Electronic Supplementary Material (ESM)

An intelligent readable and capture-antibody-independent lateral flow immunoassay based on $Cu_{2-x}Se$ nanocrystals for point-of-care detection of *Escherichia coli* O157:H7

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Experimental section

Materials and reagents

Cetyltrimethyl Ammonium bromide (CTAB), selenium dioxide (SeO_2), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), ascorbic acid (AA), tween-20, tris (hydroxymethyl) aminomethane (Tris), disodium hydrogen phosphate (Na_2HPO_4), and 5,5-dimethyl-1-neneneba pyrroline-N-oxide (DMPO) were purchased from Aladdin Biochemical Co., Ltd (Shanghai, China). 3,3',5,5' - tetramethylbenzidine (TMB) was purchased from Shanghai Sangon Biotech Co., Ltd (Shanghai, China). Monosodium phosphate (NaH_2PO_4) was purchased from Thain Chemical Technology Co., Ltd (Shanghai, China). H_2O_2 (30%) and hydrochloric acid (HCl) were purchased from Xilong Science Co., Ltd (Guangdong, China). The dialysis bag (10 KD) was purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). The culture medium used for bacterial cultivation was purchased from Guangdong Biyuntian Biotechnology Co., Ltd (Guangdong, China). *Escherichia coli* O157:H7(*E. coli* O157:H7, NCTC 12900), *Escherichia coli* (*E. coli*, ATCC 29522), *Salmonella typhimurium* (*S. typhimurium*, ATCC 14028), *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and *Bacillus subtilis* (*B. subtilis*, ATCC 6633) were purchased from Guangzhou Huankai Microbial Technology Co., Ltd (Guangdong, China). Fetal bovine serum (FBS) was purchased from Saiye Biotechnology Co., Ltd (Guangdong, China). *E. coli* O157:H7 monoclonal antibody was purchased from Keyue Zhongkai Biotechnology Co., Ltd. (Beijing, China) The simple film scribing kit, nitrocellulose (NC) film, sample pad (Ahlstrom), absorbent pad, and PVC base plate were purchased from Shanghai Jieyi Biotechnology Co., Ltd (Shanghai, China). All chemical reagents were of analytical grade and used without further purification. The solution was prepared from deionized water (≥18.2 mΩ cm^{-1}) obtained by the Milli-Q Water purification system (Milli-Q, Millipore, USA).

Experimental instruments and characterization

The morphology and dispersion of $Cu_{2-x}Se$ NCs synthesized were analyzed and characterized by an EOL 200 kV field emission transmission electron microscopy (FE-

TEM, JEM-2100F, Japan), and the element mapping images of $Cu_{2-x}Se$ NCs were recorded by an energy dispersive X-ray spectrdmeter (EDS); The hydration particle size and Zeta potential of $Cu_{2-x}Se$ NCs were determined by using the Zetasizer Nano ZS90 potential analyzer (Malvern, UK); X-ray photoelectron spectroscopy (XPS) was measured by using Thermo Scientific ESCALAB 250Xi electron spectroscopy (Thermo, USA); Obtaining SEM images using Quanta200 field emission scanning electron microscopy (SEM) (Thermo, USA); Fourier transform infrared (FTIR) spectroscopy was measured by using a Nicolet 5700 infrared spectrometer (Thermo, USA); The electron paramagnetic resonance (EPR) spectrum was obtained by using a Bruker A300 electron paramagnetic resonance spectrometer (Bruker, Germany). The peroxidase-like activity of $Cu_{2-x}Se$ NCs was verified by using a TECAN Spark enzyme marker (TECAN, Switzerland); HC-2064 high-speed centrifuge (Zhongjia, China) was used for synthesizing the $Cu_{2-x}Se$ NCs; The constant temperature mixer (Mio, China) was used for reagent mixing and incubation.

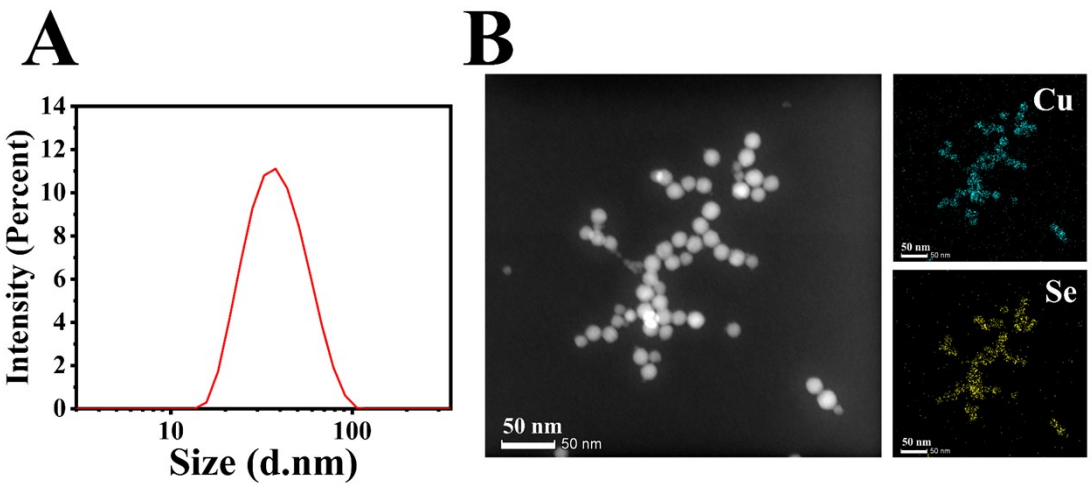

Fig. S1 (A) The hydrodynamic size of $Cu_{2-x}Se$ NCs. (B) The EDS-mapping of $Cu_{2-x}Se$ NCs.

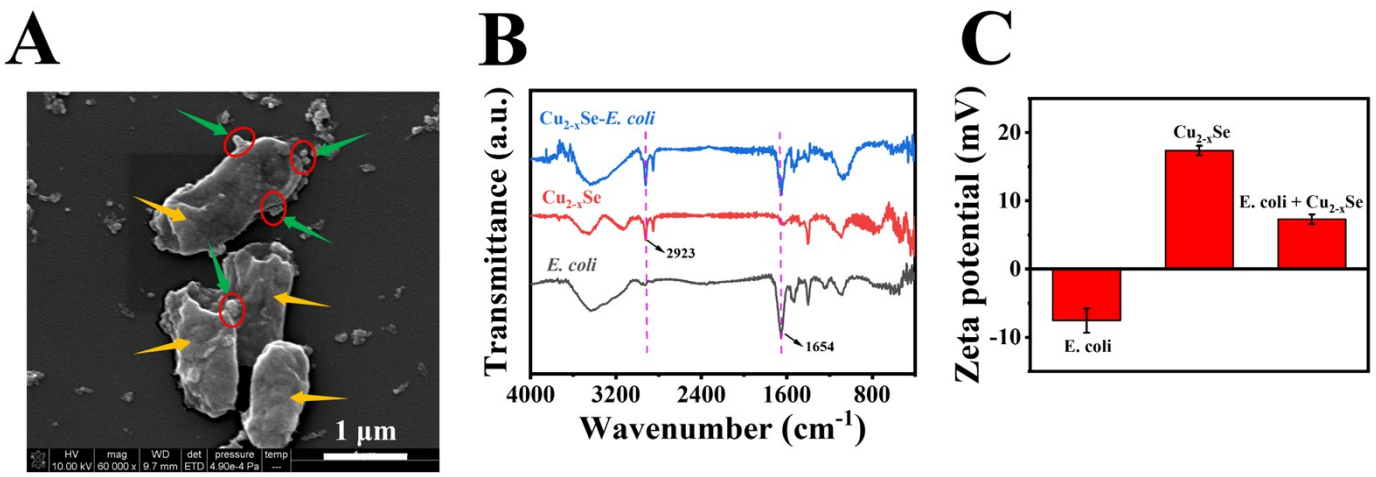

Fig. S2 (A) SEM images of $Cu_{2-x}Se$-*E. coli* O157:H7 complexes (the green arrows indicate $Cu_{2-x}Se$ NCs, the orange arrows indicate *E. coli* O157:H7, and the circle indicates that $Cu_{2-x}Se$ NCs aggregate on the surface of *E. coli* O157:H7). (B) FT-IR spectroscopy of *E. coli* O157:H7, $Cu_{2-x}Se$ NCs and $Cu_{2-x}Se$-*E. coli* O157:H7 complexes. (C) The zeta potential of *E. coli* O157:H7, $Cu_{2-x}Se$ NCs and $Cu_{2-x}Se$-*E. coli* O157:H7 complexes.

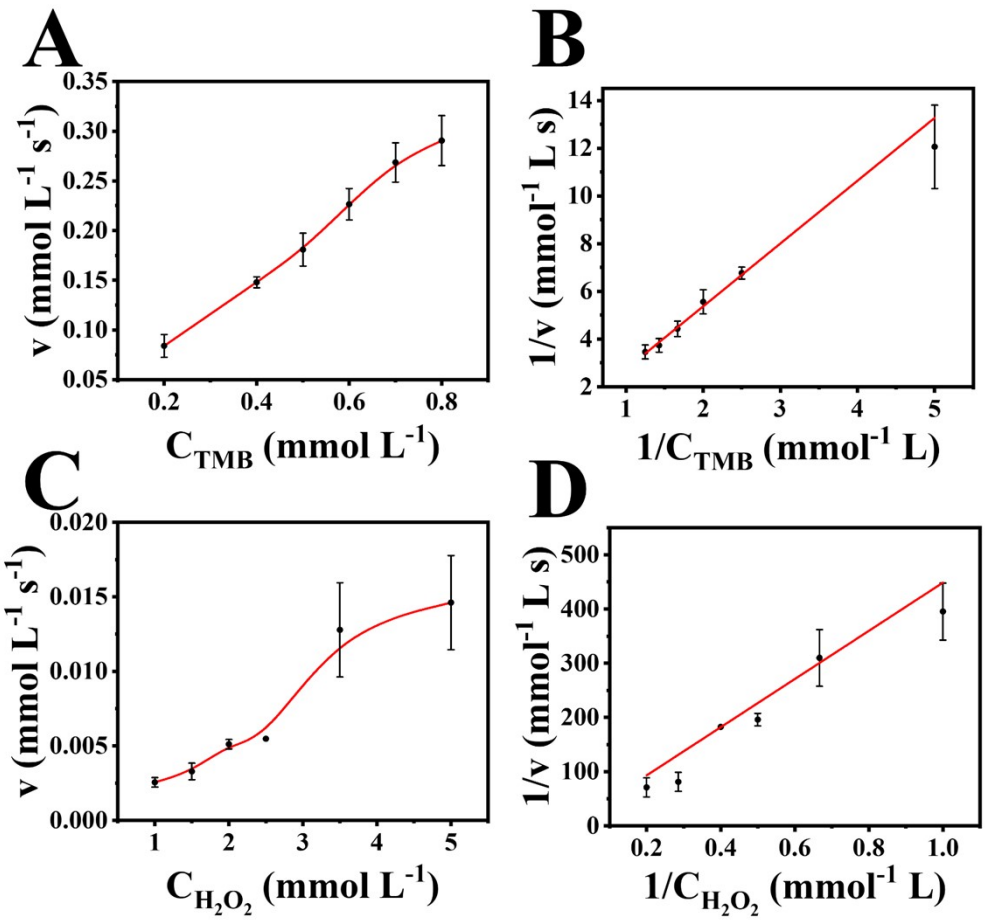

Fig. S3 (A) Michaelis-Menten curve under 2.5% H_2O_2 and various TMB concentrations, (B) Line-weaver-Burk plot for TMB. (C) Michaelis-Menten curve under 20 mmol L^{-1} TMB with various H_2O_2 concentrations, (D) Line-weaver-Burk plot for H_2O_2.

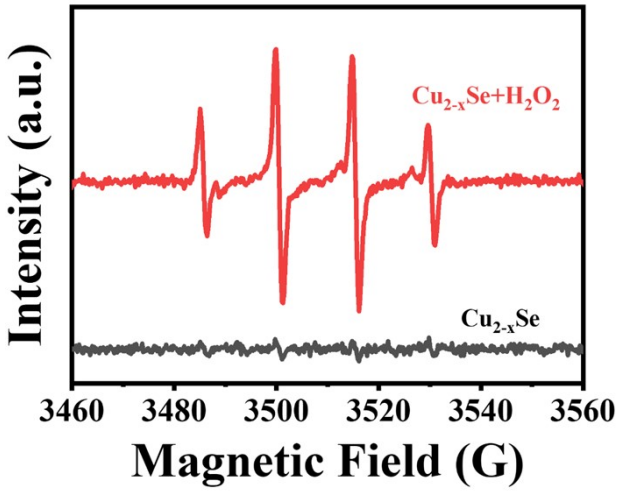

Fig. S4 EPR spectra of $Cu_{2-x}Se+H_2O_2$+DMPO and $Cu_{2-x}Se$+DMPO.

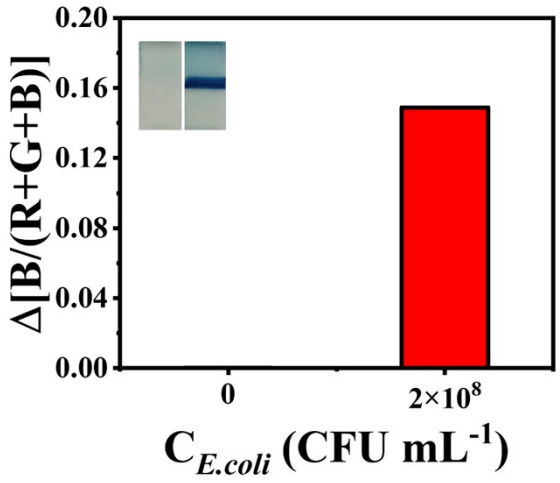

Fig. S5 Test strip signal strength of samples with or without *E. coli* O157:H7.

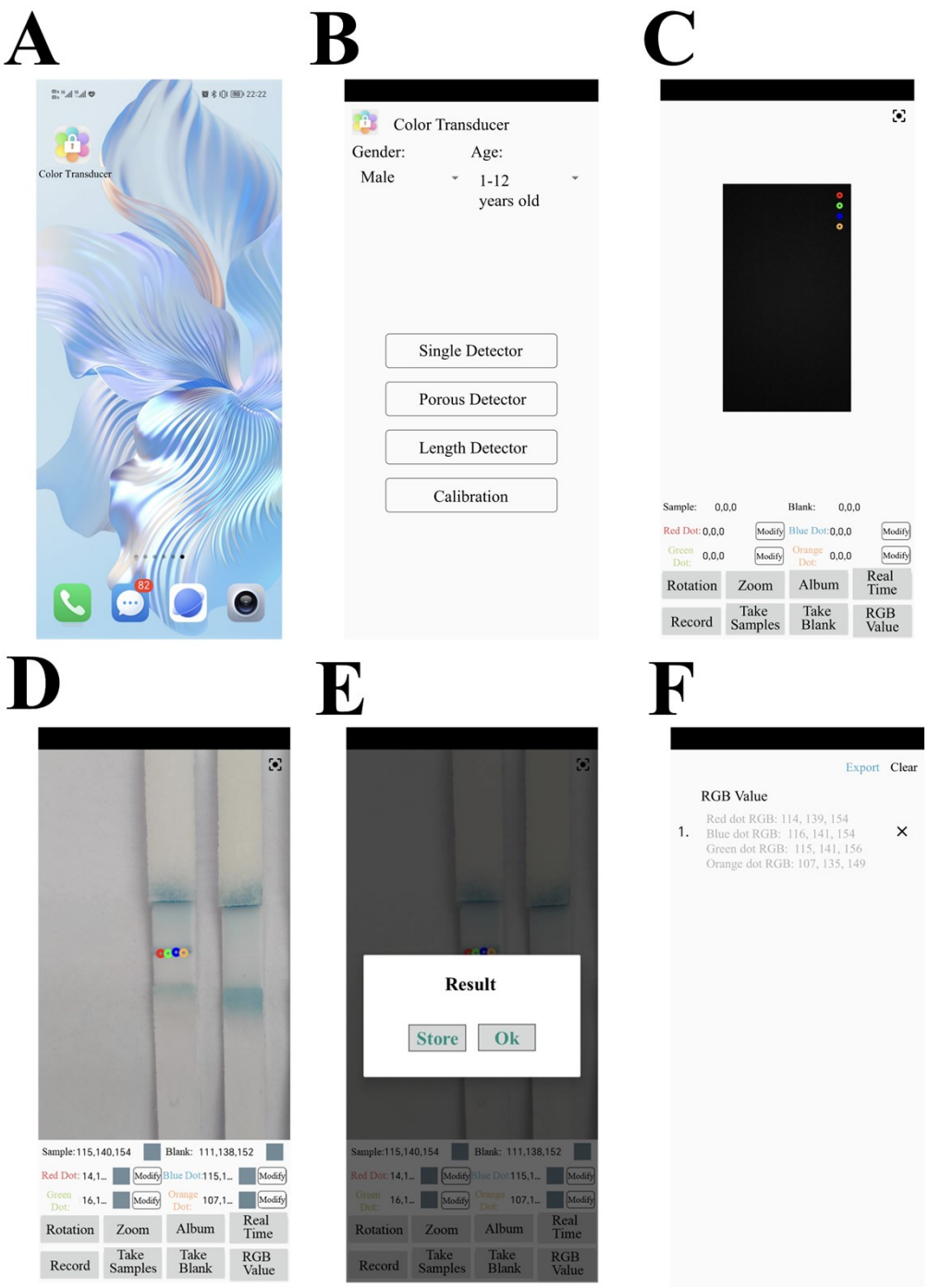

Fig. S6 Operation steps for analyzing the RGB signal strength on the T-line of the test strip using the Color Transducer App.

Table S1 Comparison of kinetic parameters between $Cu_{2-x}Se$ NCs and other catalysts

Catalyst	Substance	K_m (mmol L^{-1})	V_{max} (μmol s^{-1})	Reference
HRP	TMB	0.434	10.00×10^{-2}	1
	H_2O_2	3.70	8.71×10^{-2}	
AgPt-Fe_3O_4	TMB	0.442	4.763×10^{-2}	2
	H_2O_2	3.463	2.076×10^{-2}	
FeS_2	TMB	8.202	0.184	3
	H_2O_2	0.242	0.490	
R-Fe_3O_4/Au	TMB	0.13	6.22	4
	H_2O_2	5.34	24.10	
Au@Hg^0/WO_3 HNFs	TMB	-	-	5
	H_2O_2	145.58	220.75	
$Cu_{2-x}Se$ NCs	TMB	1.23	467.34	This work
	H_2O_2	1.80	4.05	

Table S2 Performance comparison with other methods for detecting *E. coli* O157:H7

Methods	Type	Linear range (CFU mL^{-1})	Detection limit (CFU mL^{-1})	Reference
Colorimetry	Aptamer-modified AuNPs decorated on polystyrene microparticles (PS-Au)	10^8-10^3	10^3	6
Fluorescence	CQDs magnetic nanoparticles (CQDs-MNPs)	1×10^6-5×10^2	4.87×10^2	7
	BCD@SiO_2@AuNC	10^7-10^3	1.50×10^2	8
Photothermal	MPBA induced aggregation of MPBA-AuNPs	10^9-10^6	1.97×10^4	9
LFIA	AuNPs	10^8-10^6	10^6	10
	$CoFe_2O_4$ nanoparticles	10^8-10^3	10^3	11
	Fluorescent microsphere	1×10^6-5×10^3	3.98×10^3	12
	Lac dye	10^8-10^6	10^6	13
	Mannose modified Prussian blue	10^8-10^2	10^2	14
	Au−PMBA nanocrabs	10^7-10^3	10^3	15
	$Cu_{2-x}Se$ NCs	4.16×10^7-4.16×10^5	2.65×10^5	This work

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