Electronic Supplementary Information (ESI)

A dual-signal-amplified electrochemical biosensor for sensitive and accurate detection of cancer cells

Yujuan Wu[a], Yi Wang[a], Rongxiang He[c], YuLi*[b], Yali Ben*[a]

[a]School of Medicine, Jianghan University, Wuhan 430056, China.

[b]Department of dermatology, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Huangshi 435000, China.

[c]School of Electronic and Electrical Engineering, Wuhan Textile University, Wuhan 430200, China.

* liyu295@hotmail.com, benyali@jhun.edu.cn (corresponding author)

Optimization of experimental conditions

1. **Optimization of HRP-to-Ab2 Ratio for Conjugation with Carboxylated Multi-Walled Carbon Nanotubes (MWCNTs) on the Nanofiber Microchip.**
Different ratios of horseradish peroxidase (HRP) to antibody (Ab2) specifically 50:1（Fig. S1 A）, 100:1（Fig. S1 B）, 200:1（Fig. S1 C）, 300:1（Fig. S1 D）, and 400:1（Fig. S1 E） were used to conjugate with carboxylated MWCNTs on the cell-captured nanofiber microchip to construct the biosensor. Electrochemical measurements showed that the peak current increased with the HRP-Ab2 ratio up to 200:1（Fig. S1 C）, beyond which a decline was observed at 400:1（Fig. S1 E）. This decrease is attributed to inhibited binding interactions among excess HRP, carboxylated MWCNTs, and specific antibodies, leading to reduced capture efficiency of circulating tumor cells. Based on these results, the optimal conjugation ratio of HRP to Ab2 was determined as 200:1. The entire fabrication process of the antibody conjugates and biosensor construction utilized this optimized ratio to ensure peak sensor performance.

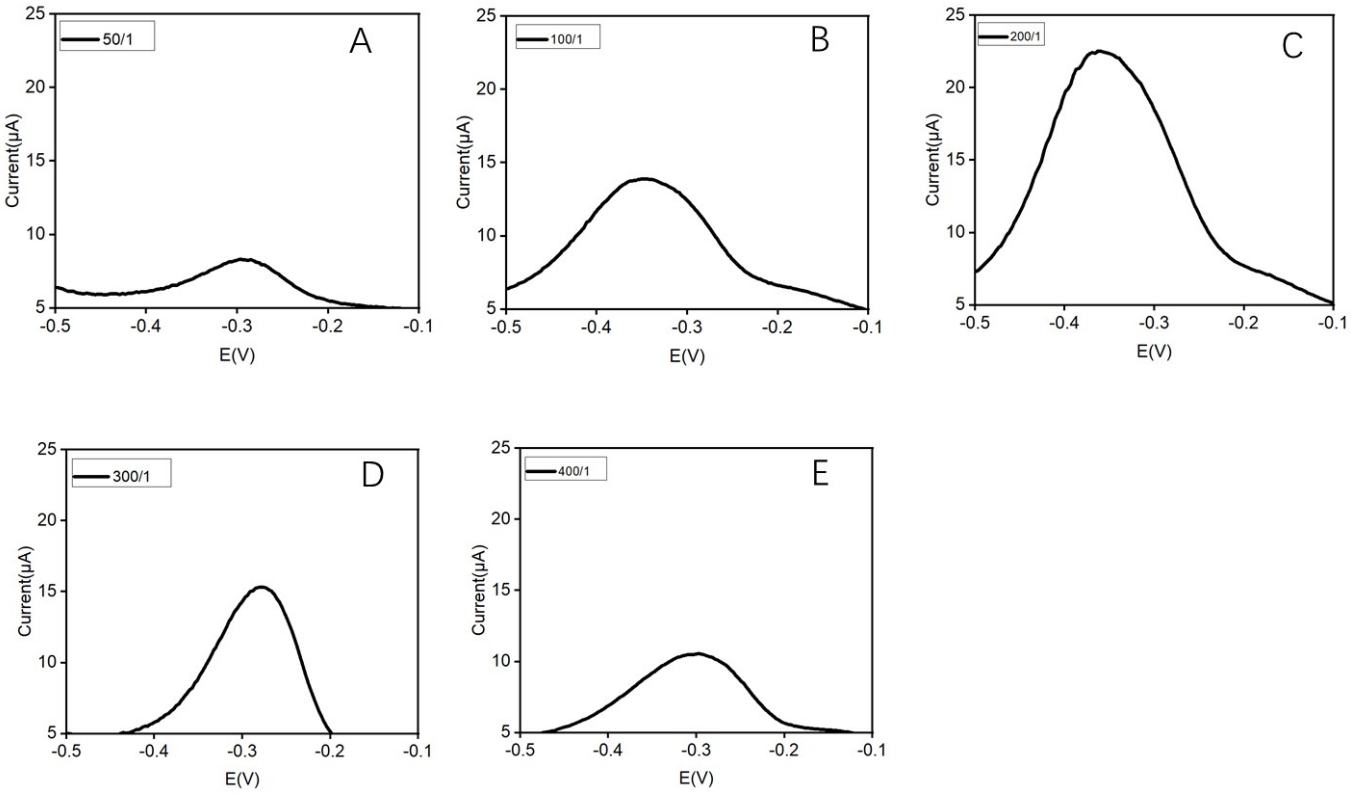

Fig.S1.Electrochemical sensor response for varying ratios of HRP to Ab2

2. Optimization of Tumor Cell Incubation Time for Enhanced Electrochemical Performance of the Immunosensor.

Tumor cells were incubated at gradient time points of 10（Fig. S2 A）, 20（Fig. S2 B）, 30（Fig. S2 C）, 40（Fig. S2 D）, 50（Fig. S2 E）, 60（Fig. S2 F）, 70（Fig. S2 G）, and 80（Fig. S2 H）minutes to determine the optimal incubation duration. Electrochemical measurements were conducted using an electrochemical workstation. As shown in Figure S1, the peak current increased gradually with incubation time from 10 to 40 minutes, reaching a maximum at 40 minutes（Fig. S2 D）. Beyond 40 minutes, although incubation times were extended further, no significant changes in peak current were observed. Based on these results, an incubation time of 40 minutes（Fig. S2 D） was selected for tumor cell incubation in the assembly of the immunosensor.

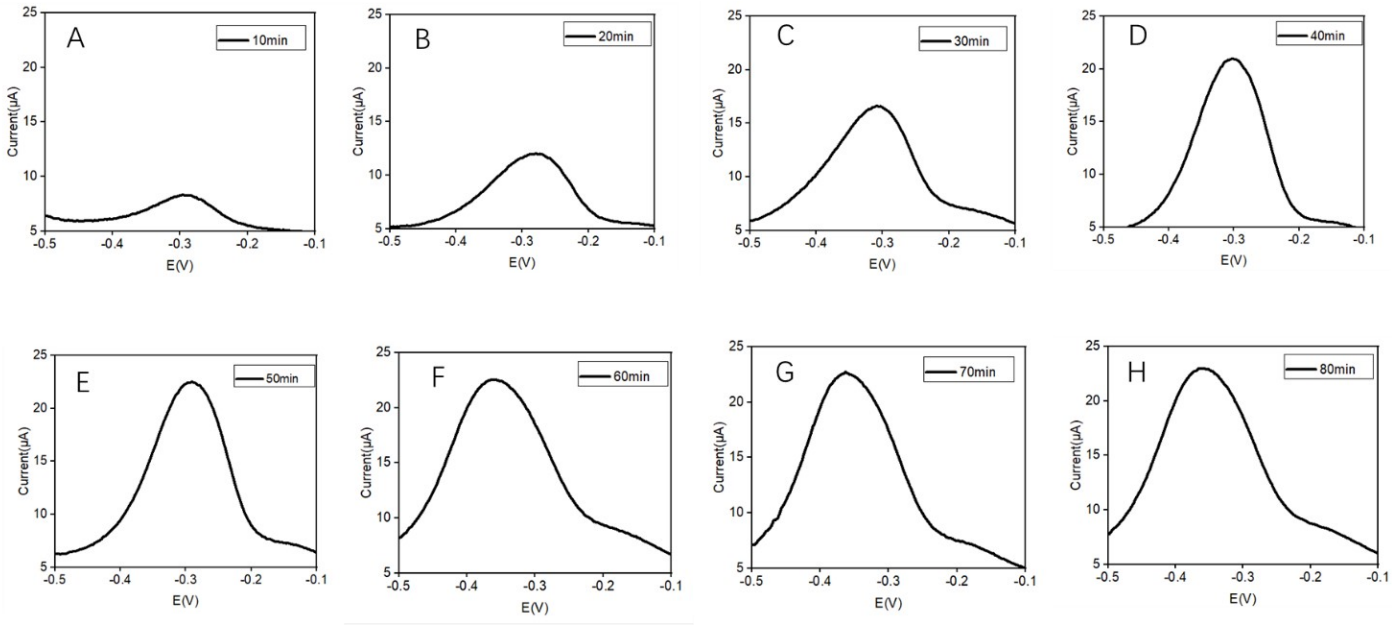

Fig.S2.Electrochemical sensor response for different cell incubation times (10-80 min)

3. Optimization of Incubation Time for Antibody Conjugates to Maximize Immunosensor Electrochemical Performance.

To determine the optimal incubation time for the HRP-MWCNTs-COOH-Ab_2 conjugate, a gradient of incubation durations was tested, and the resulting electrochemical responses were measured using an electrochemical workstation. Specifically, the conjugate was incubated for 10（Fig. S3 A）, 20（Fig. S3 B）, 30（Fig. S3 C）, 40（Fig. S3 D）, 50（Fig. S3 E）, 60（Fig. S3 F）, 70（Fig. S3 G）, and 80（Fig. S3 H） minutes. The peak current increased gradually between 10 and 50 minutes, reaching a maximum at 50 minutes（Fig. S3 E）. Further extension of the incubation time beyond 50 minutes resulted in no appreciable change and the peak current entering a stable plateau phase. This behavior suggests that, as the incubation time increases, a greater number of antibody conjugates bind to the target cells, leading to an amplified response peak current. However, once the binding capacity of the cells approaches saturation, the peak current stabilizes. Therefore, 50 minutes（Fig. S3 E） was determined to be the optimal incubation time for the antibody conjugate. In this study, both tumor cells and antibody conjugates were incubated for 50 minutes during the immunosensor assembly process.

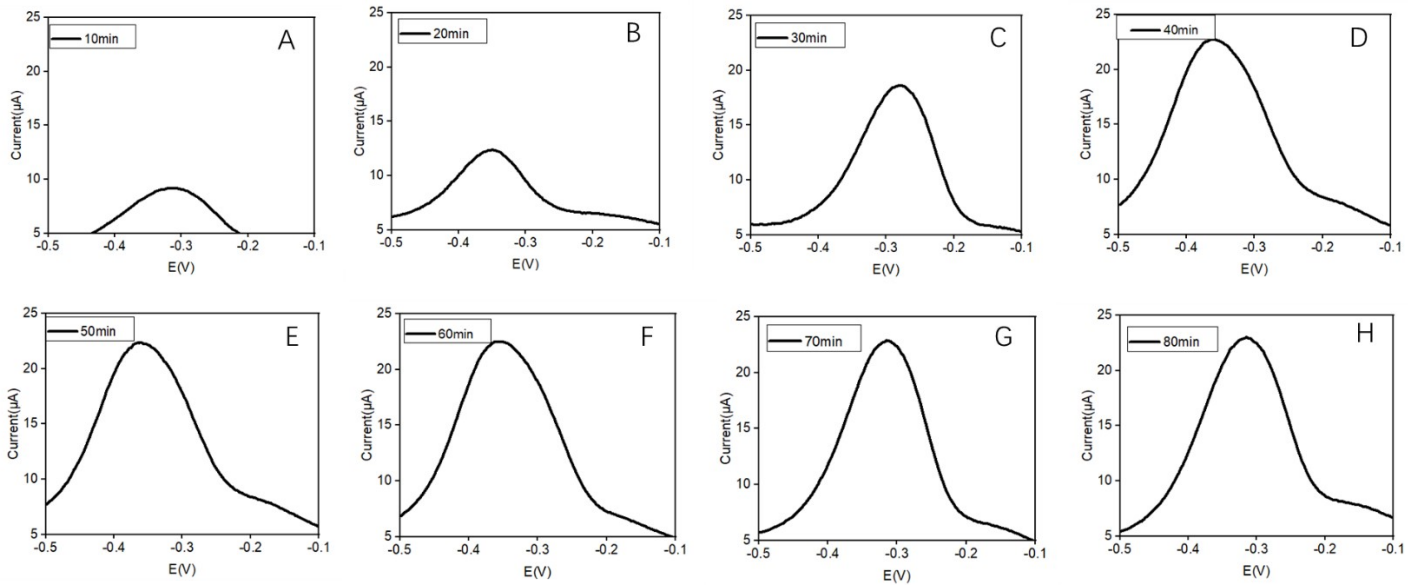

Fig.S3.Electrochemical sensor response for different antibody incubation times (10- 80 min)