

Sandwich-Type Electrochemiluminescence Immunosensor Based on CDs@dSiO₂ Nanoparticles as Nanoprobe and Co-Reactant

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(A)

Certificate of Analysis

Catalog #: α -HCG Lot #: 220608
Description: **MAb to α HCG**
Monoclonal Antibody to human chorionic gonadotropin
Host Animal: Mouse, Hybridization of Sp2/0 myeloma cells with spleen cells from Balb/c mice.
Source: Ascites
Immunogen: Purified human HCG
Format: Purified, Liquid, Protein solution in 0.01M PBS
Purification: >90% pure, By SDS-PAGE.
Caprylic acid method and ammonium sulfate method
Affinity Constant: Not determined.
Concentration: 10 mg/ml (OD280)
Titer:
Buffer: PBS, pH 7.4
Preservative: None
Applications: Immune colloidal gold immunochromatography
Storage: Stored at -20°C for two years. Stored at 2-8° for two weeks.
Avoid repeated freeze-thaw cycles.
Warning: For research only

Q.C. Audit Test Results:

Visual Quality	PASS
Concentration	10mg/ml
Purity	PASS
Application Evaluation	PASS

Quality Representative:

Date: 2022.11.02

(B)

Certificate of Analysis

Catalog #: β -HCG Lot #: 220608
Description: **MAb to β HCG**
Monoclonal Antibody to human chorionic gonadotropin
Host Animal: Mouse, Hybridization of Sp2/0 myeloma cells with spleen cells from Balb/c mice.
Source: Ascites
Immunogen: Purified human HCG
Format: Purified, Liquid, Protein solution in 0.01M PBS
Purification: >90% pure, By SDS-PAGE.
Caprylic acid method and ammonium sulfate method
Affinity Constant: Not determined.
Concentration: 10mg/ml (OD280)
Titer:
Buffer: PBS, pH 7.4
Preservative: None
Applications: Immune colloidal gold immunochromatography
HCG specifically recognises human chorionic gonadotropin.
Negative results were obtained from all tests conducted with 500 mIU/ml hLH, 1000 μ IU/ml hTSH.
Storage: Stored at -20°C for two years. Stored at 2-8° for two weeks.
Avoid repeated freeze-thaw cycles.
Warning: For research only

Q.C. Audit Test Results:

Visual Quality	PASS
Concentration	10mg/ml
Purity	PASS
Application Evaluation	PASS

Quality Representative:

Date: 2022.11.02

Figure S1. The certificate of analysis of HCG-Ab₁ and HCG-Ab₂.

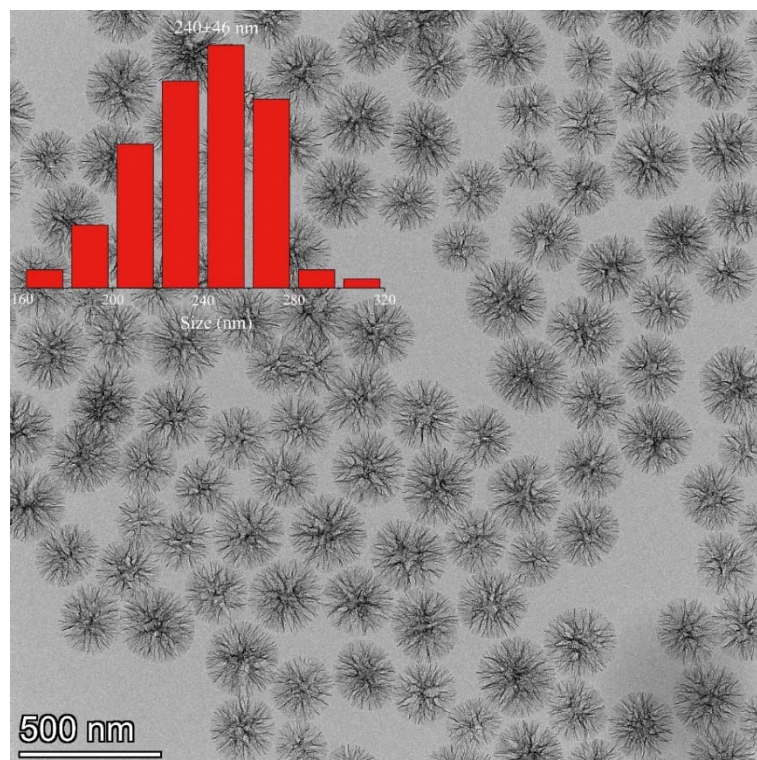


Figure S2. TEM image of dSiO₂ nanospheres at lower magnification. And the corresponding particle size distribution (insert).

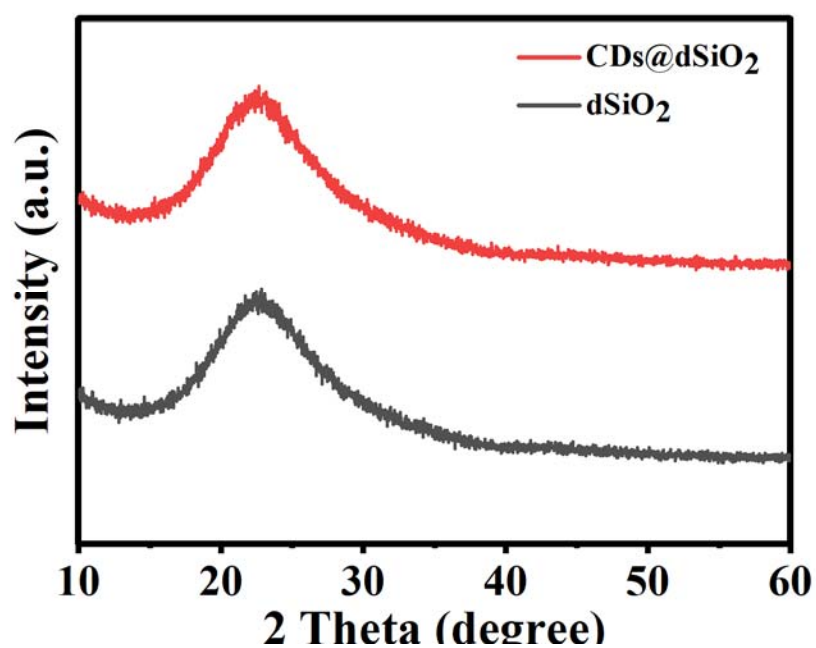


Figure S3. Powder X-ray Diffraction Pattern of dSiO₂ NPs (black curve) and CDs@dSiO₂ NPs (red curve).

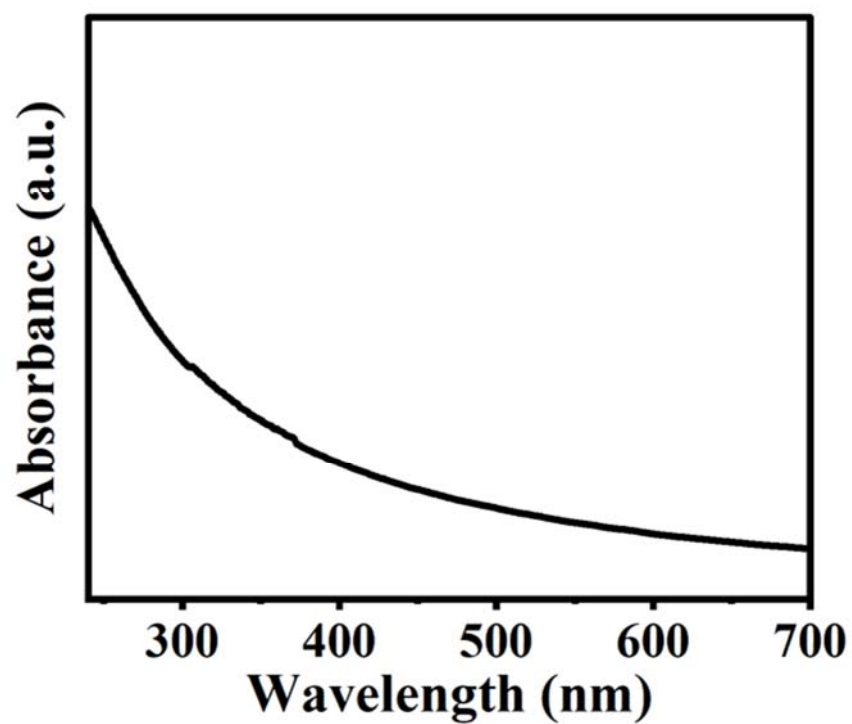


Figure S4. UV-vis absorption spectra of dSiO₂ NPs.

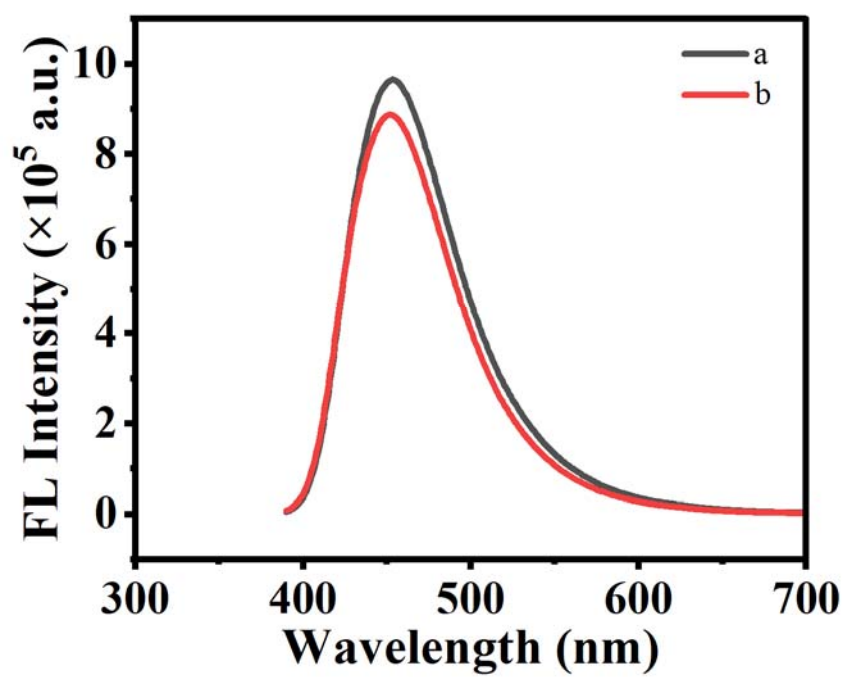


Figure S5. Fluorescence spectrum of (a) CDs and (b) residual CDs in supernatant.

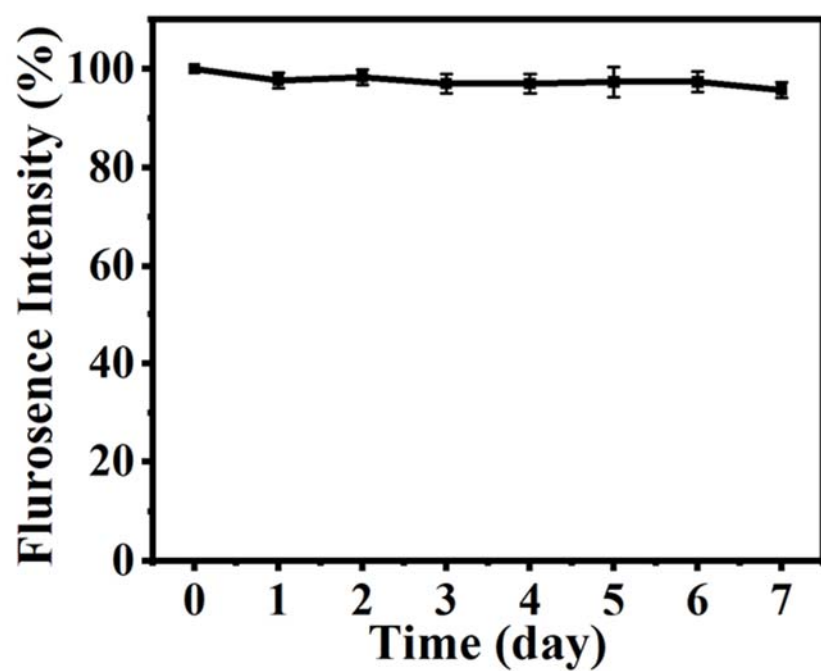


Figure S6. Fluorescent stabilities of CDs@dSiO₂ NPs against storage time.

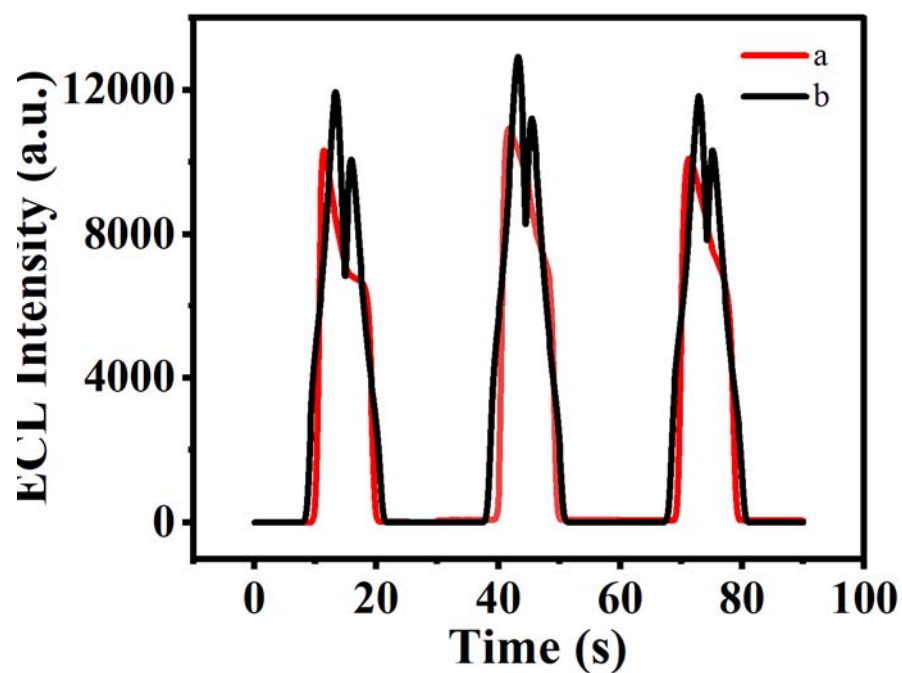


Figure S7. ECL curves of (a) CDs (15.62 mg/mL) and (b) TPrA (1.43 mg/mL) in the solution of 0.1 M PBS (pH = 7.4) containing 50 μ M Ru(bpy)₃²⁺. The voltage of PMT was set at 600 V.

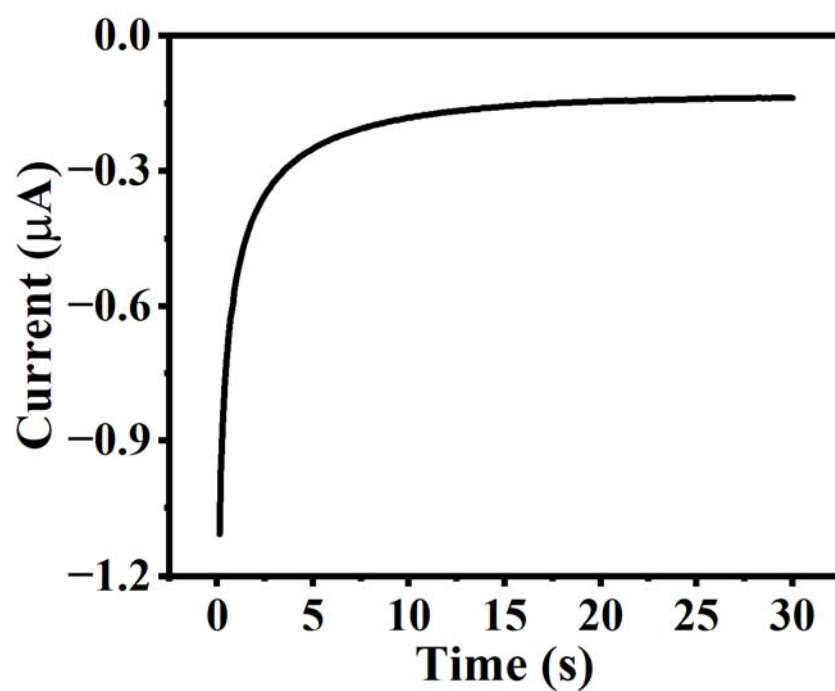


Figure S8. The current-time curve of electrodeposited Au NPs.

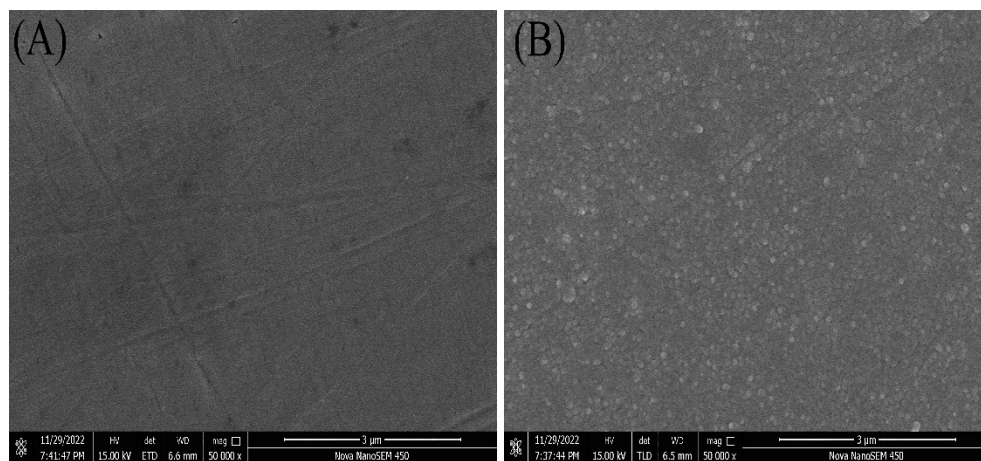


Figure S9. SEM image of electrode surface (A) GCE, (B) Au NPs-GCE.

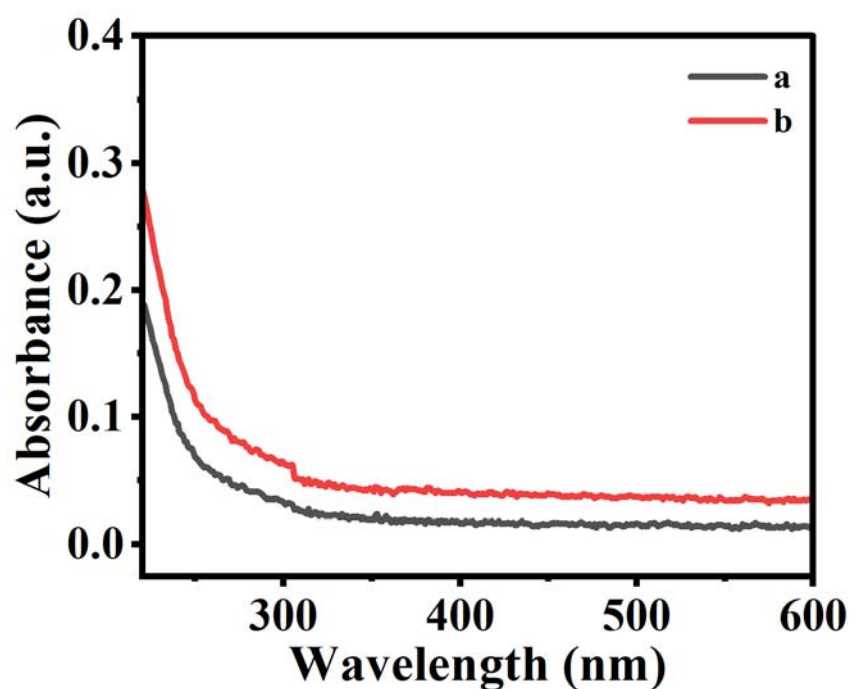


Figure S10. UV-vis absorption spectrum of (a) HCG-Ab₁ (0.016 mg/mL) and (b) a solution contains the excess HCG-Ab₁ of the constructed immunosensor.

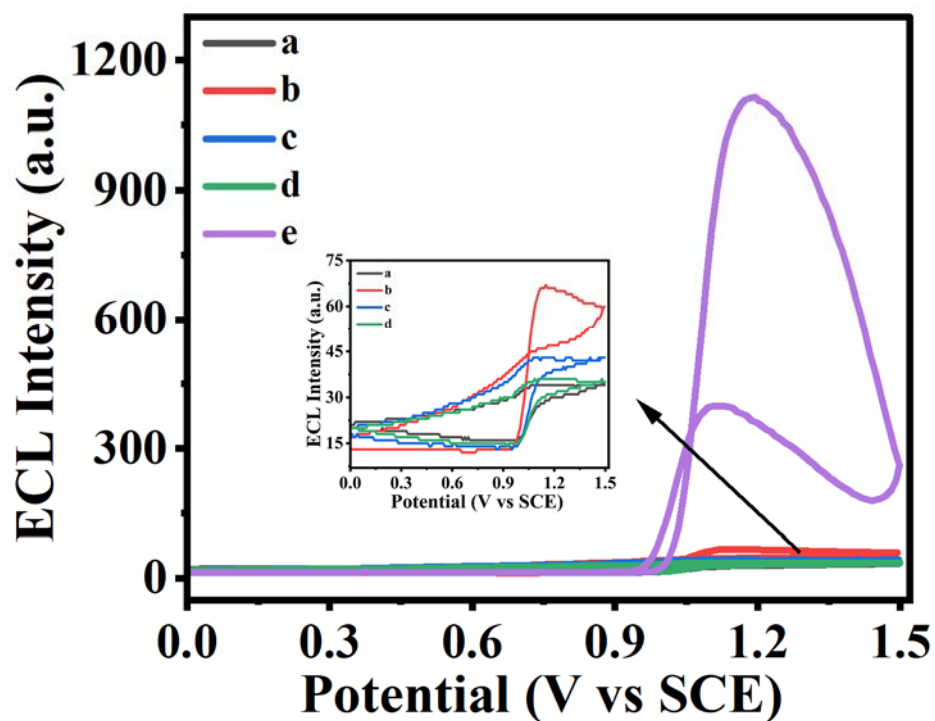


Figure S11. The ECL spectra of electrodes modified with different substances (a) GCE, (b) Ab₁/Au/GCE, (c) BSA/Ab₁/Au/GCE, (d) Ag/BSA/Ab₁/Au/GCE and (e) Ab₂-CDs@dSiO₂ NPs / Ag/BSA/Ab₁/Au/GCE in a solution of 0.01 M PBS (pH = 7.4) containing 50 μ M Ru(bpy)₃²⁺. The voltage of PMT was set at 800 V.

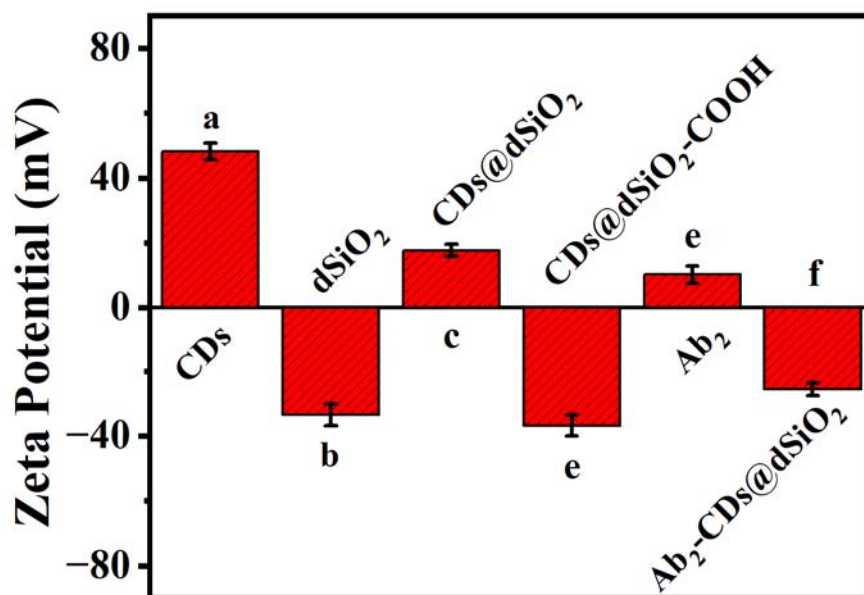


Figure S12. Zeta potential of (a) CDs, (b) dSiO₂, (c) CDs@dSiO₂, (d) CDs@dSiO₂-COOH, (e) Ab₂, and (f) CDs@dSiO₂-Ab₂ in the solution of 0.01 M PBS (pH = 7.4).

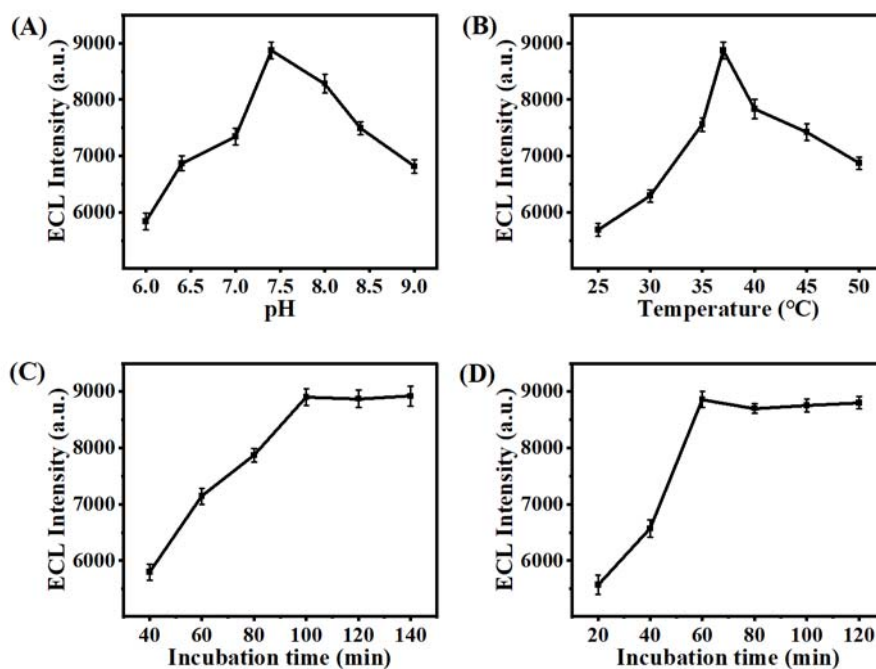


Figure S13. Optimization of (A) pH of PBS. (B) Incubation temperature. (C) Incubation time for Ab₁ bind to the electrode. (D) Specific binding time between the Ag and Ab₁. (Error bars: SD, n = 3). The voltage of PMT was set at 800 V.

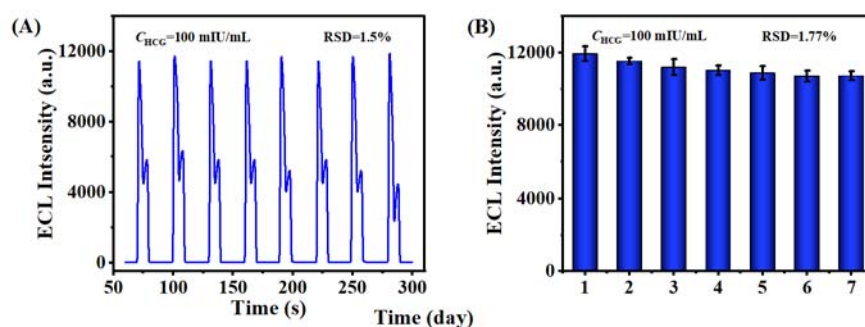


Figure S14. (A) Performance stability and (B) Storage stability of the ECL immunosensor. (Error bars: SD, $n = 3$). The voltage of PMT was set at 800 V.

Table S1. Comparison of analytical performances with other reported HCG bioassays.

Detection Method	Linear Range	Detection Limit	Ref.
Immunochromatographic strip	0.5-500 mIU/mL	0.22 mIU/mL	[1]
Surface-enhanced Raman scattering	0.5~200 mIU/mL	0.18 mIU/mL	[2]
Chemiluminescence	0.1~10 mIU/mL	0.06 mIU/mL	[3]
Electrochemiluminescence	0.001~500 mIU/mL	0.0003 mIU/mL	[4]
Electrochemiluminescence	0.001~500 mIU/mL	0.00033 mIU/mL	[5]
Electrochemiluminescence	0.0005~500 mIU/mL	0.00019 mIU/mL	Herein

Table S2. Analytical results for HCG detection in human serum specimens.

Sample	Initial Human Serum	Added (mIU/mL)	Measured (mIU/mL)	Average (mIU/mL)	RSD (%)	Recovery (n=3, %)
Serum 1	<LOD	5.00	4.48	5.72	4.4	100.1
			4.87			
			4.48			
Serum 2	<LOD	15.00	13.36	14.15	6.7	99.94
			13.88			
			15.20			
Serum 3	<LOD	25.00	24.4	25.43	2.3	100
			25.87			
			26.02			

References

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