Supporting Information

The Combined Detection of Ovarian Cancer Biomarkers HE4 and CA125 by a Fluorescence and Quantum Dot Dual-signal Immunoassay

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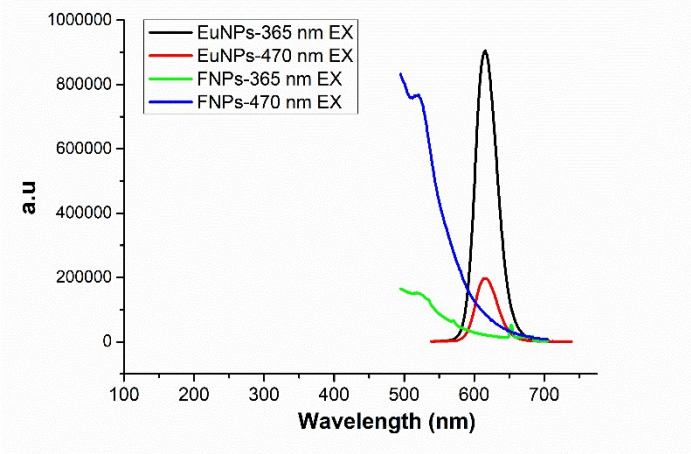


Fig. S1 Corresponding photoluminescence emission spectra of FNPs, FNPs-HE4-Ab2, QDNBs and QDNBs-CA125-Ab2.

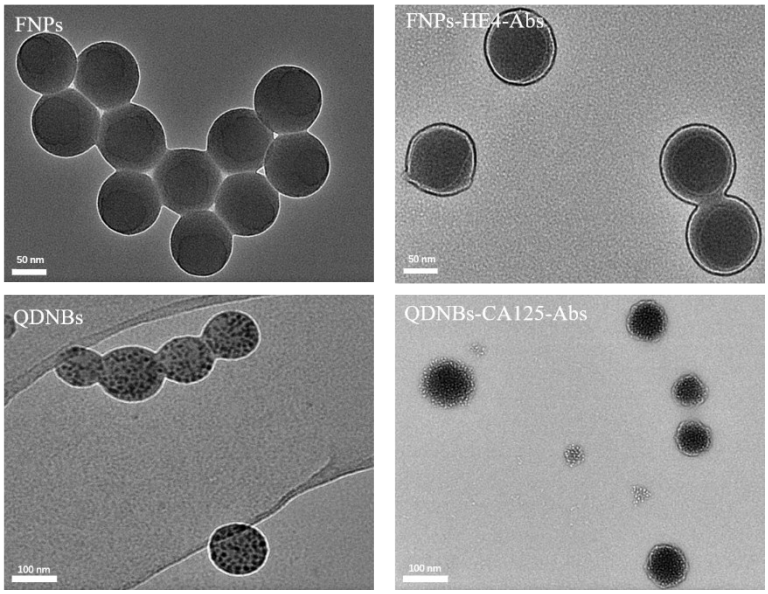


Fig. S2 Transmission electron microscope (TEM) images of FNPs, FNPs-HE4-Ab2, QDNBs and QDNBs-CA125-Ab2.

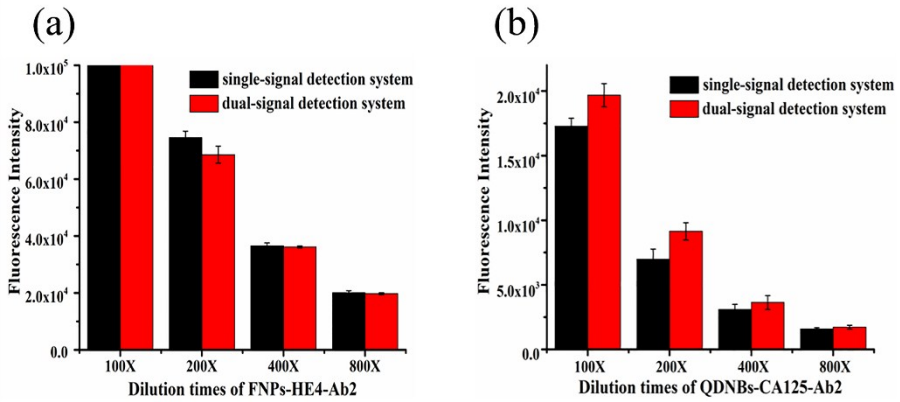


Fig. S3 Fluorescence intensity of FNPs-HE4-Ab2 in combined detection and single detection. (c) Fluorescence intensity of QDNBs-CA125-Ab2 in combined detection and single detection.

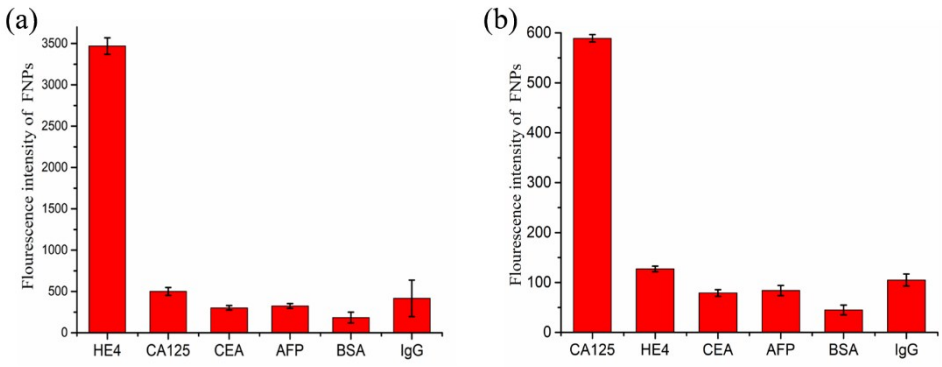


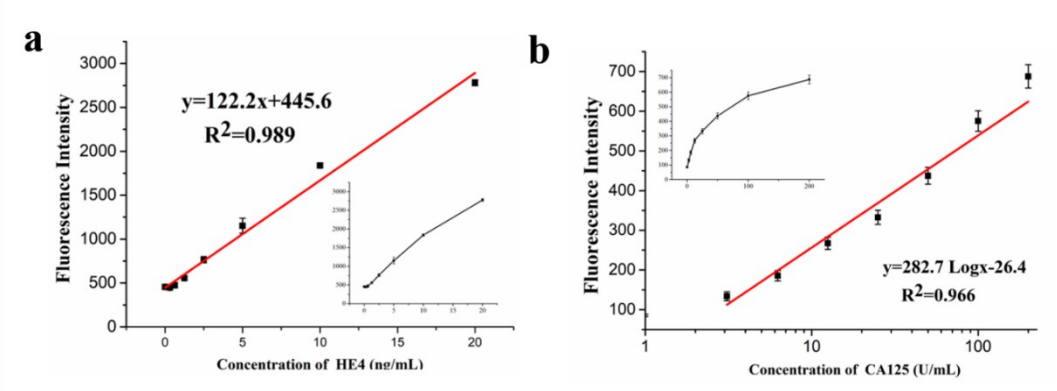
Fig. S4 Selectivity of the single-signal system-based immunoassay. The selectivity of the single-signal system-based immunoassay for HE4 (a) and CA125 (b), 5 ng mL-1 for HE4, 100 U mL^{-1} for CA125 and 50 ng mL^{-1} for other proteins.

Procedure of single-signal system-based immunoassay for HE4 and CA125 detection

In this modified ELISA method, polystyrene microplates were modified with capture antibody of HE4 and CA125 in coating buffer (0.05 mol/L CBS) at 37 °C for 45 min，respectively. After washing the microplates three times with wash buffer (1% Tween-20 in 0.01 mol/L PBS), the microplates were blocked with blocking buffer (5 mg/mL skim milk in PBS) at 4 °C overnight. Subsequently, the microplates were washed three times with wash buffer and drying, which can be stored at -20℃ for a long time. When the microplates are used for testing, first wash it with wash buffer back to

room temperature, then different concentration HE4 and CA125 was added, respectively. After 60 min, the microplates were washed three times with wash buffer, and FNPs-HE4-Ab and **QDNBs-C**A125-Ab was added at 37 °C for 45 min. Lastly, three washing steps were performed, we used the multi-mode microwell reader simultaneous collected the corresponding data of fluorescence and quantum dots. Different concentration of HE4 and CA125 from 0-20 ng/mL and 0-200 U/mL were added to the single-signal detection system, and the fluorescence intensity of FNPs and QDNBs were measured using the synergy H1 hybrid multi-mode microwell reader, respectively. The results are shown in the **Fig. S5a-b**: the linear relationship of fluorescence intensity was determined according to the standard curves of HE4 and CA125 concentrations.

Fig. S5



Calibration curve of the single-signal system for HE4 (a)and CA125 (b) single detection.

Table S1. Comparison of the dual-signal system-based immunoassay and reported other methods of HE4 and CA125 detection.

Analytical methods	Analyte	LDR	LOD	Reference
Electrochemical	Just CA125	10-100 U/mL	5.5 U/mL	[1]
Nonenzymatic colorimetric immunodetection	Just CA125	0.1-100 U/mL	0.1 U/mL	[2]
Microchip ELISA	Just HE4	1-100 ng/mL	19.5 ng/mL	[3]
Dual-signal system-based immunoassay	Simultaneous immunoassay of HE4 and CA125	1.25-20 ng/mL 12.5-200 U/mL	0.16 ng/mL 9.4 U/m	This work.

Reference

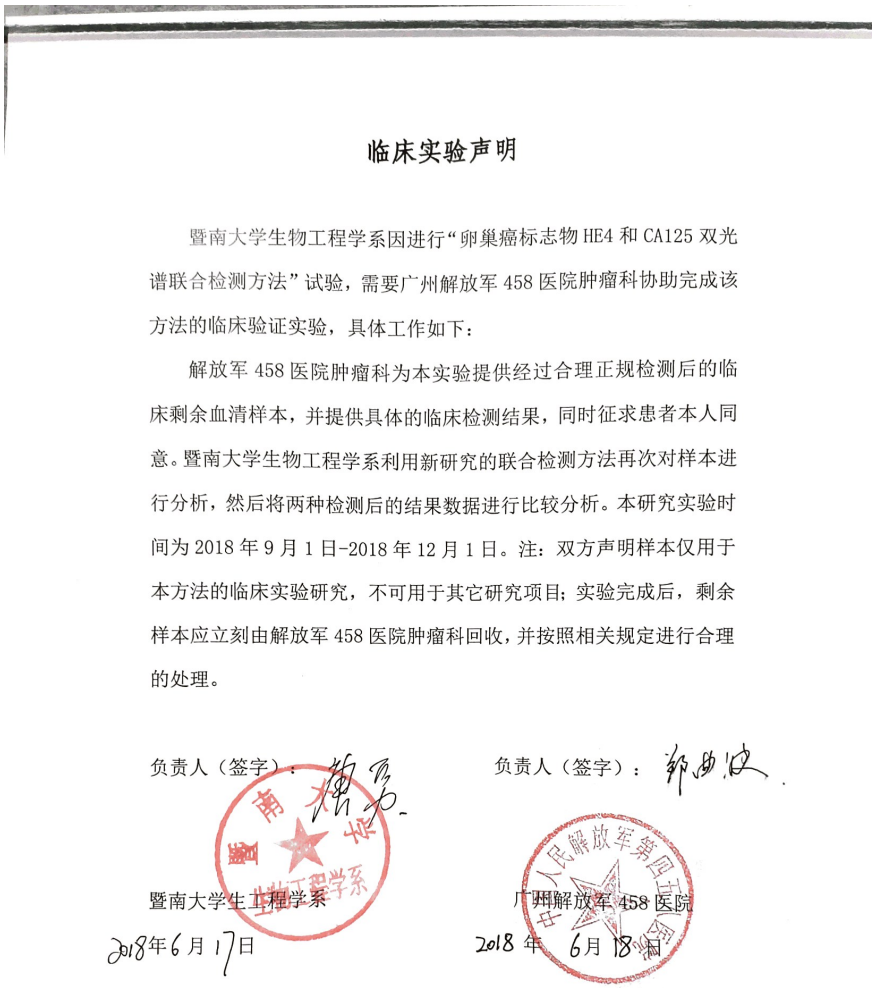
1. Torati SR, Kasturi KC, Lim B, Kim C (2017) Hierarchical gold nanostructures modified electrode for

electrochemical detection of cancer antigen CA125. Sensors and Actuators B: Chemical 243:64-71

2. Zhao Y, Zheng Y, Zhao C, You J, Qu F (2015) Hollow PDA-Au nanoparticles-enabled signal amplification for sensitive nonenzymatic colorimetric immunodetection of carbohydrate antigen 125. Biosensors and Bioelectronics 71:200-206

3. Wang S, Zhao X, Khimji I, Akbas R, Qiu W, Edwards D, Cramer DW, Ye B, Demirci U (2011) Integration of cell phone imaging with microchip ELISA to detect ovarian cancer HE4 biomarker in urine at the point-of-care. Lab on a Chip 11 (20):3411-3418

Appendix



临床实验声明

暨南大学生物工程学系因进行“卵巢癌标志物 HE4 和 CA125 双光谱联合检测方法”试验，需要广州解放军 458 医院肿瘤科协助完成该方法的临床验证实验，具体工作如下：

解放军 458 医院肿瘤科为本实验提供经过合理正规检测后的临床剩余血清样本，并提供具体的临床检测结果，同时征求患者本人同意。暨南大学生物工程学系利用新研究的联合检测方法再次对样本进行分析，然后将两种检测后的结果数据进行比较分析。本研究实验时间为 2018 年 9 月 1 日-2018 年 12 月 1 日。注：双方声明样本仅用于本方法的临床实验研究，不可用于其它研究项目；实验完成后，剩余样本应立刻由解放军 458 医院肿瘤科回收，并按照相关规定进行合理的处理。

负责人（签字）：

暨南大学生工程学系

2018年6月17日

负责人（签字）：

广州解放军 458 医院

2018 年 6月 18日