

Supporting Information

Personal glucose sensor for point-of-care early cancer diagnosis

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Experimental Section

Materials: Bovine serum albumin (BSA), invertase from baker's yeast, sucrose and Tween 20 were received from Sigma (St. Louis, MO). Sulfo-NHS-LC-biotinylation kit and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Pierce Biotechnology (Rockford, IL, USA). Streptavidin-coated magnetic beads (MyOne™ Streptavidin C1) were obtained from Invitrogen Corp. (Oslo, Norway). Primary CEA antibody (Ab₁), biotin-conjugated secondary CEA antibody (biotin-Ab₂), CEA, AFP, CA125, mouse IgG and the commercial CEA ELISA kit were all ordered from Biocell Co., Ltd (Zhengzhou, China). Human serum samples were provided by the 9th people's hospital of Chongqing (Chongqing, China). The PGS (Contour™TS) and test strips were from Bayer Healthcare LLC (Mishawaka, IN).

Preparation of biotin-invertase: It was reported that the reaction with amines of invertase would not affect the enzyme natural activity.¹⁻³ Biotin was conjugated to the amines of invertase by using a sulfo-NHS-LC-biotinylation kit. In brief, the sulfo-NHS-

LS-biotin (67 μL , 10 mM) was added to an invertase solution (1 mL, 5mg mL⁻¹). The mixture was incubated at room temperature for 1 h, followed by removal of excess biotin reagent and collection of the resulting biotin-invertase with a desalting column.

PGS-based CEA assay protocol: An aliquot (100 μL , 10 mg mL⁻¹) of the streptavidin coated magnetic beads (STV-MB) were washed twice with PBS and incubated with 100 μL PBS buffer containing biotin-invertase (0.5 mg mL⁻¹) and biton-Ab₂ (5 μg mL⁻¹) for 30 min. Then the invertase/Ab₂-MB conjugates were separated, washed with PBS and resuspended in PBS-BSA (1 % BSA) for further use.

The 96 well polystyrene microplate (NUNC, Roskilde, Denmark) was first coated with Ab₁ antibody (10 μg mL⁻¹, 100 μL , in carbonate buffer, PH 9.6) at 4°C overnight. The wells were then washed with 200 μL PBST (0.01 % Tween-20) for three times and blocked with 200 μL PBS-BSA. Next, 100 μL of CEA solution at different concentration or serum samples were added to the wells. After incubation at 37 °C for 1 h, the wells were washed with PBST for three times. Subsequently, the invertase/Ab₂-MB solution (100 μL) was incubated with the wells at 37 °C for 1 h, followed by washing with PBST. Finally, the sucrose solution (0.5 M, 100 μL) were added to the wells and allowed to react at 37 °C for 0.5 h. The enzymatically produced glucose was then monitored by the PGS.

References:

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