Supplementary Information

Fluorescence immunoassay for targeted determination of trace *Listeria monocytogenes* based on immunomagnetic separation and CdZnTe quantum dots indication

Shan Liang, Li Ji, Yingying Zhong, Tiantian Wang, Huiyi Yang, Qing-Lan Li*, Xiangguang Li* and Suqing Zhao*

Department of Pharmaceutical Engineering, School of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, People's Republic of China

***Corresponding Author**

Dr. Qing-Lan Li

E-mail addresses: qlli19@gdut.edu.cn

Prof. Xiangguang Li

E-mail addresses: xgl@gdut.edu.cn

Prof. Suqing Zhao

E-mail addresses: sqzhao@gdut.edu.cn

E-mail addresses for other authors

2111906111@mail2.gdut.edu.cn (Shan Liang), grana233@163.com (Ji Li), gdutchemzyy@163.com (Yingying Zhong), ttwang2314163620@163.com (Tiantian Wang), iyiuhgnay@163.com (Huiyi Yang)

Evaluation of the anti-*L. monocytogenes* pAbs

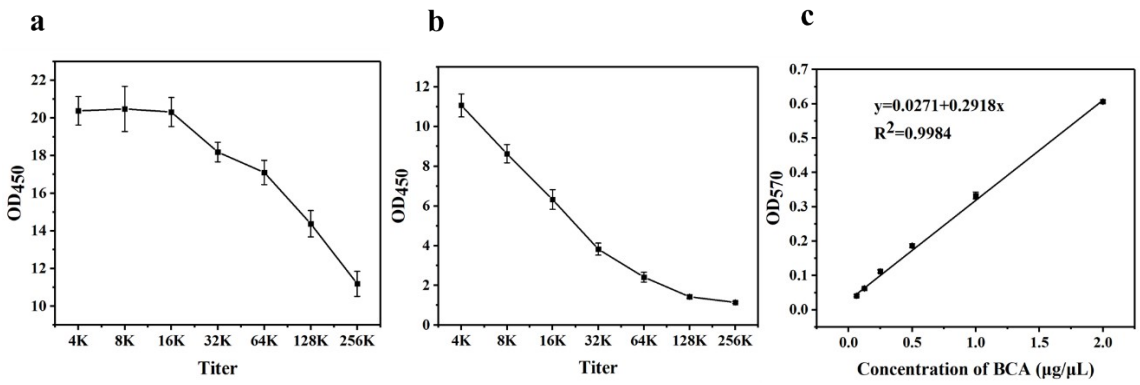

Fig. S1. The titer of the anti-*L. monocytogenes* (a) rabbit antibody and (b) guinea pig antibody after the fourth injection (n=3) by the method of iELISA method. (c) The standard protein curve of BCA. The concentration of anti-*L. monocytogenes* rabbit and guinea pig antibody is 10 μg/μL, respectively.

Characterization of Fe_3O_4 NPs

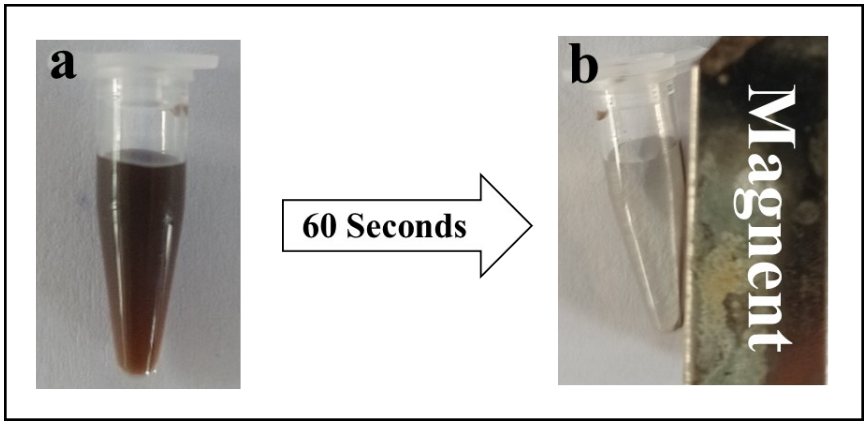

Fig. S2. Photographs of Fe_3O_4 nanoparticles in water taken before (a) and after (b) magnetic separation.

Preparation of CdZnTe QDs and CdZnTe QDs/pAb2

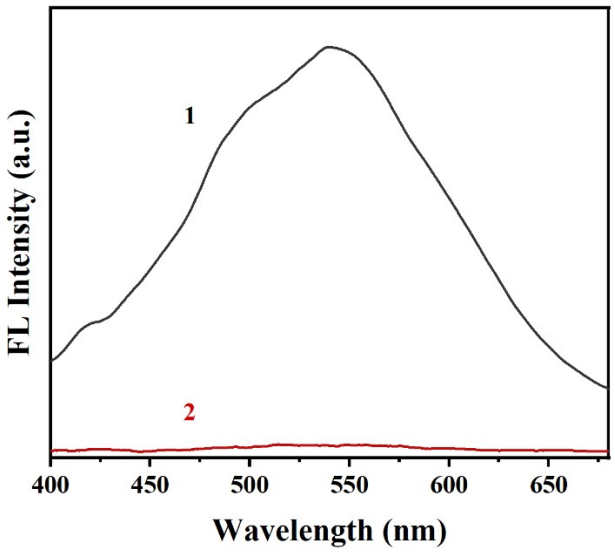

Fig. S3. Fluorescence spectra of CdZnTe QDs in deionized water (Line 1) and the supernatant of CdZnTe QDs after centrifugation (Line 2).

Optimization of the concentration ratio of Fe_3O_4 NPs to pAb1 and CdZnTe QDs to pAb2

To optimize the concentration ratio of Fe_3O_4 NPs to pAb1, Fe_3O_4 NPs (2.9 mg mL^{-1}) with different volumes (50, 100, 200, 300, 400, 500, 600 and 700 μL) was mixed with 10 μL pAb1 (10.0 mg mL^{-1}) and the mixture was stirred for 24 hours at 4 ℃. After washing three times with PBS (10 mM, pH 7.4), Fe_3O_4 NPs/pAb1 was collected with centrifuging at 10000 rpm/min for 15 min. Then, the Fe_3O_4 NPs/pAb1 was been added to HRP-goat anti-guinea pig secondary antibodies diluted 1: 5000 with PBS and cultivated at 37 °C for 1 hour. Finally, the mixture was washed three times under the same conditions as before and the optical density (OD) values at

a wavelength of 450 nm were measured by a Thermo Scientific microplate reader.

With the purpose to obtain the optimal concentration ratio of CdZnTe QDs to pAb2, 120 mg of EDC·HCl and 18 mg of NHS were mixed with CdZnTe QDs (5 mL, 6.9 mg mL^{-1}) with vigorous shaking for 15 minutes at room temperature. After that, 10 μL of pAb2 (10.0 mg mL^{-1}) was added into the above mixture with different volumes (50, 100, 200, 300, 400, 500 and 600 μL) which was stirred for another 24 h at 4 °C. Finally, the obtained CdZnTe QDs/pAb2 were washed by centrifugation (10000 rpm, 5 minutes) and dispersed in HRP-goat anti-rabbit secondary antibodies diluted 1: 5000 with PBS and cultivated at 37 °C for 1 hour. The optical density (OD) values at a wavelength of 450 nm were measured by a Thermo Scientific microplate reader.

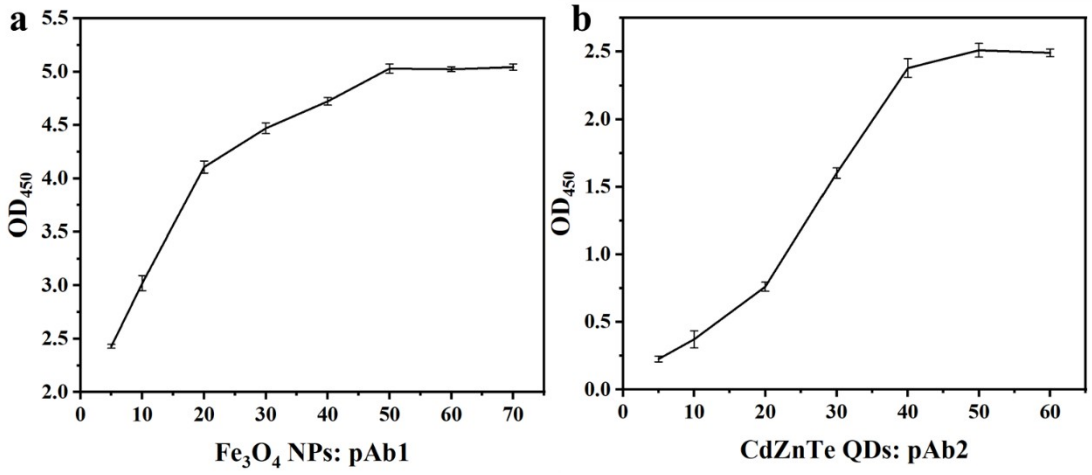

Fig. S4. The optimization of volume ratio of Fe_3O_4 NPs to pAb1 (a) and CdZnTe QDs to pAb2 (b).

As shown in Fig. S4, the OD value of the antigen-antibody complex

gradually increased with the volume ratio of Fe_3O_4 NPs: pAb1 and CdZnTe QDs: pAb2, and plateaued starting at 50, respectively. The results indicated that the optimal volume ratio of Fe_3O_4 NPs to pAb1 and CdZnTe QDs to pAb2 was 50. When the binding rate peaked, the binding site was filled up and no more coupling took place.

The stability of the Fe_3O_4 NPs/ pAb1 and QDs/pAb2

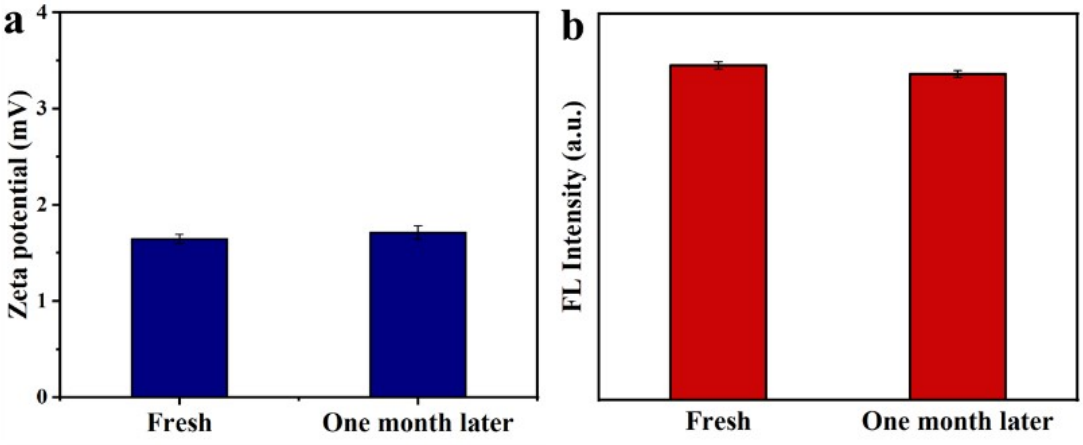

Fig. S5. The zeta potential (mV) of the Fe_3O_4 NPs/pAb1 sample (a) and FL intensity of the CdZnTe QDs/pAb2 sample (b), freshly prepared and stored for one month, respectively.

Characterization of Fe_3O_4 and Fe_3O_4 NPs/pAb1

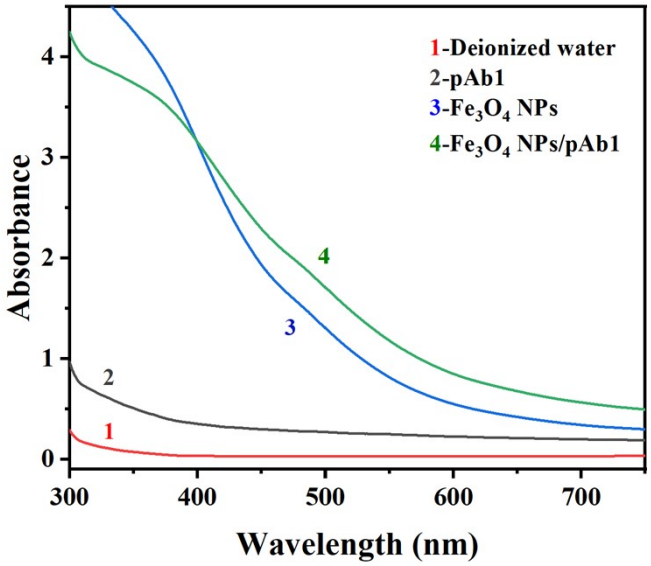

Fig. S6. UV-vis absorption spectra of the deionized water (line 1), the pAb1 (10.0 mg mL^{-1}, line 2) and the Fe_3O_4 NPs (2.725 mg mL^{-1}, line 3) and the Fe_3O_4 NPs/pAb1 (line 4).

As shown in Fig. S6, the UV-vis absorption spectra of the free pAb1, Fe_3O_4 NPs and Fe_3O_4 NPs/pAb1 exhibited no characteristic UV-vis peak.

Characterization of CdZnTe QDs and CdZnTe QDs/pAb2

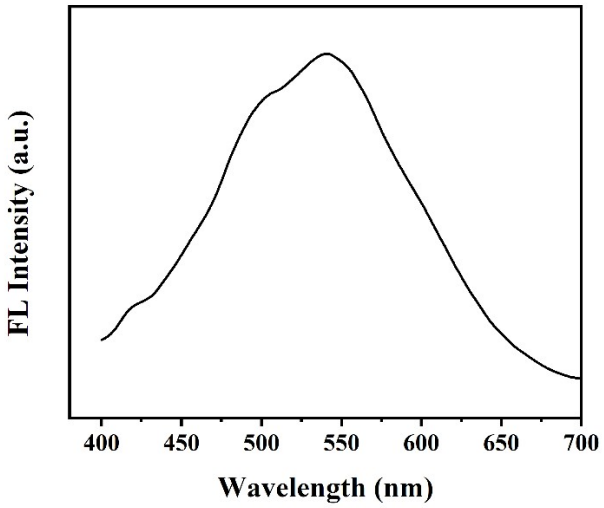

Fig. S7. Fluorescence spectrum of CdZnTe QDs/pAb2

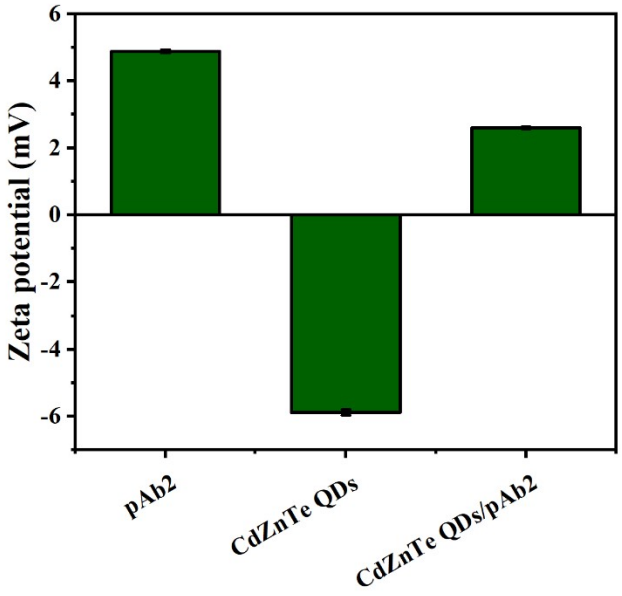

Fig. S8. Zeta potentials of the pAb2, the CdZnTe QDs and the CdZnTe QDs/ pAb2.