# Supplementary information for

# Graphene Oxide and Exonuclease-aided Amplification Immuno-Sensor for Antigen Detection

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## Experimental section

### Materials

All oligonucleotide with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

(1) 5'- GGAGTAAATGTTGGAGAACAGTATC - biotin -3' (biotin DNA);

(2) 5'- CTGTTCTCCAACATTTACTCC- FAM -3' (FAM-DNA);

Capture antibody, biotin antibody and antigen (AFP, IgG, PSA, CEA) were purchased from Shanghai Linc-Bio Science Co (Shanghai, China). Exonulease III was purchased

from Fermentas (Canada). Bovine serum albumin (BSA) and bovine hemoglobin were purchased from Biosharp. Streptavidin was obtained from Amresco (USA). Tris (hydroxymethyl) aminomethane hydrochloride (Tris) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Graphene oxide (GO) was purchased from Sinocarbon Materials Technology Co., Ltd. The human serum sample was supplied by The Zhongnan Hospital of Wuhan University.

All other chemicals not mentioned here were of analytical-reagent grade or better. 18.2 MΩ·cm at 25 °C water purified by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA) was used throughout.

**Instruments**

Fluorimetric spectra were obtained with a RF-5301PC spectrophotometer (Shimadzu, Japan) equipped with a 150 W xenon lamp (Ushio Inc, Japan). Absorption spectra were recorded on a UV-2550 spectrometer (Shimadzu, Japan).

**Immuno probe preparation procedure for AFP detection**

The 96-well microplate was coated with 100 μL capture antibody solution (20 μg/ml) for 5 h. Then the substrate was blocked with 200 μL blocking buffer (Tris 10 mM, NaCl 150 mM, BSA 1%, pH 8.0) for 1h. Subsequently, a 50 μL AFP standards or samples and 10 μL biotin antibody (0.1mg/ml) solution was injected. After incubation for 30 min at 37 °C, the resulting mixture was washed three times with washing buffer (Tris 10 mM, NaCl 150 mM, Tween 0.01%, pH 8.0). Then 10 μL streptavidin (0.5 mg/ml) and 90 μL Tris-HCl buffer (Tris 10 mM, NaCl 150 mM, $MgCl_2$ 5 mM, pH 8.0) were added. After

incubation for 30 min at 37 °C, the resulting mixture was washed three times with washing buffer. Afterward, 100 μL biotin DNA ($10^{-8}$M) solution was added and incubated for 30 min at 37 °C and washed by the washing buffer for three times. Subsequently, 100 units of Exo III and 6 nM FAM-DNA target to a final volume of 200 μL, followed by incubating at 37 °C for 100 min. Finally, the reaction solution was taken out, and then added 4 μg/ml GO and Tris-HCl buffer solution to a total volume 500 μL. The fluorescence signals of the supernatant were measured with scanning fluorescence spectrometry. Excitation wavelength was 485 nm and the scan range is from 490 nm to 650 nm.

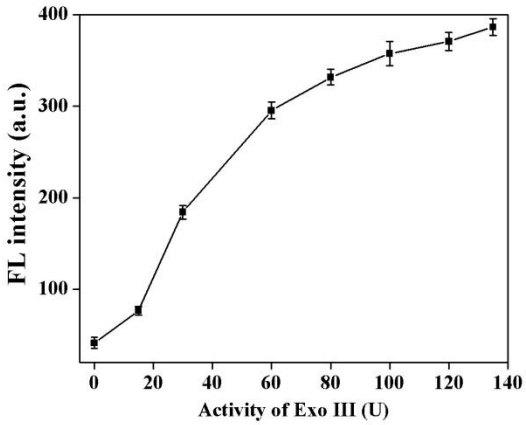


Fig. S1 Effects of concentration of Exo III for detection on the 100 pg/ml AFP. The reaction time was 120 min.

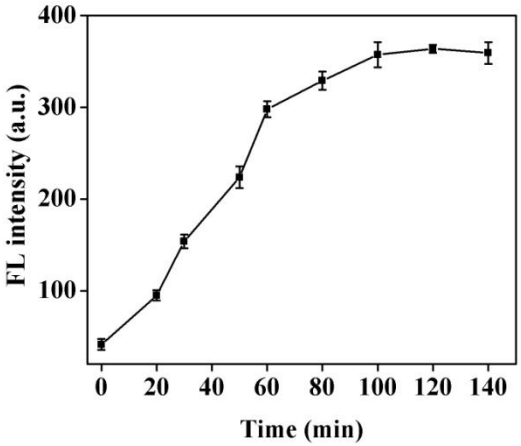


Fig. S2 Effects of the reaction time for detection on the 100 pg/ml AFP. The concentration of Exo III was 100 U.

Table S1 Determination of AFP added in normal human serum with this proposed immunosensor.

| Sample number | Add (ng/ml) | Found (ng/ml) | Recovery % |
|---|---|---|---|
| **1** | 1 | 0.976 | 97.6 |
| **2** | 2 | 2.104 | 105.2 |
| **3** | 3 | 2.786 | 92.9 |
| **4** | 4 | 4.140 | 103.5 |
| **5** | 5 | 4.857 | 97.1 |