Electronic Supplementary Information

An ultrasensitive electrochemical immunosensor for determination of estradiol

using coralloid Cu₂S nanostructure as labels

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Fig. S2 Electrochemical characterization of the bioconjugation between Cu_2S and BSA-E2; a:

SWV of Cu₂S, b: SWV of Cu₂S-BSA-E2.

Optimization of conditions

Some parameters in this experimental system were identified. Free E2 and Cu₂S-BSA-E2 bioconjugates were used together to perform an indirect competitive immunoassay. Free E2 and Cu₂S-BSA-E2 were captured by the anti-E2 antibody. Nonspecific binding was blocked with appropriate blocking buffer. In the experiment, Cu₂S-BSA-E2 was sensitive to pH. Then the influences of pH on the performance of the immunosensor were investigated. Fig. S2 shows that the best SWV current response of the immunosensor was obtained at pH 6.6. Thus, pH 6.6 of the PBS buffer were selected as one of the optimized conditions.



Fig. S2 Effect of pH on the response of the immunosensor.

Construction	Analytical Methods	Linear range (pg/mL)	LOD (pg/mL)	References
E2-6- carboxymethyloxime- BSA/SPCE	Competitive immunoassay between alkaline phosphatase labeled anti- estradiol and E2, p-aminophenyl phosphate as enzyme substrate, Amperometry	2.44-2500	0.15	Butler et al., 2006
E2-6- carboxymethyloxime- BSA/SPCE	Competitive immunoassay between E2 + anti-estradiol and alkaline phosphatase labeled anti-IgG, p- aminophenyl phosphate as enzyme substrate, Amperometry	6.1-25000	1.25	Butler et al., 2006
Anti-estradiol/Au nanoparticle thiolated Protein G-scaffold/Gold electrode	Competitive immunoassay between E2 and BSA-E2, Fe(CN) ₆ ^{3-/4-} as redox probe, Square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS)	-1200 (SWV) -1000 (EIS)	18 (SWV) 26 (EIS)	Liu et al., 2010
Anti-estradiol/ bismuth- coated carbon electrodes	Competitive immunoassay between E2 and CdSe labeled BSA-E2, Cd ²⁺ released by acid dissolution, Differential pulse anode stripping voltammetry	50-1000	50	Chaisuwan et al., 2013
ethylenediamine-core poly (amidoamine)-gold nanoparticles/ graphene- polyaniline composites/GCE	Competitive immunoassay between E2 and horseradish peroxidase-graphene oxide-anti-estradiol, Differential pulse voltammetry	40-7000	20	Li et al., 2013
Anti-estradiol/ Gold electrode	Competitive immunoassay between E2 and Cu ₂ S labeled BSA-E2, Square wave voltammetry	25-7500	7.5	This work

Table S1	Comparison	between	the	proposed	assay	and	other	reported	competitive	immunoa	ssay
for e	stradiol detec	ction									

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X. Liu, P. A. Duckworth and D. K. Wong, Biosensors and Bioelectronics, 2010, 25, 1467-1473.

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Water samples	Estradiol added Estradiol found				
	(pg/mL)	(<i>n</i> =5, pg/mL)	KSD (%)	Recovery (%)	
Tap water	50	48	1.5	96	
	100	103	3.2	103	
	250	244	2.7	97.6	
Lake water	50	47	2.3	94	
	100	104	1.8	104	
	250	256	3.4	102.4	

Table S2. Detection of E2 in water samples