Supporting Information

Washing-Free Displacement Immunosensor for Cortisol in Human Serum Containing Numerous Interfering Species

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Preparation of DI-Modified ITO Electrodes. 70 μL of PBSB containing 100 μg/mL of DI was dropped on bare ITO electrodes for 2 h at 4 °C and then washed with rinsing buffer.

Procedure of Displacement Assay with Washing. 70 μL of TBSB containing 10 μg/mL anti-cortisol IgG-DI conjugate was dropped onto ITO electrodes modified with cortisol BSA and BSA (cortisol-BSA:BSA = 1:10). The electrodes were maintained in the treated state for 30 min at 25 °C, followed by washing with rinsing buffer. For the displacement assay, 70 μL of PBS containing 1 nM cortisol was dropped onto the electrodes. This state maintained for 30 min at 25 °C, followed by washing with rinsing buffer. Finally, chronocoulograms were obtained at an applied potential of 0.05 V after an incubation period of 10 min at 25 °C in PBS containing 10 μM $Os(bpy)_2Cl_2$ and 1.0 mM NADH.

Procedure of Competitive Assay with Washing. 70 μL of TBSB containing 10 μg/mL anti-cortisol IgG-DI conjugate and 1 nM cortisol was dropped onto ITO electrodes modified with cortisol BSA and BSA (cortisol-BSA:BSA = 1:10). The electrodes were maintained in the treated state for 30 min at 25 °C, followed by washing with rinsing buffer. Finally, chronocoulograms were obtained at an applied potential of 0.05 V after an incubation period of 10 min at 25 °C in PBS containing 10 μM $Os(bpy)_2Cl_2$ and 1.0 mM NADH.

Procedure of Washing-Free Competitive Assay. 1 mL of TBSB containing 1 μg/mL anti-cortisol IgG-DI conjugate, 1 nM cortisol, 10 μM $Os(bpy)_2Cl_2$, and 1.0 mM NADH was dropped onto ITO electrodes modified with cortisol BSA and BSA (cortisol-BSA:BSA = 1:10). The electrodes were maintained in the treated state for 10 min at 25 °C. Finally, chronocoulograms were obtained at an applied potential of 0.05 V.

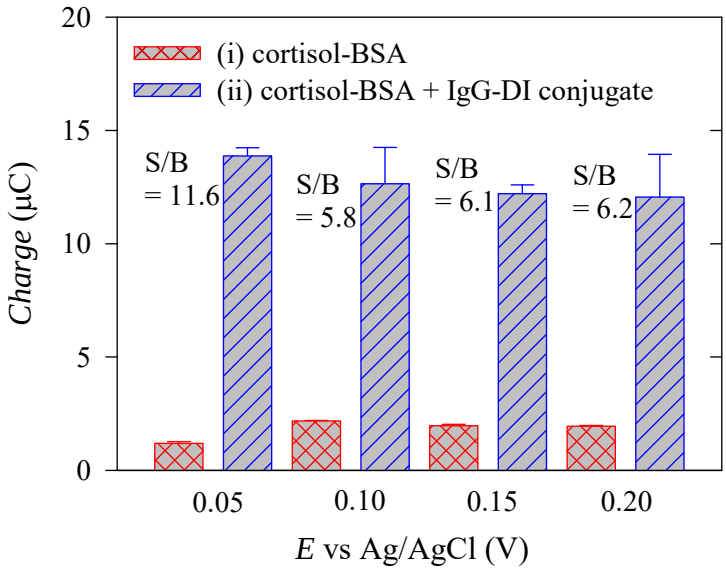

Figure S-1. Histogram of the charge values measured at 100 s in the chronocoulograms obtained at different applied potentials (0.05 V, 0.10 V, 0.15 V and 0.2 V) after an incubation period of 10 min at 25 °C in PBS containing 10 µM $Os(bpy)_2Cl_2$ and 1.0 mM NADH.

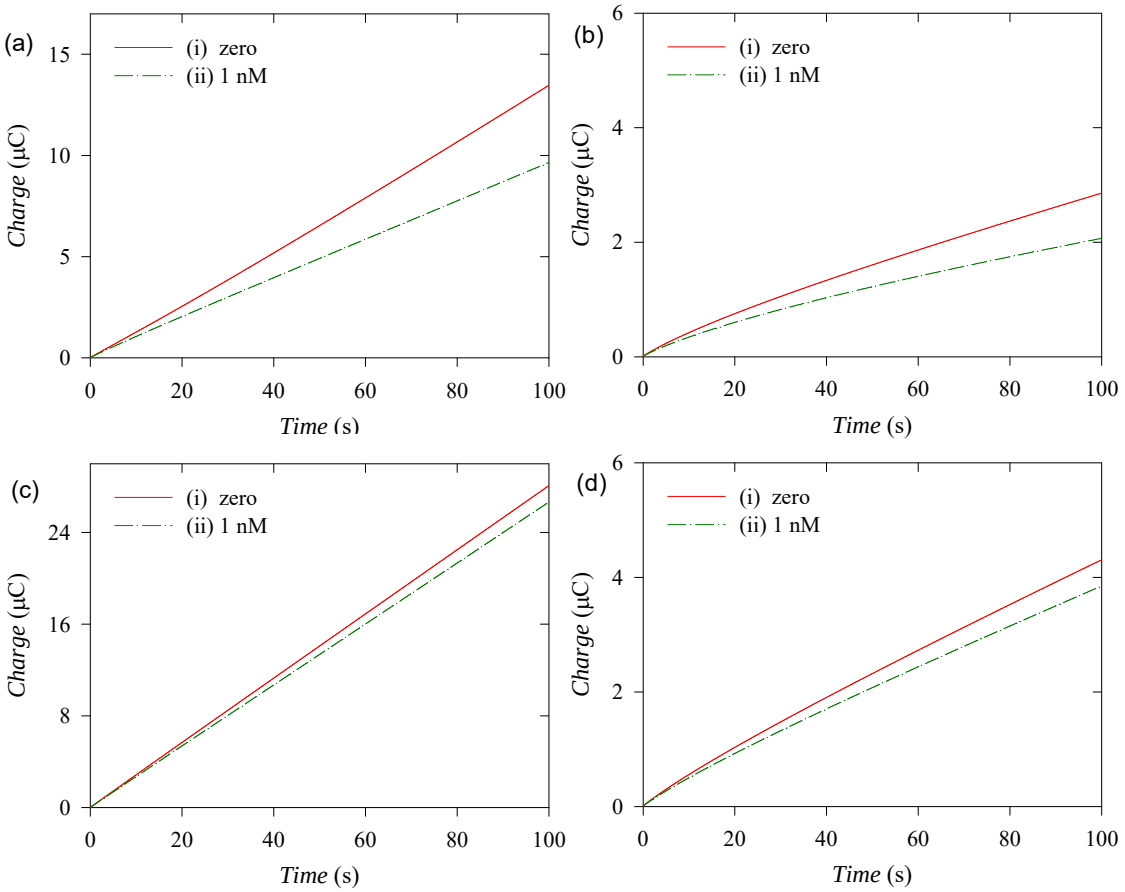

Figure S-2. Chronocoulograms obtained at an applied potential of 0.05 V after an incubation period of 10 min at 25 °C in PBS containing 10 µM $Os(bpy)_2Cl_2$ and 1.0 mM NADH, for the detection of zero and 1 nM cortisol using four different methods: (a) washing-free displacement assay, (b) displacement assay with washing, (c) washing-free competitive assay, and (d) competitive assay with washing.

Table S-1. Comparison data for chronocoulograms shown in Figure 5. The charge values measured at 100 s were compared.

Condition (cortisol-BSA: BSA)	Bare (μC)	Harsh washing (μC)	Zero (μC)	100 μM cortisol (μC)
1:0	1.08	2.14	8.89	7.93
1:3	1.29	3.16	11.2	8.16
1:10	1.23	2.17	13.5	6.59
1:30	1.07	2.41	12.3	7.68

Table S-2. Comparison of the developed method with other detection methods.

Ref.	Method	Preparation period (after sample injection)	Detection limit	Detection range
This work	Chronocoulometry	12 min	30 pM	30 pM − 1 μM
1	Square Wave Voltammetry	-	10 μM	10 – 80 μM
2	Cyclic Voltammetry	35 min	28 pM	28 pM – 1.4 μM
3	Luminescence	30 min	100 pM	100 pM – 10 μM
4	Fluorescence	3 h	300 pM	300 pM – 30 nM
5	Electrochemical Impedance Spectroscopy	30 min	1 pM	1 pM – 10 nM
6	Fluorescence	3 h	28 nM	28 nM – 3 μM
7	Enzyme Immunoassay	Overnight	1 pM/well	1 – 280 pM/well

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