Supporting Information

General Strategy to Fabricate Electrochemiluminescence Sandwich-Type Nanoimmunosensors Using CdTe@ZnS Quantum Dots as Luminescent Labels and Fe_3O_4@SiO_2 Nanoparticles as Magnetic Separable Scaffolds

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Contents

Synthesis of water-soluble CdTe cores and CdTe@ZnS QDs. CdTe quantum dots capped with MPA were synthesized by hydrothermal method according to the literature with slight modifications[1]. Briefly, 67 μL of MPA and 91.3 mg of $CdCl_2 \cdot 2.5H_2O$ were dissolved in 40 mL of H_2O. After adjusted the pH to 11.0 using 1 M NaOH solution, 1 mL of 0.04 M NaHTe solution (produced by reaction of oxygen-free $NaBH_4$ solution with Te power under N_2 atmosphere) was injected into above solution. Then the solution was stirred vigorously and refluxed at 100℃. By controlling the heating time, CdTe cores with different sizes were attained. The resulting products were precipitated by ethanol with centrifugation at 10000 rpm for 10 min, and then purified three times with ethanol. The purified CdTe QDs were stored in the dark at 4℃ for late use.

CdTe@ZnS core-shell QDs were prepared as reported before with slight modifications[2-4]. The purified CdTe QDs with different sizes were used as cores to grow ZnS layers directly. In a typical experiment, 40 mg of as-prepared CdTe was added to the 50 mL solution (pH=10) containing 1 mM $ZnCl_2$ and 4 mmol MPA. MPA was used as both the capping agent and sulphur source. The solution was heated to 100℃ under open-air conditions and refluxed 20 min. Then core-shell QDs were precipitated with ethanol, centrifuged and dried under vacuum. The purified CdTe@ZnS QDs were stored in the dark at 4℃ for late use.

Optimization of experimental conditions. A series of optimization experiments were conducted based on sandwiched immunoassay toward 50 ng/mL CEA. As illustrated in Figure S7A, the optimal pH was 7.4, which may be attributed to the break of Ab and Ag linkage and weak bioactivity in acid or alkaline solution[5]. Thus, PBS (pH 7.4) was selected as the buffer solution for further studies.

The amount of $QD_{650\ nm}$ added during labeling process also influences the ECL intensity of the immunosensor. As shown in Figure S7B, ECL intensity increased and reached to a maximum at 500 μL, meaning a saturated binding between QDs and Ab_2. Then, ECL intensity exhibited a downtrend when the addition amount of $QD_{650\ nm}$ exceeded 500 μL, which is probably because too much QDs would cause aggregate formation, and then induce less available QDs-Ab_2. Hence, 500μL was chosen as an appropriate amount for labeling process.

The effect of the amount of sandwich nano-composite modified on electrode was also investigated and shown in Figure S7C. When the modified amount was less than 7.2 μL, ECL intensity increased with modified amount increased. Further increment of modified amount caused a gradual decline, owing to the fact that more magnetic immunosensors are prone to gather together and thus block the luminescence. Therefore, 7.2 μL was chosen as the optimal modified amount of sandwich nano-composite on electrode.

The incubation time was found to greatly affect the analytical performance of the proposed immunoassay. Figure S7D show that the ECL signal increased with the increase of incubation time and reached a plateau at 1 h both in the incubation with Ag and QD_{650nm}-Ab_2. This means the binding of the antigen and antibody was saturated at 1h, and less incubation time would affect the extent of immunoreaction resulting low ECL response. So, 1h was selected as the optimum time in two incubation steps.

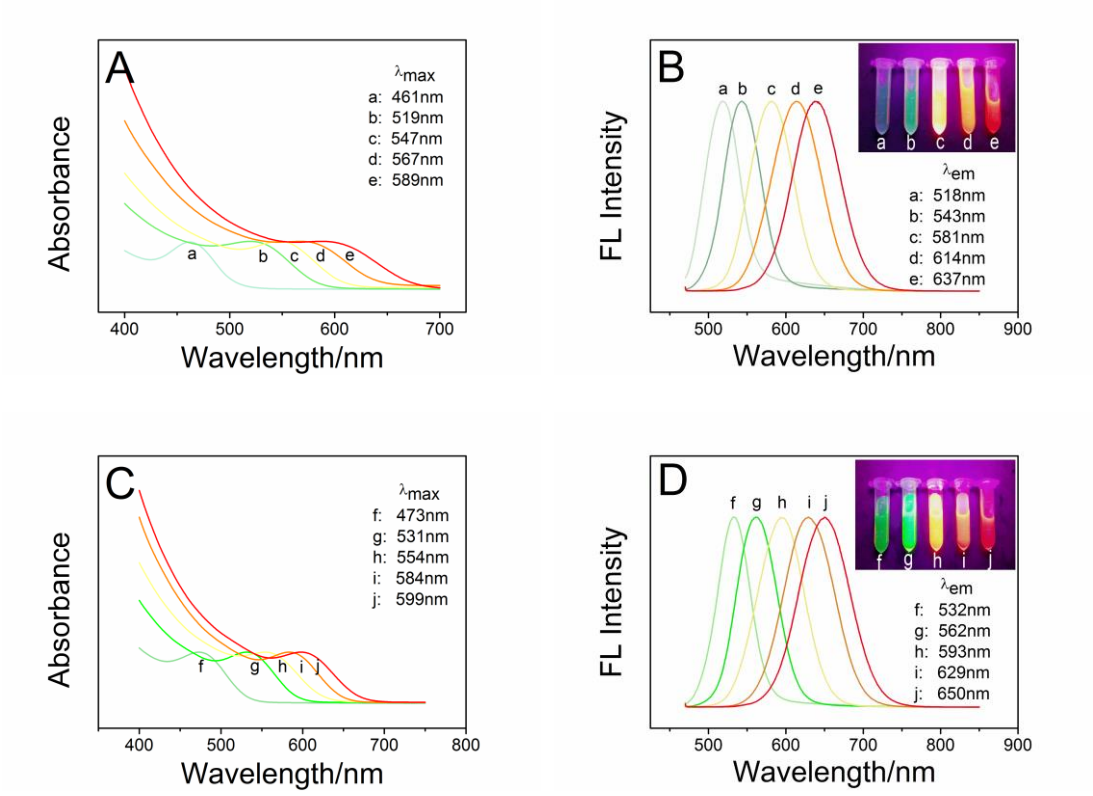

Figure S1. Normalized UV-vis absorption of CdTe cores with different sizes (A) and fluorescence emission spectra at the excitation wavelength of 475 nm (B): (a) CdTe-1, (b) CdTe-2, (c) CdTe-3, (d) CdTe-4, and (e) CdTe-5; Normalized UV-vis absorption (C) and fluorescence emission spectra (D) of CdTe@ZnS QDs with different core sizes: (f) CdTe@ZnS-1, (g) CdTe@ZnS-2, (h) CdTe@ZnS-3, (i) CdTe@ZnS-4 and (j) CdTe@ZnS-5. Inset (B) and (D): photographs of aqueous solutions of CdTe cores and CdTe@ZnS QDs under the radiation of a 365 nm UV light, respectively.

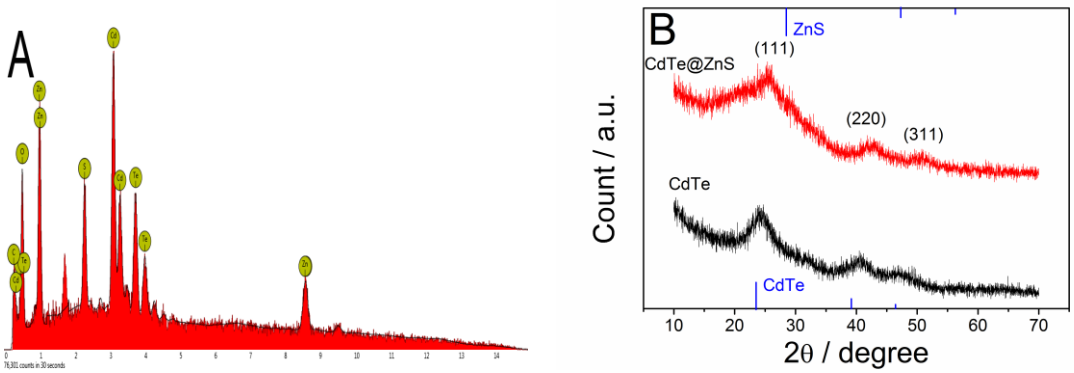

Figure S2. (A) EDS of CdTe@ZnS-5, and (B) XRD of CdTe-5 and CdTe@ZnS-5.

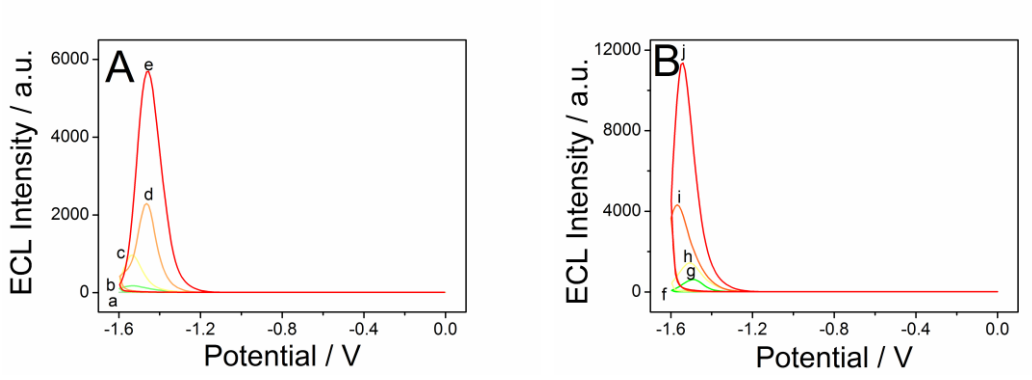

Figure S3. ECL intensity vs. potential curves of CdTe QDs with different sizes on GCE (A): (a) CdTe-1, (b) CdTe-2, (c) CdTe-3, (d) CdTe-4, and (e) CdTe-5; and corresponding CdTe@ZnS/GCE (B): (f) CdTe@ZnS-1, (g) CdTe@ZnS-2, (h) CdTe@ZnS-3, (i) CdTe@ZnS-4 and (j) CdTe@ZnS-5.

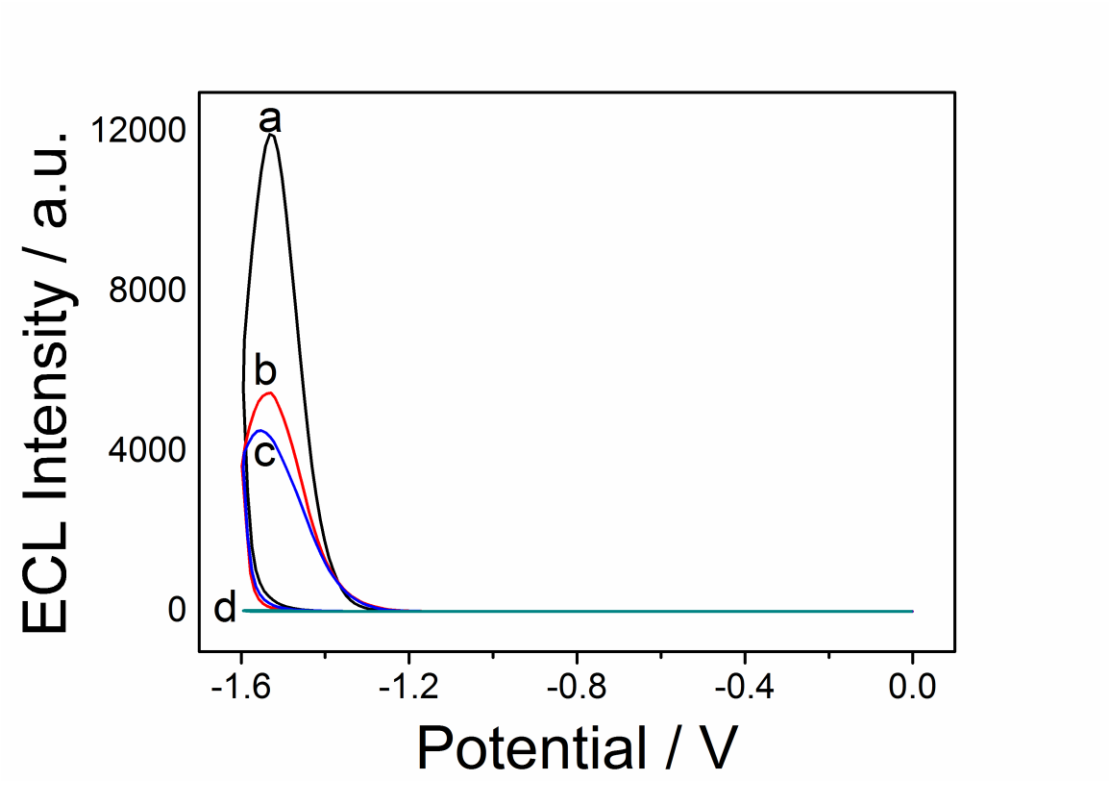

Figure S4. ECL intensity vs. potential curves of (a) CdTe@ZnS-5, (b) CdTe-5, (c) CdTe-5+ZnS and (d) ZnS.

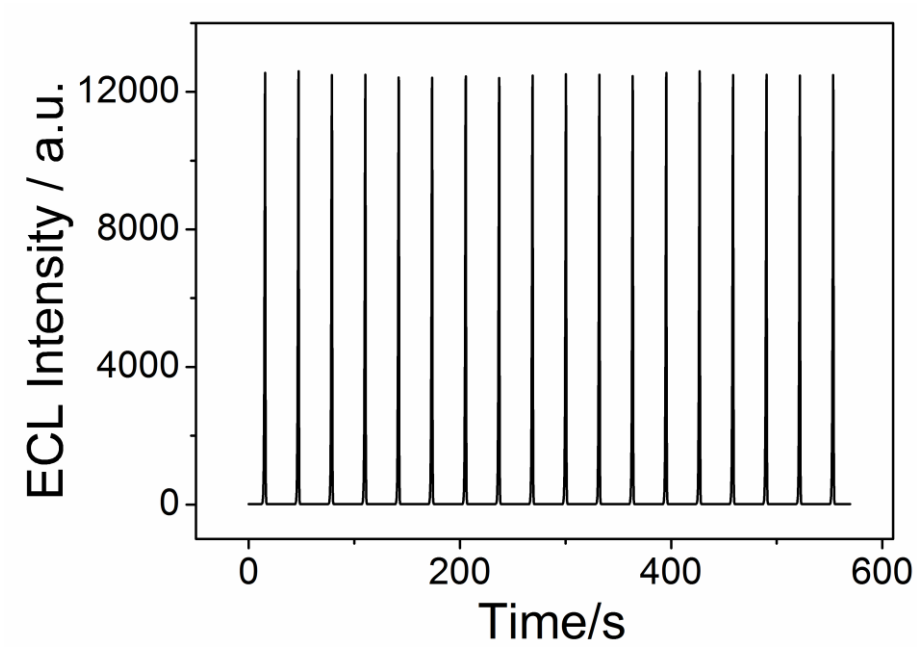

Figure S5. Stability of the ECL emission of CdTe@ZnS-5/GCE under continuous cyclic potential scanning for 18 cycles. The voltage of PMT was set at 400V.

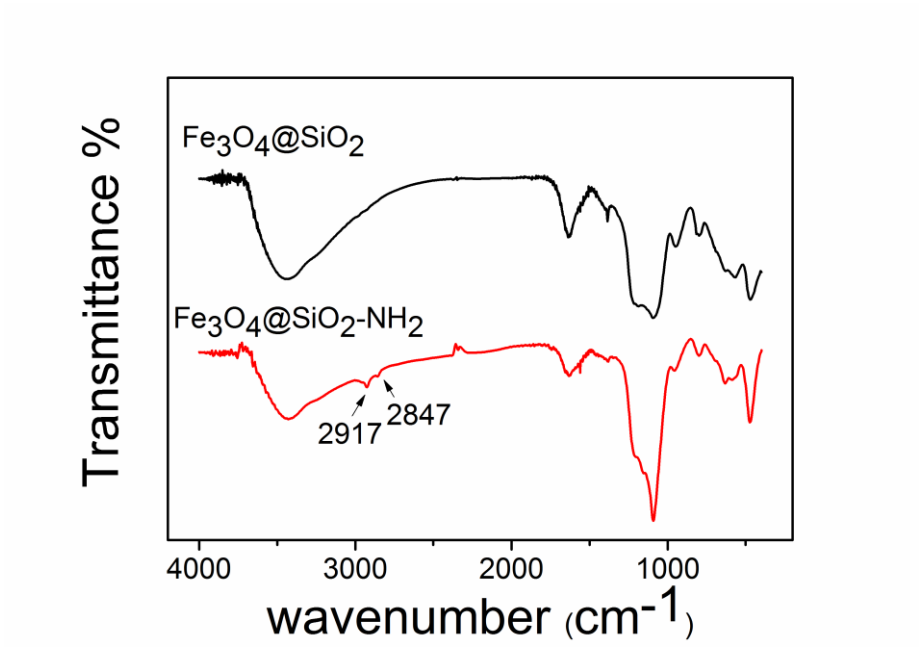

Figure S6. FT-IR spectra of the Fe_3O_4@SiO_2 nanoparticles before and after surface modification with APTES.

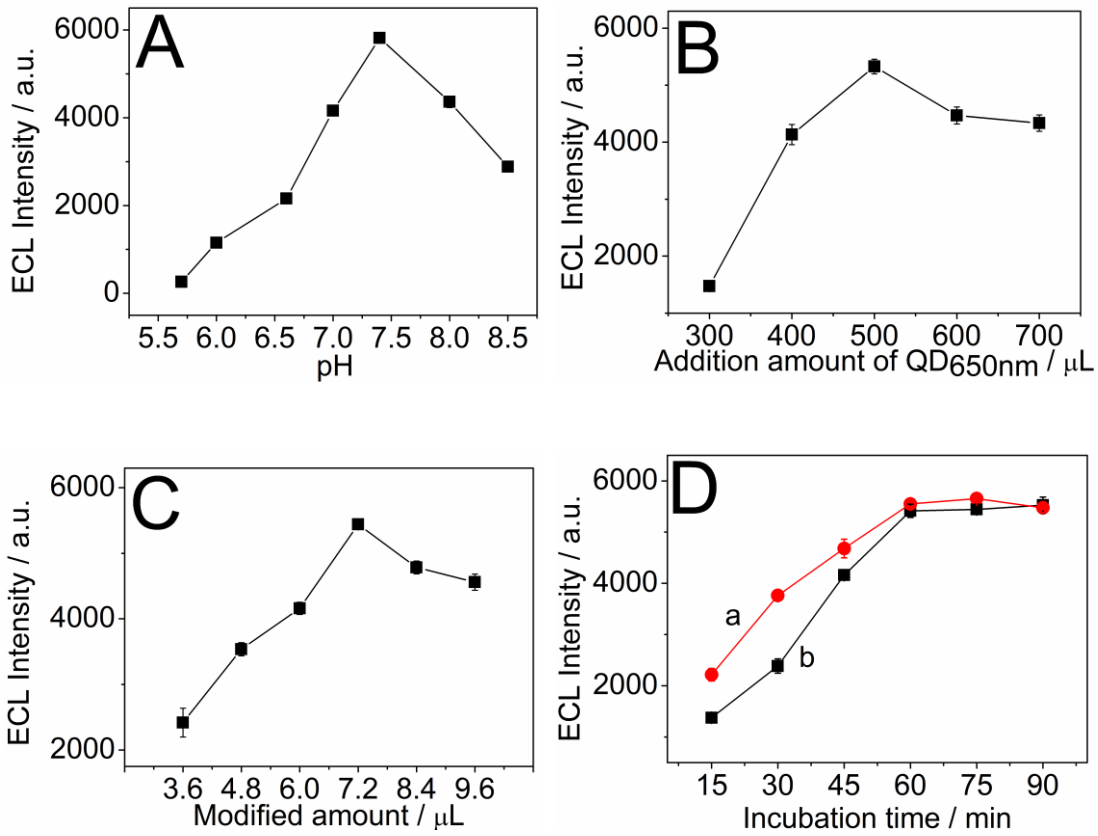

Figure S7. Effects of pH (A), the amount of $QD_{650\ nm}$ added during labeling (B), the amount of the sandwich nano-composite modified on electrode (C), and incubation time (D) on ECL intensity. (a) 1st incubation with Ag, (b) 2nd incubation with QD_{650nm}-Ab_2.

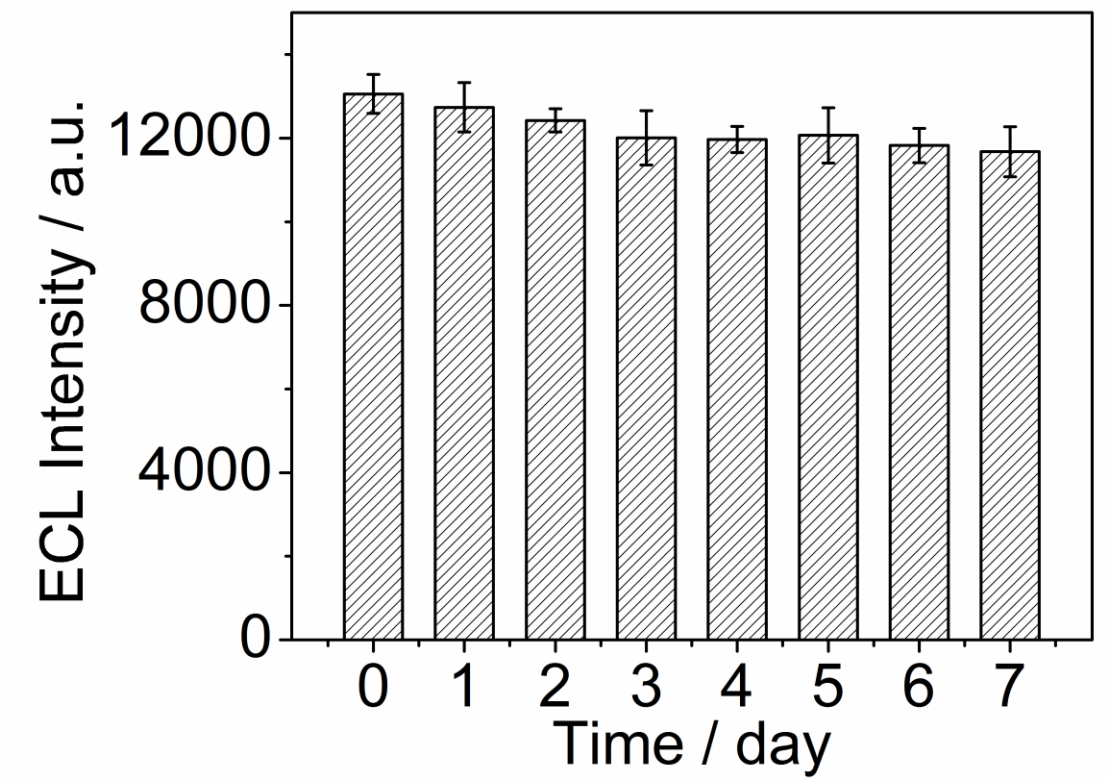

Figure S8. Stability of the immunosensor stored at 4℃ in the dark.

Table S1. Comparison of different immunosensors for CEA detection.

Materials, Methods	Linear ranges	LOD	References
Hydrazide-modified graphene QDs/AuNPs, ECL	0.02-80 ng mL^{-1}	0.01 ng mL^{-1}	6
GO/MWCNTs-COOH/Au@ CeO_2, ECL	0.05-100 ng mL^{-1}	0.02 ng mL^{-1}	7
Au@Ag nanorods /NH_4CoPO_4, ECL	0.1 pg mL^{-1}-380 ng mL^{-1}	30 fg mL^{-1}	8
Graphene-polymer nanotags, CV	5 pg mL^{-1}-40 ng mL^{-1}	1 pg mL^{-1}	9
Cu^{2+} doped chitosan-PAA nanospheres, SWV	0.1-100 ng mL^{-1}	0.02 ng mL^{-1}	10
Thiol-derivative-nanogold and gold modified ITO,	0.5-80 ng mL^{-1} (UV-vis)	2 pg mL^{-1}	11
UV-vis/EIS	0.05-80 ng mL^{-1} (EIS)	1 pg mL^{-1}	
Polyaniline derivative-Au/Pd, SWV	0.01-100 ng mL^{-1}	8.1 pg mL^{-1}	12
This method	0.01-125 ng mL^{-1}	3.0 pg mL^{-1}	-

Table S2. Recovery tests of the proposed immunosensor in real samples.

Initial CEA concentration in the serum (ng/mL)	Added CEA concentration (ng/mL)	Measured concentration after addition (ng/mL)	Average value (ng/mL)	RSD (%)	Recovery (%)
1.86	5	6.91, 6.48, 6.83, 6.18, 6.41	6.56	4.63	95.66
	10	11.96, 12.18, 12.44, 12.72, 12.48	12.36	2.37	104.18
	20	21.28, 23.03, 21.81, 22.84, 23.01	22.39	3.58	102.44
3.13	5	8.74, 8.46, 8.01, 8.49, 8.54	8.45	3.17	103.91
	10	12.51, 13.19, 12.73, 12.82, 13.47	12.94	2.96	98.58
	20	23.06, 23.54, 23.18, 23.03, 21.14	22.79	4.14	98.53

REFERENCES

(1) Zou, L.; Gu, Z.; Zhang, N.; Zhang, Y.; Fang, Z.; Zhu, W.; Zhong, X. Ultrafast synthesis of highly luminescent green- to near infrared-emitting CdTe nanocrystals in aqueous phase. *J. Mater. Chem.* **2008**, *18*, 2807-2815.

(2) Liu, Y. F.; Yu, J. S. In situ synthesis of highly luminescent glutathione-capped CdTe/ZnS quantum dots with biocompatibility. *J. Colloid Interface Sci.* **2010**, *351*, 1-9.

(3) Adegoke, O.; Nyokong, T. Probing the sensitive and selective luminescent detection of peroxynitrite using thiol-capped CdTe and CdTe@ZnS quantum dots. *J. Lumin.* **2013**, *134*, 448-455.

(4) Liu, H.; Liu, D.; Fang, G.; Liu, F.; Liu, C.; Yang, Y.; Wang, S. A novel dual-function molecularly imprinted polymer on CdTe/ZnS quantum dots for highly selective and sensitive determination of ractopamine. *Anal. Chim. Acta* **2013**, *762*, 76-82.

(5) Yuan, R.; Tang, D. P.; Chai, Y. Q.; Zhong, X.; Liu, Y.; Dai, J. Y. Ultrasensitive potentiometric immunosensor based on SA and OCA techniques for immobilization of HBsAb with colloidal Au and polyvinyl butyral as matrixes. *Langmuir* **2004**, *20*, 7240-7245.

(6) Dong, Y.; Wu, H.; Shang, P.; Zeng, X.; Chi, Y. Immobilizing water-soluble graphene quantum dots with gold nanoparticles for a low potential electrochemiluminescence immunosensor. *Nanoscale* **2015**, *7*, 16366-16371.

(7) Pang, X.; Li, J.; Zhao, Y.; Wu, D.; Zhang, Y.; Du, B.; Ma, H.; Wei, Q. Label-Free Electrochemiluminescent Immunosensor for Detection of Carcinoembryonic Antigen Based on Nanocomposites of GO/MWCNTs-COOH/Au@CeO_2. *ACS Appl. Mater. Interfaces* **2015**, *7*, 19260-19267.

(8) Zhang, Y.; Lu, F.; Yan, Z.; Wu, D.; Ma, H.; Du, B.; Wei, Q. Electrochemiluminescence immunosensing strategy based on the use of Au@Ag nanorods as a peroxidase mimic and NH_4CoPO_4 as a supercapacitive supporter: Application to the determination of carcinoembryonic antigen. *Microchim. Acta* **2015**, *182*, 1421-1429.

(9) Feng, X. B.; Gan, N.; Zhang, H. R.; Yan, Q.; Li, T. H.; Cao, Y. T.; Hu, F. T.; Yu, H. W.; Jiang, Q. L. A novel strategy for multiplexed immunoassay of tumor markers based on electrochemiluminescence coupled with cyclic voltammetry using graphene-polymer nanotags.

Electrochim. Acta **2015**, *170*, 292-299.

(10) Rong, Q.; Feng, F.; Ma, Z. Metal ions doped chitosan-poly(acrylic acid) nanospheres: Synthesis and their application in simultaneously electrochemical detection of four markers of pancreatic cancer. *Biosens. Bioelectron.* **2016**, *75*, 148-154.

(11) Zeng, H.; Agyapong, D. A. Y.; Li, C.; Zhao, R.; Yang, H.; Wu, C.; Jiang, Y.; Liu, Y. A carcinoembryonic antigen optoelectronic immunosensor based on thiol-derivative-nanogold labeled anti-CEA antibody nanomaterial and gold modified ITO. *Sens. Actuators, B* **2015**, *221*, 22-27.

(12) Wang, L.; Feng, F.; Ma, Z. Novel electrochemical redox-active species: one-step synthesis of polyaniline derivative-Au/Pd and its application for multiplexed immunoassay. *Sci. Rep.* **2015**, *5*, 16855. DOI: 10.1038/srep16855.