

Supplementary data

An Enzymatic Biosensor for the Detection of D-2-Hydroxyglutaric Acid in Serum and Urine

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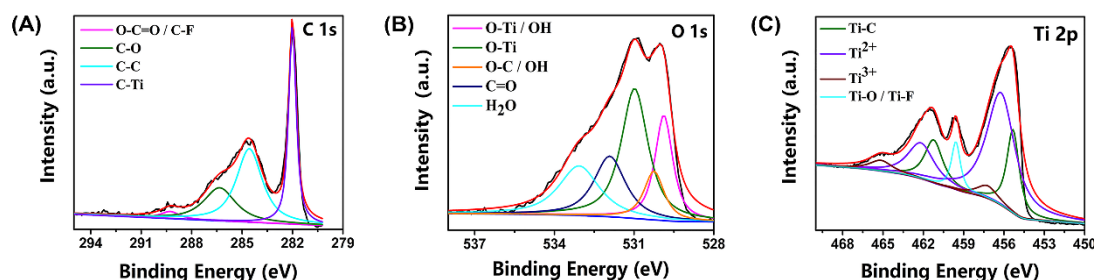


Figure S1. Curve-fitted XPS spectrum related to (A) C 1s, (B) O 1s and (C) Ti 2p.

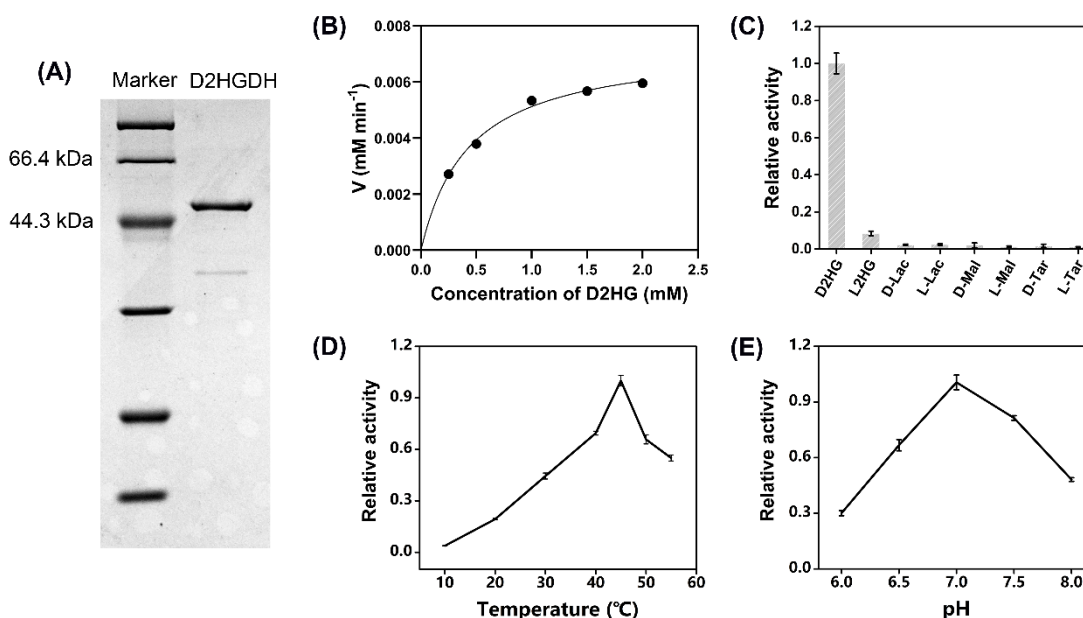


Figure S2. (A) SDS-PAGE of purified RsD2HGDH; (B) enzymatic reaction rates of RsD2HGDH with different substrate concentrations; (C) selectivity of recombinant RsD2HGDH for various substrates, including D/L2HG, D/L-lactate, D/L-malate and D/L-tartrate; (D) relative activity of RsD2HGDH at different temperatures; (E) relative activity of RsD2HGDH at different pHs.

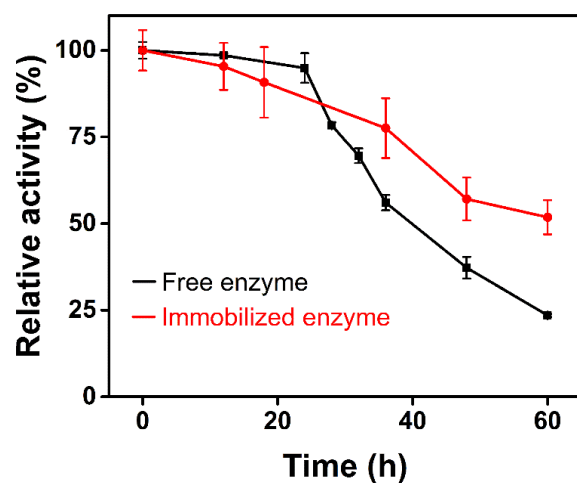


Figure S3. The thermal stability of free *RsD2HGDH* and immobilized *RsD2HGDH* at 25 °C.

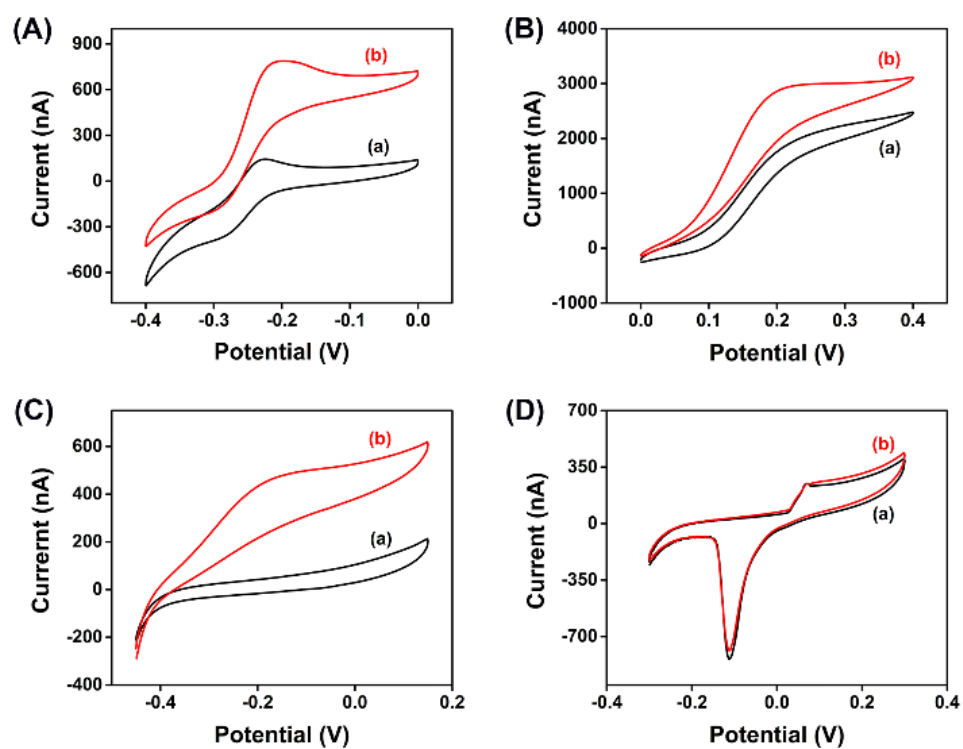


Figure S4. CV curves of enzymatic electrodes prepared with different electron mediators, (A) MB, (B) FC, (C) VK₃ and (D) NQ, with (a) 0 μM and (b) 1 mM D2HG in 50 mM PB (pH 7.0) with 0.1 M KCl at a scan rate of 10 mV s⁻¹ vs. Ag/AgCl.

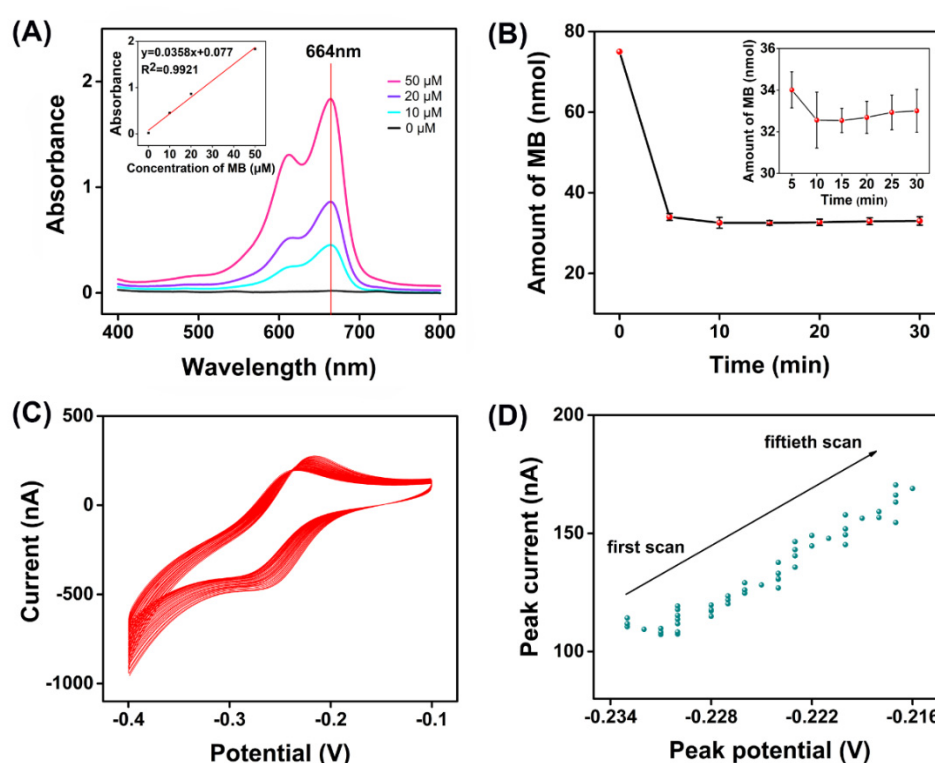


Figure S5. (A) Absorbance of full wavelength scanning with different concentrations of MB. Insert: calibration plot of absorbance value and MB concentration; (B) immobilization amount of MB on electrode surface at different elution times; (C) continuous cyclic voltammetry of the $R_sD2HGDH/MB/MXene/AuSPE$ in 50 mM PB (pH 7.0) with 0.1 M KCl at a scan rate of 10 mV s^{-1} vs. $Ag/AgCl$; (D) the peak current and peak potential of continuous cyclic voltammetry.

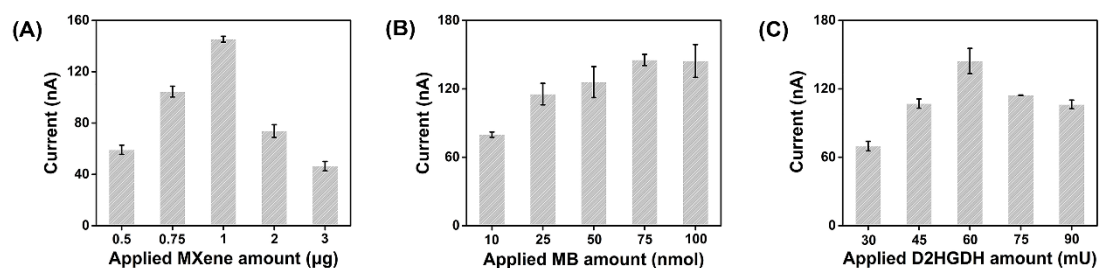


Figure S6. Optimization of the preparation conditions of biosensor, including the amount of (A) MXene, (B) MB and (C) $R_sD2HGDH$ in 50 mM PB (pH 7.0) with 0.1 M KCl containing $50 \mu\text{M}$ D2HG. Applied potential: -0.23 V vs. $Ag/AgCl$.

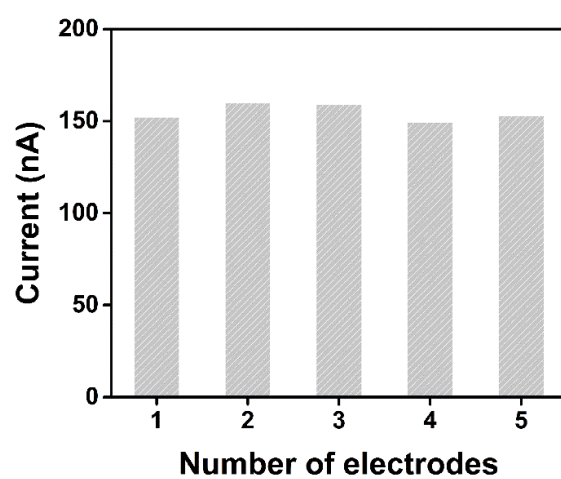


Figure S7. Repeatability of D2HG biosensor in 50 mM PB (pH 7.0) with 0.1M KCl containing 50 μ M D2HG. Applied potential: -0.23 V vs. Ag/AgCl.