Supporting Information

Bioanalytical method for NAD^+ detection in blood plasma utilizing solution-phase *Candida boidinii* formate dehydrogenase and electrochemical detection

Wichit Taron,[a] Tharinda Kasemphong,[b] Pachanuporn Sunon,[a] Keerakit Kaewket,[a] Nuntaporn Kamonsutthipaijit,[c] James R. Ketudat-Cairns,[b] Gun Bhakdisongkhram,[d] Warut Tulalamba,[e,f] Supakmongkon Sanguansuk,[g] Vip Viprakasit,[e,h] Kamonwad Ngamchuea[b]*

[a] Institute of Research and Development, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand

[b] School of Chemistry, Institute of Science, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand

[c] Synchrotron Light Research Institute, 111 University Avenue, Suranaree, Muang District, Nakhon Ratchasima 30000, Thailand

[d] School of Medicine, Institute of Medicine, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand

[e] Siriraj Thalassemia Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

[f] Research Division, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

[g] Research & Development Laboratory, ATGenes Limited Company, Bangkok, Thailand

[h] Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

*Corresponding author: Kamonwad Ngamchuea, School of Chemistry, Institute of Science, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand. Email: kamonwad@g.sut.ac.th. Tel: +66 (0) 44 224 637

S1 UV-visible spectra of CbFDH enzymatic reactions

As discussed in the main text, UV-visible spectrophotometry (200–400 nm) was used to monitor enzymatic reactions involving CbFDH, formate, and NAD^+, verifying the formation of NADH. The corresponding spectra are shown in Figure S1 below, with a detailed discussion provided in the main text.

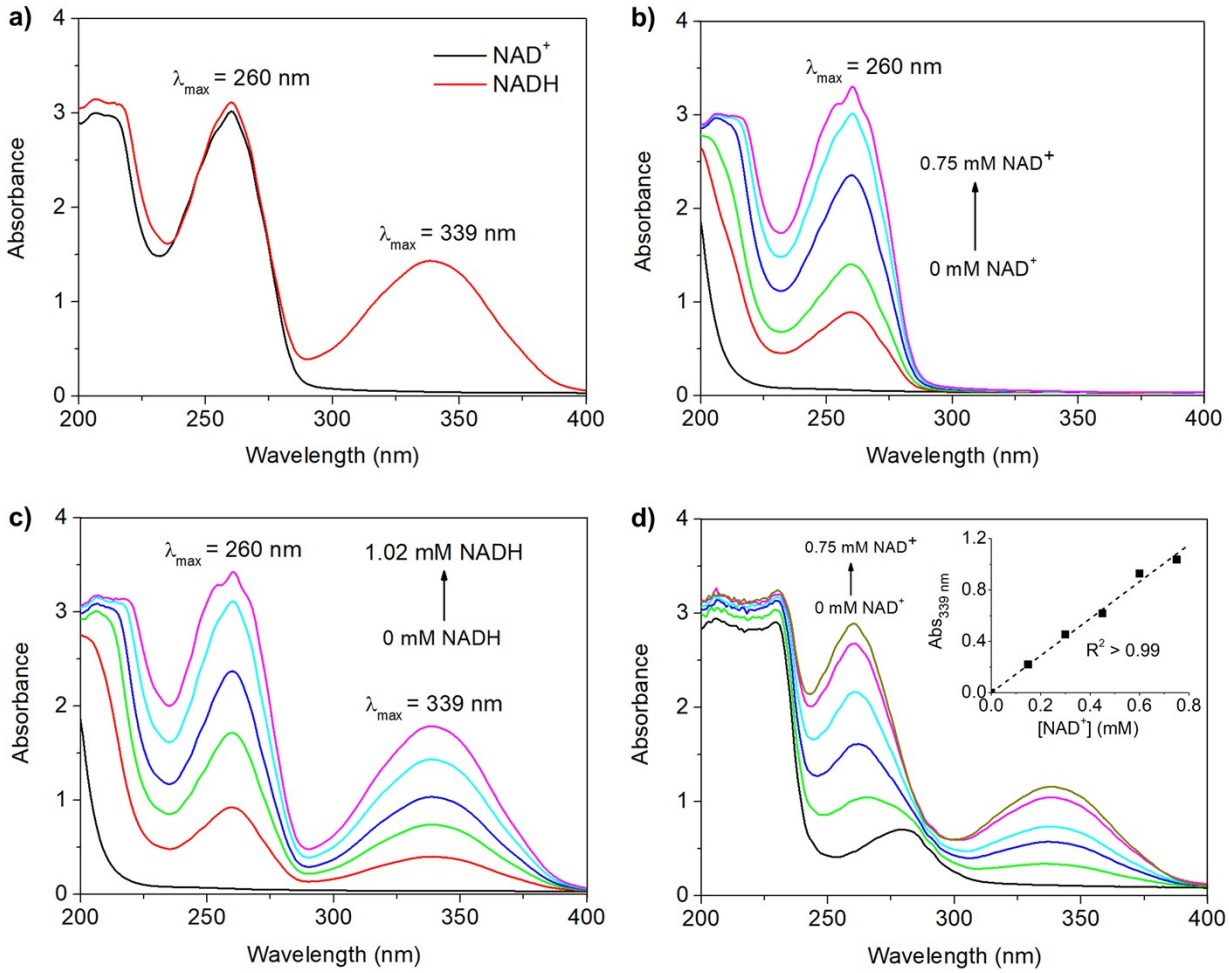

Figure S1: UV-vis spectra of **a)** 0.6 mM NAD^+ vs. 0.8 mM NADH; **b)** varying concentrations of NAD^+ (0, 0.15, 0.30, 0.45, 0.60, and 0.75 mM); **c)** varying concentrations of NADH (0, 0.20, 0.40, 0.60, 0.80, and 1.02 mM); **d)** a mixture of 320 mM formate, 3 U mL^{-1} CbFDH, and varying concentrations of NAD^+ (0, 0.15, 0.30, 0.45, 0.60, and 0.75 mM) in 100-fold diluted blood plasma.

S2 Analytical performance for non-enzymatic detection

Figures 2b and 2c in the main text display cyclic voltammograms of NAD^+ and NADH, respectively, over various concentrations under non-enzymatic conditions at a scan rate of 25 mV s^{-1}. For NAD^+, the electrochemical response exhibits a linear range from 0.43 μM to 10 mM, with a sensitivity of 7.10 ± 0.17 μA mM^{-1}, a limit of detection ($3s_b/m$) of 0.13 μM, and a limit of quantification ($10s_b/m$) of 0.43 μM. Meanwhile, NADH shows an oxidation response with a linear range of 0.57 μM to 12 mM, achieving a sensitivity of 9.98 ± 0.10 μA mM^{-1}, a limit of detection ($3s_b/m$) of 0.17 μM, and a limit of quantification ($10s_b/m$) of 0.57 μM.

S3 Tafel analysis

To assess the electron transfer kinetics, Tafel analysis was performed according to equation 2 in the main text. The plot of ln *I* vs. *E* (Figure S2) was constructed using current values within 15–50% of the peak current in order to minimize the influence of mass transport.[35] The slope of this plot yielded a value of $n' + \beta_{n'+1}$ of 0.53 ± 0.01, indicating that the initial electron transfer is the rate-determining step. The corresponding apparent anodic transfer coefficient $\beta_{n'+1}$ for this step was therefore 0.53 ± 0.01, as discussed in the main text.

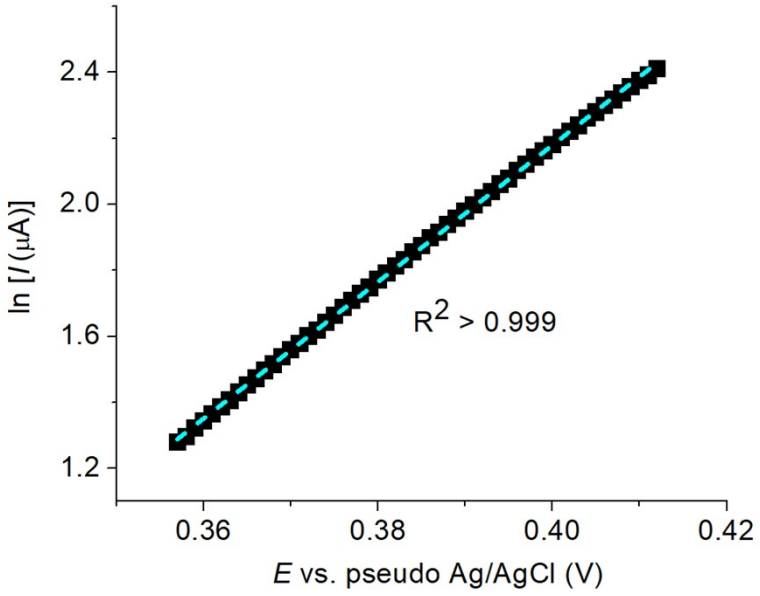

Figure S2: The plot of ln *I* vs. *E* in the Tafel analysis of 5 mM NADH in 0.1 M PBS pH 7.6 and 0.1 M KCl at a scan rate of 10 mV s^{-1}. Current values used in the analysis were taken within 15–50% of the peak current.

S4 Scan rate studies: log I_p versus log υ plot

The plot of log I_p vs. log υ (Figure S3) exhibited a slope of 0.46 ($R^2 > 0.999$), which closely approximates the theoretical value of 0.5. This agreement supports the conclusion that the NADH oxidation at graphite SPEs is predominantly diffusion-controlled, as discussed in the main text.

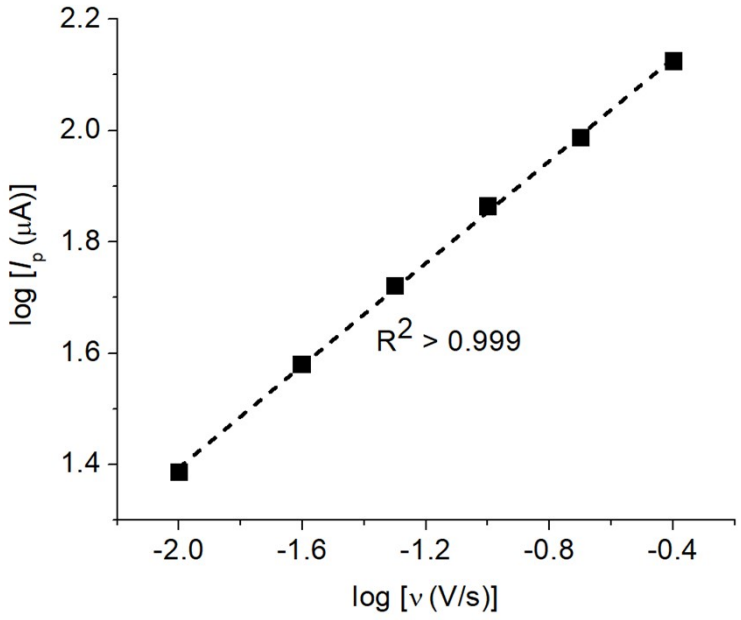

Figure S3: log I_p vs. log υ plot of 5 mM NADH in 0.1 M PBS pH 7.6 and 0.1 M KCl, at scan rates ranging from 10 to 400 mV s^{-1}.