18th National and 3rd International Conference of Iranian Biophysical chemistry

هجدهمین همایش ملی و سومین همایش بین المللی بیوشیمی فیزیک ایران

25-26 Des, 2024, University of Hormozgan

۶-۵ دی ماه ۱۴۰۳، دانشگاه هرمزگان

CRISPR/Cas-based impedimetric biosensor for detection of SARS-CoV-2

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Abstract

In this study, we designed a CRISPR/Cas-based impedimetric biosensor which operates as follows: The 20-mer poly adenine probes are immobilized on the surface of a gold working electrode, and their free thiolated tails are bound with gold magnetic nanoparticles. We designed a single guide RNA (sgRNA) targeting the conserved region of ORF1ab in the SARS-CoV-2 virus. In the presence of target, the sgRNA binds to the target sequence and activates Cas12a. The collateral nuclease activity of Cas12a, once activated, cleaves the immobilized probe. Consequently, the gold magnetic nanoparticles are released and adsorbed onto the gold electrode surface using an external magnet. The absorption of nanoparticles increases the physical surface area of the gold electrode, facilitating redox ion electron transfer, and decreasing the charge transfer resistance. We utilized a low-cost polytetrafluoroethylene setup equipped with three electrodes for the impedimetric detection of target nucleic acid. The amplification-free setup demonstrates high specificity and sensitivity for detecting SARS-CoV-2 samples with a detection limit of 8.3 fM and a linear responce range for concentrations from 0.7 to 175 pM. Due to its simplicity and low reagent cost, this electrochemical biosensing platform, utilizing CRISPR/Cas and gold magnetic nanoparticles, shows great potential as a reliable biosensor for detection of nucleic acid-based targets.

Key words: Biosensor, Impedimetric detection, SARS-CoV-2, CRISPR/Cas