

Supplementary Materials

**Co-amplification of luminol-based electrochemiluminescence
immunosensor based on multiple enzyme catalysis of bimetallic oxides**

CoCeO_x and NiMnO₃ for the detection of CYFRA21-1

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Fig. S1 elemental distribution images of CoCeO_x@Au; (B) Ce element; (C) O element; (D) Co element; (E) Au element.

Fig. S2 UV absorption spectra of NiMnO₃@Au (black), UV absorption spectra of NiMnO₃@Au-luminol (red), and UV absorption spectra of luminol (blue)

Fig. S3 XPS spectra of the as-prepared CoCeO_x composites. (A) Survey XPS spectra; (B) Ce(u': Ce³⁺ 3d_{3/2}; u'': Ce³⁺ 3d_{5/2}; v': Ce⁴⁺ 3d_{3/2}; v'': Ce⁴⁺ 3d_{5/2}) spectra; (C) Co spectra; and (D) O spectra.

Fig. S4 (A) Color development reaction: No. 1 TMB solution; No. 2 CoCeO_x solution; No. 3 color development reaction experiment without H₂O₂; No. 4 color development reaction experiment with H₂O₂; (B) Graph of the effect of CoCeO_x@Au on luminol-H₂O₂ system.

Fig. S5 (A) Color development reaction: No. 1 TMB solution; No. 2 NiMnO₃ solution; No. 3 TMB+NiMnO₃ solution; (B) Color development reaction: No. 1 color development reaction experiment in air; No. 2 color development reaction experiment after passing 15 min N₂; No. 3 color development reaction experiment after passing 30 N₂ for 30 min; (C) Color development reaction: No. 1 TMB solution; No. 2 color development reaction experiment without H₂O₂ after passing 30 min N₂; No. 3 color development reaction experiment with H₂O₂ after passing 30 min N₂.

Fig. S6 Long-term stability of ECL immunosensor

Table S1. Comparison of the performance of the proposed and referenced immunosensor for CYFRA21-1 detection.

5. Reference

1. Experiment section

1.1 Reagents and apparatuses

Reagents : Cerium (III) nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, 99.5%) and Manganese sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$, AR) were obtained from Aladdin Database Company (Shanghai, China). Cobalt (II) acetate tetrahydrate ($(\text{CH}_3\text{COO})_2\text{Co} \cdot 4\text{H}_2\text{O}$, 99.9%), nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), terephthalic acid (H_2BDC , 99%), absolute ethanol ($\text{C}_2\text{H}_5\text{OH}$, 99.8%), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) Chloroauric acid (HAuCl_4 , 1 %), and sodium borohydride (NaBH_4) were obtained from Macklin Reagent Database Company (Beijing, China). N, N-dimethylformamide (DMF), were obtained from Sinopharm Reagent Database Corporation (Shanghai, China). potassium permanganate (KMnO_4) was bought from Laiyang Economic and Technological Development Zone Fine Chemical Plant. urea ($\text{CO}(\text{NH}_2)_2$) was purchased from Sinopharm Group Chemical Reagent Co., and Bovine serum albumin (BSA) (96 – 99%) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Cytokeratin 19 fragment antigen (CYFRA21-1) and CYFRA21-1 antibody (Ab) were bought from Nanjing Kingsray Technology Co. Ultrapure water used for all experiments was purified by a purification system.

Apparatus: Electrochemical signals were recorded with a CHI 760D workstation (RST 5200F Zhengzhou Seris Instrument Technology Co., Ltd.). ECL signals were detected with an MPI-F electrochemiluminescence analyzer (Xi'an Re/Max Analytical Instruments Co., Ltd.). Scanning electron microscopy (SEM) images were obtained by a field emission scanning electron microscope (Zeiss, Germany). x-ray diffraction (XRD) mapping was done by a D8 focusing diffractometer (Bruker AXS, Germany). Fourier transform infrared (FT-IR) spectrograms were recorded with a VERTEX70 spectrometer (Bruker, Germany). The UV spectra were measured by a TU1900 double-beam UV-visible spectrophotometer. A conventional three-electrode cell system was used during the experiments, with a modified glassy carbon electrode (GCE, 4.0 mm diameter) as the working electrode, a platinum wire as the auxiliary electrode, and

Ag/AgCl (saturated KCl solution) as the reference electrode.

2. The calculation of the detection limit

The limits of detection are calculated with reference to the literature that has been reported¹. Parallel determinations were performed on 10 blank samples and the detection limits were calculated according to the following equation.

$$I_L = I_B + k \cdot S_B$$

Where I_B indicates the average ECL intensity, S_B is the standard deviation, and k is a numerical factor chosen according to the signal-to-noise ratio, which is taken as 3 here. The calculated I_L was substituted into the working curve, and the final detection limit was obtained as 0.3 pg/mL.

3. Preparation and storage of serum samples.

Blood samples were provided by the hospital of the University of Jinan. Prior to the experiment, 3 mL of blood samples were centrifuged in a 5 mL centrifuge tube at 4000 rpm/min for 10 min, and the supernatant was the prepared serum sample. The serum samples were stored in a refrigerator at 4 °C until use. For testing, the serum samples were equally divided into several groups, one of which was confirmed by the constructed immunosensor for its concentration. Standards of different concentrations were added to the remaining fractions and their concentrations were determined after thorough mixing.

4. Figures and Table

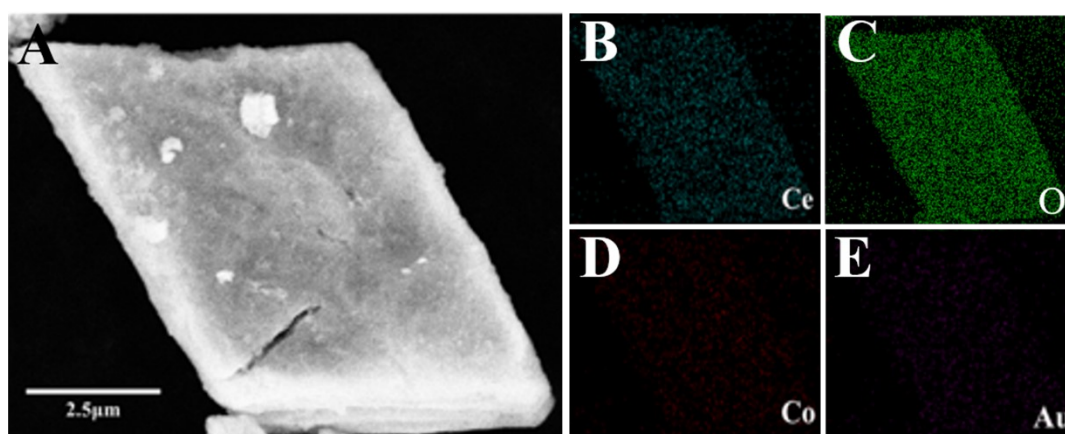


Fig. S1 elemental distribution images of CoCeO_x@Au; (B) Ce element; (C) O element; (D) Co element; (E) Au element.

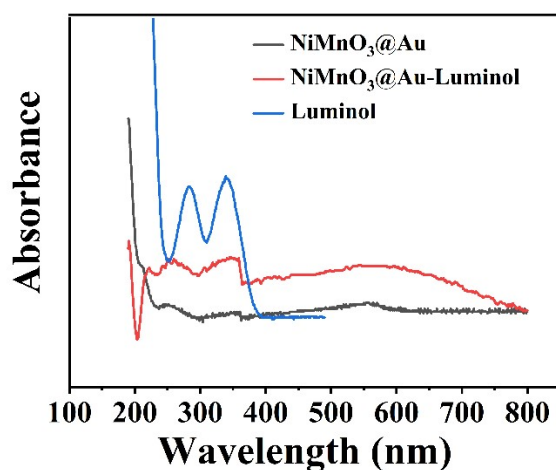


Fig. S2 UV absorption spectra of NiMnO₃@Au (black), UV absorption spectra of NiMnO₃@Au-luminol (red), and UV absorption spectra of luminol (blue)

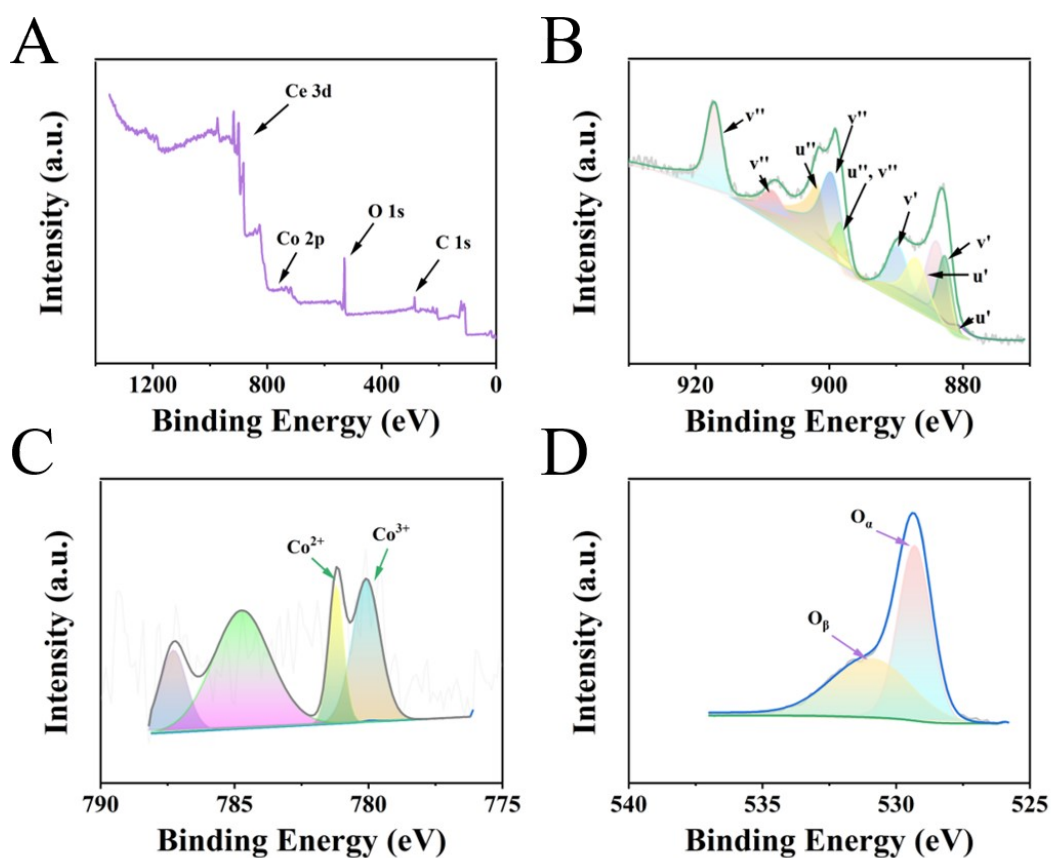


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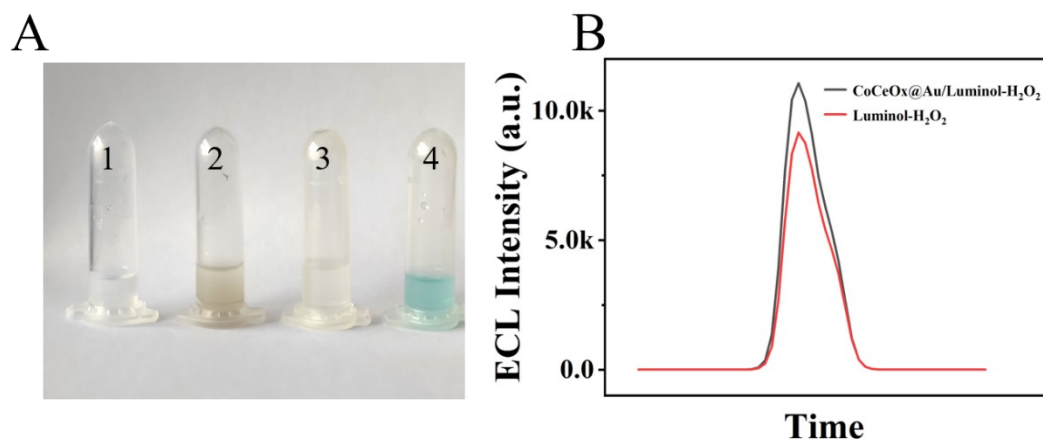


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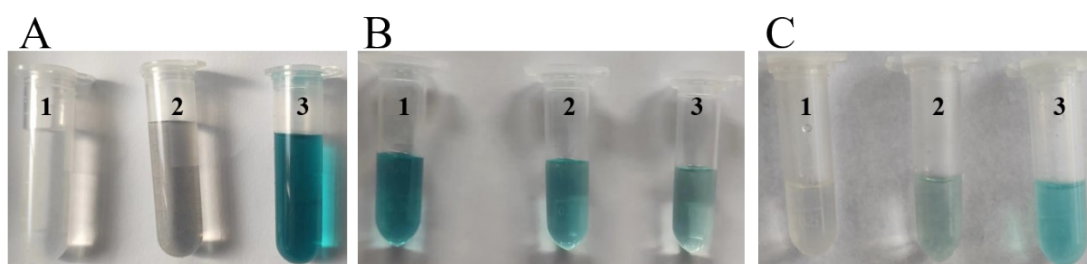


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100 μ L acetate buffer (pH = 4.9) was added to 1 mL TMB (1 mM in H₂O). This was followed by the addition of 500 μ L of NiMnO₃ dispersion (50 μ g/mL). The resulting solution was turned into blue in 2 minutes at room temperature.

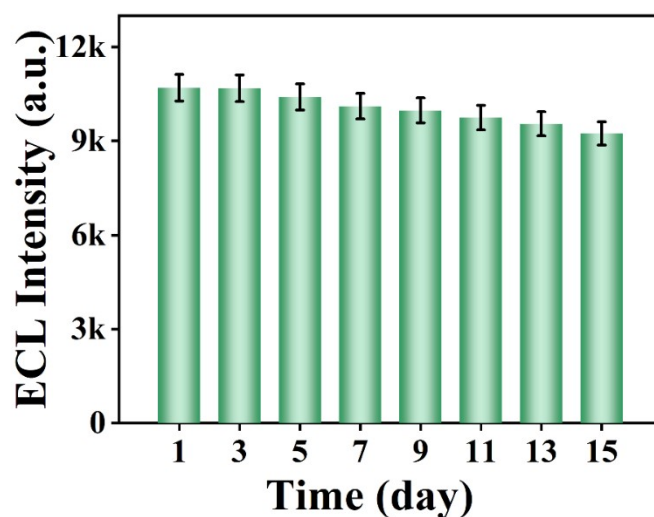


Fig. S6 long-term Stability of ECL immunosensor

After calculation, the RSD for the first 15 days is 5.26%, and the RSD for the first 7 days is 2.64%, indicating that the storage performance of the immunosensor is well.

Table S1. Comparison of the performance of the proposed and referenced immunosensor for CYFRA21-1 detection

Methods	Linear range (ng/mL)	The detection limit (ng/mL)	Reference
fluorescence analysis	0.18–100	0.18	2
electrochemical	0.50–200	0.18	3
controlled release sensor	1.3–160	0.79	4
electrochemiluminescent immunoassay	0.010–100	0.0026	5
electrochemical ratiometric method	0.00050–50	0.00039	6
electrochemiluminescent immunoassay	0.10–50	0.0060	7
electrochemiluminescent immunoassay	0.0010–150	0.00030	This work

Reference

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