

Label-Free and Homogeneous Electrochemical Biosensor for Flap Endonuclease 1 Based on the Target-Triggered Difference in Electrostatic Interaction between Molecular Indicators and Electrode Surface

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1. Experimental section

1.1. *Materials and reagents.*

T4 DNA Ligase, Bst DNA polymerase (large fragment) with corresponding buffer solutions, deoxynucleotide (dNTPs) were obtained from New England BioLabs (Beijing, China). FEN1 in conjunction with buffer solutions was bought from NEB (Beijing, China). Exonuclease I and III (Exo I and III) were purchased from Thermo Fisher Scientific (Shanghai, China). SYBR Green I (10,000×) was supplied by Xiamen Biovision Biotechnology Co., Ltd. (Xiamen, China). 20× PBS buffer was acquired from Aladdin Chemistry Co. (Shanghai, China). Methylene blue (MB) was sourced from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

1.2. *Cell Culture and Protein Extraction.*

Human immortalized epidermal cells (HaCaT) and human gastric adenocarcinoma cells (AGS) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin, in a humidified atmosphere with 5% CO₂ at 37 °C. The cellular extracts were harvested by RIPA according to the protocol. Finally, the obtained total protein extracts were diluted and stored at -20 °C before using for further FEN1 detection.

2. Supplementary figures

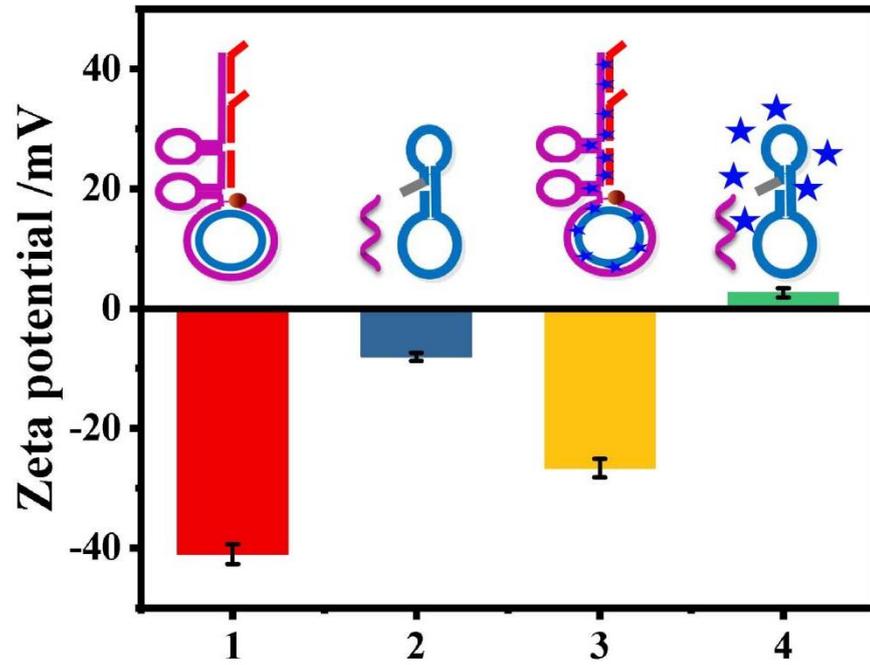


Figure S1. Zeta potential characterization of this biosensor.

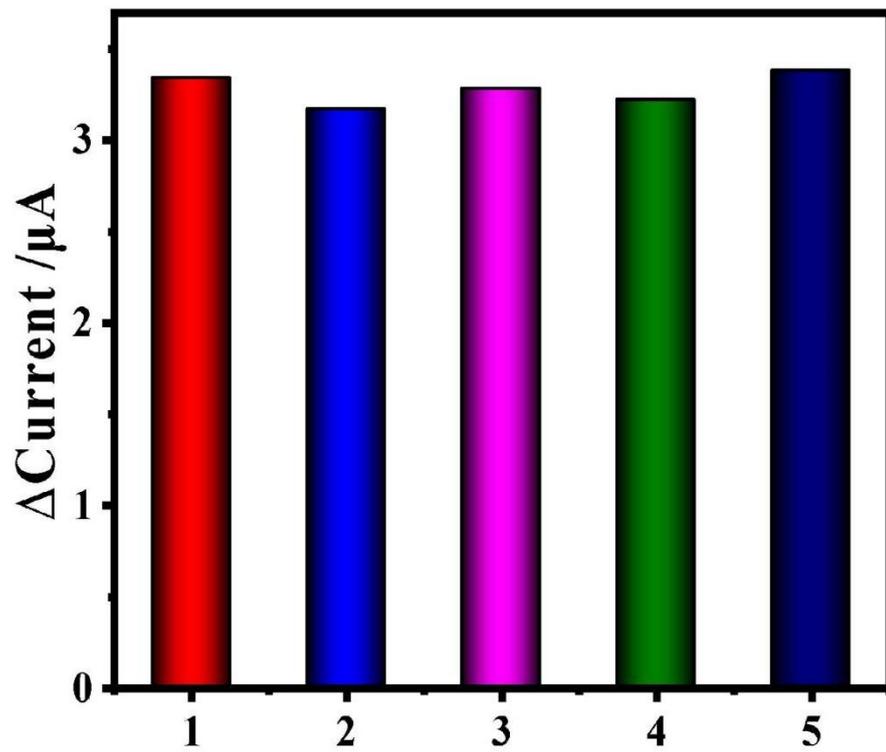


Figure S2. Stability of the proposed biosensor.