Supplementary Information

A novel colorimetric immunoassay strategy using iron(III) oxide magnetic nanoparticle as a label for signal generation and amplification

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1. Optimization of the concentration of AA/BPT

The effect of AA and BPT concentration on the color development was investigated to obtain the optimal signal intensity. We assumed that the microwell bottom was fully covered by a monolayer of the magnetic nanoparticle(MNP). Thus the maximum amount of the nanoparticle in each microwell was determined to be about 1.085 μg according to its average diameter (9.7nm) and density (5.24g cm^{-3}). A same amount of MNP at 1.085 μg was injected into each microwell, followed by addition of AA/BPT at different concentrations for color development. As shown in Fig. S1, the absorbance of the BPT-Fe^{2+} complex gradually raise along with the increase of AA and BPT concentration. The absorbance gets a plateau when AA is not less than 970 mM and BPT is not less than 7 mM (as shown in the green rectangle). This result indicates that γ-Fe_2O_3 MNPs are completely dissolved and converted into soluble red BPT-Fe^{2+} complex when AA and BPT concentration is higher than the cut-off value. Therefore, 970mM AA and 7mM BPT were utilized as the optimal concentration in the following experiments.

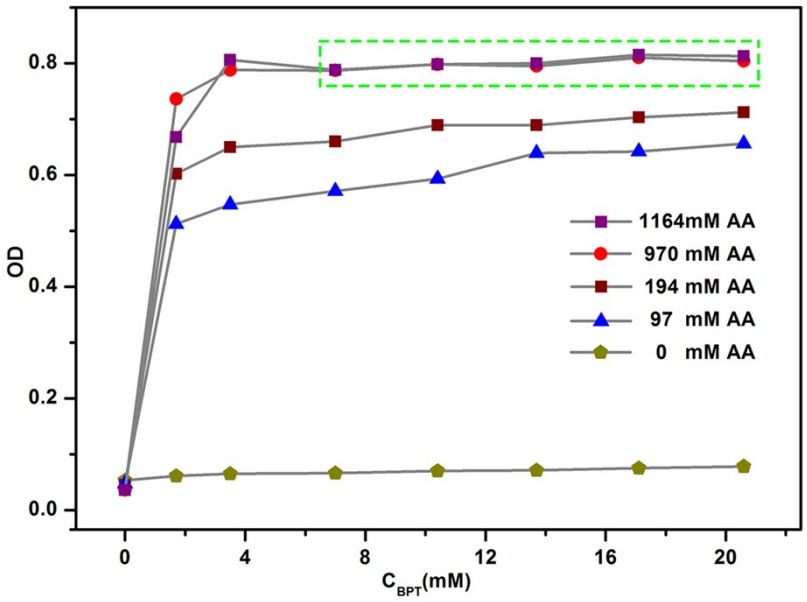

Fig. S1 UV-Vis absorbance as a function of AA and BPT concentration.

2. Optimization of the concentration of MNP-CA-Ab2

We studied the influence of MNP-CA-Ab2 concentration on signal to noise ratio in the sandwich immunoassay. As shown in Fig. S2, the absorbance at 535nm significantly increases when the concentration of MNP-CA-Ab2 is increased. The signal is very weak at four times dilution. Although the highest signal is observed from the original MNP-CA-Ab2 solution, the background signal is too high, resulting in a poor signal to noise ratio. The highest signal to noise ratio is observed from the group with twice dilution.

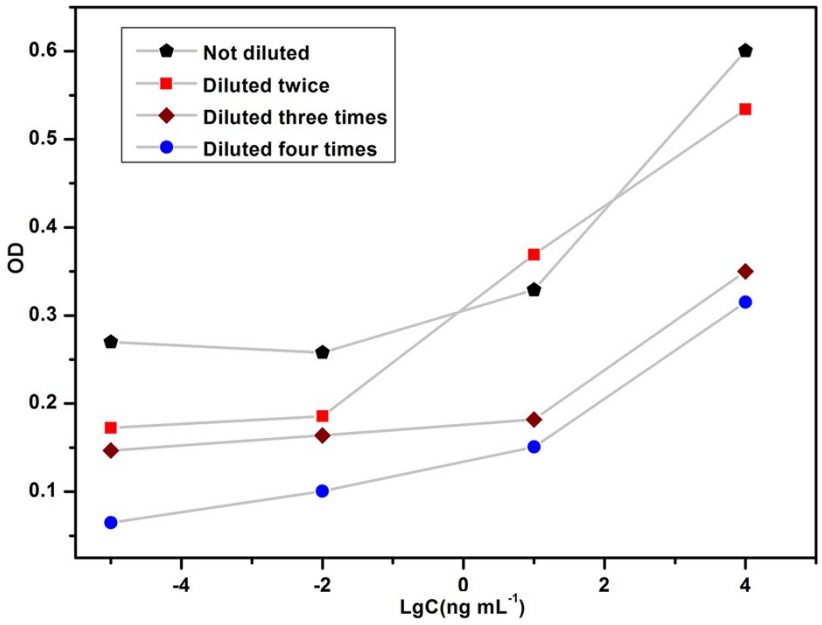

Fig. S2 Optimization of MNP-CA-Ab2 concentration. Four rabbit IgG concentrations

at 0, 10pg mL^{-1}, 10ng mL^{-1}, 10μg mL^{-1} were tested in this experiment.

3. Selectivity of the colorimetric immunoassay

The selectivity was evaluated by performing CEA detection in PBS and 10% fetal bovine serum(FBS), in which there are many interferences including proteins, amino acids, nucleic acids, carbohydrates, and small molecules. Fig. S4 shows the results of CEA detection at 0 and 100ng mL^{-1}. As for both 0 and 100ng mL^{-1}, relative lower signal intensities are observed from 10% FBS in comparison with PBS group. It apparently results from that a high level of protein in 10% FBS passivates the surface of microplate, thus reducing the nonspecific adsorption of CEA during immunoassay. The lower background absorbance and the higher signal to noise ratio from 10% FBS group demonstrates the good selectivity of the developed colorimetric immunoassay.

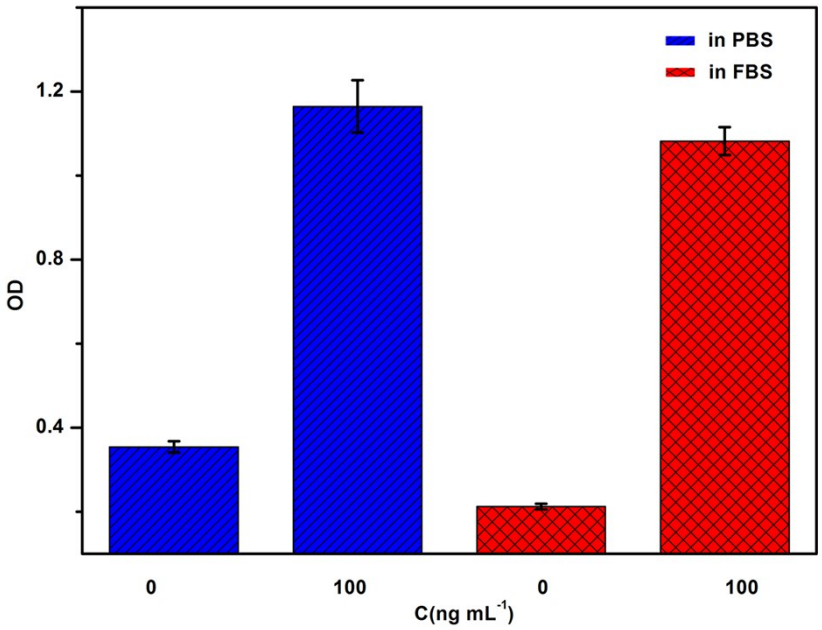

Fig. S3 UV-Vis absorbance from CEA detection in PBS and 10% FBS. CEA at concentrations of 0 and 100ng mL^{-1} was tested in this experiment.