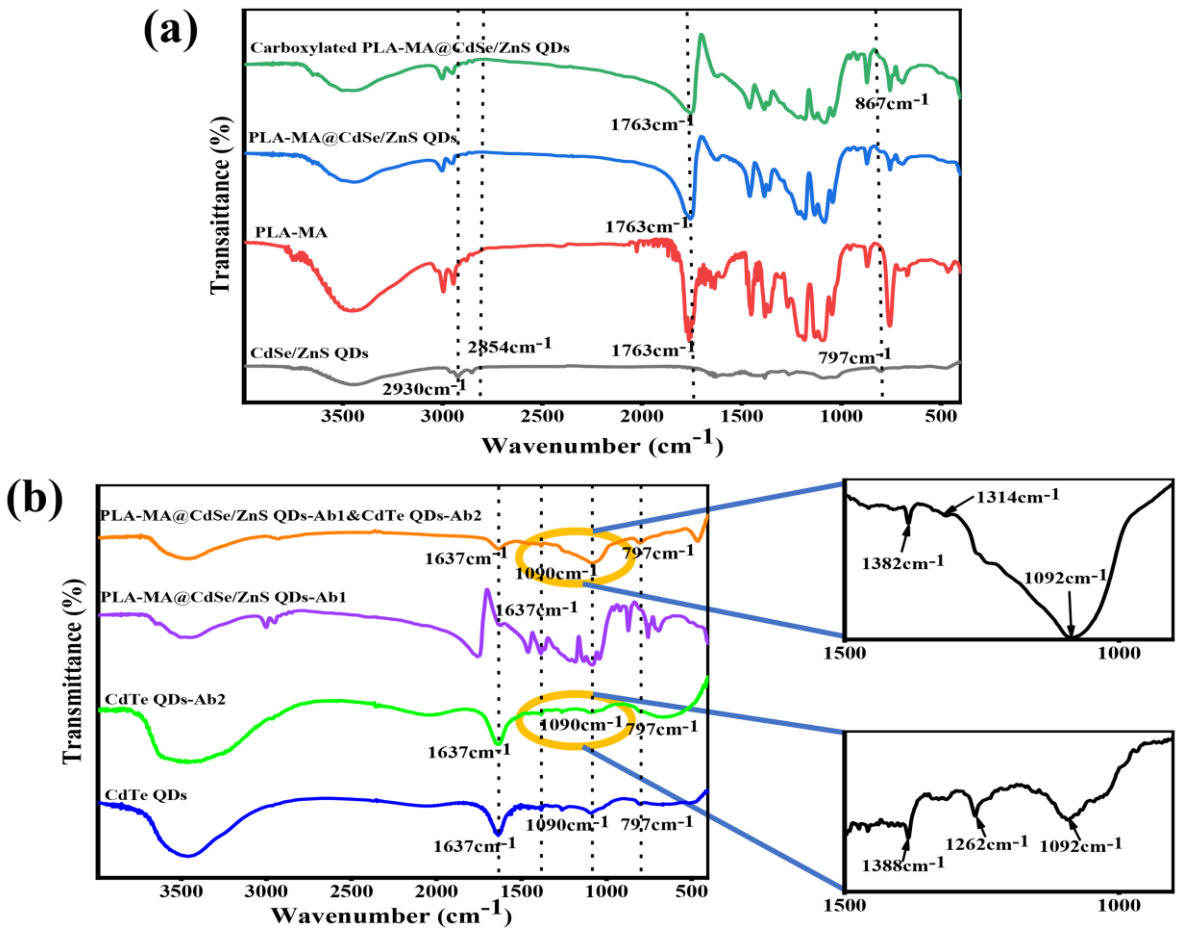


Fig. S6 FT-IR spectra of QDs in each experimental process: (a) FT-IR spectra of CdSe/ZnS QDs in various stages of tumor marker immunodetection experiments; (b) FT-IR spectra of CdTe QDs at various stages of the tumor marker immunodetection experiment.

Table S2. The relationship between the detection limit and the dosage of primary antibody in single factor detection of four tumor markers.

Coating	LOD			
	CA125	CA199	CA724	CEA
2.5 μg	2.2 KU/L	4.2 KU/L	2.13 KU/L	0.322 KU/L
5 μg	1.81 KU/L	0.923 KU/L	1.64 KU/L	0.138 KU/L
10 μg	1.62 KU/L	0.920 KU/L	1.06 KU/L	0.132 KU/L

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