1 ## Supporting Information

2 ## Au/Fe₃O₄-Based Nanozymes with Peroxidase-Like Activity

3 ## Integrated in Immunochromatographic Strips for Highly Sensitive

4 ## Biomarker Detection

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19 **1. The synthesis of pFe₃O₄ NPs**

20   Typically, $FeCl_3 \cdot 6H_2O$ (2.14 mM, 0.50 g), carbamide (15.0 mM, 0.90 g) and PAA

21 (0.5 mM, 1.00 g) were dissolved in ethylene glycol (20 mL). After magnetic stirring

22 and sonication treatment for 30 min respectively, the dark yellow transparent solution

23 was transferred to a Teflon-lined stainless-steel autoclave. It was heated at 200°C for 8

1 h and then cooled down to room temperature. After that, the black precipitate was rinsed

2 with water and ethanol three times each and then redispersed in water, stored at 4 ℃

3 for use.

2. XPS analysis

To further confirm the composition and chemical configuration of the obtained

nanoparticles, XPS analysis was carried out. Figure S1 shows the Fe 2p, O 1s and Au

4f, belongs to Fe_3O_4 NPs or Fe_3O_4/Au NPs were observed. For the Fe 2p XPS spectrum

(Fig. S1a), the peaks corresponding to $Fe2p_{3/2}$ and $Fe2p_{1/2}$ of Fe (III) were located at

710.8 and 724.5 eV, respectively. The Au 4f can be separated into 83.5 and 87.5 eV

corresponding to the characteristic of the zero valence peaks of elemental Au (Fig. S1c).
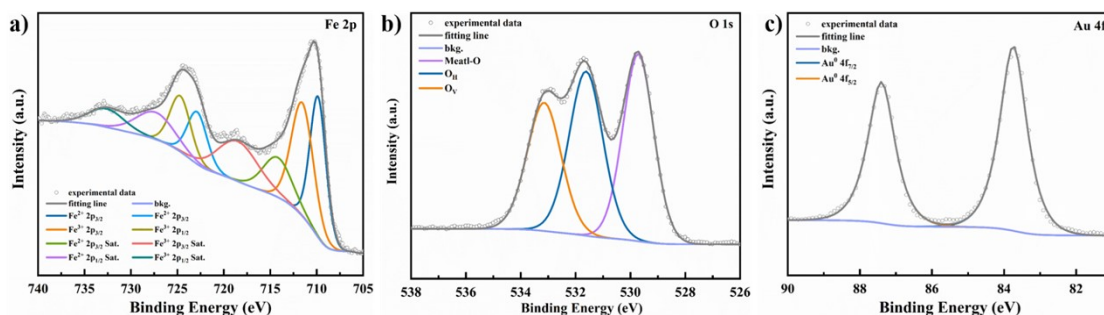
Fig. S1 The precise valence states for different elements. (a) Fe 2p, (b) O 1s, (c) Au 4f

3. Parameter optimization of catalytic reaction

Similar to HRP, the pH value, H_2O_2 concentration and DAB concentration of the

reaction system effect the catalytic activity of nanomaterials greatly. In this work, the

optimum conditions for catalytic reaction of three nanomaterials were determined by

the absorption value in 461 nm. In the first 500 s of the reaction, the adsorption was

plotted when pH value ranges pH 3.0-7.5, H_2O_2 concentration is 0.05-1.0 mM and DAB

concentration is 0.1~1.0 mM, and the maximum adsorption was taken as 100%.
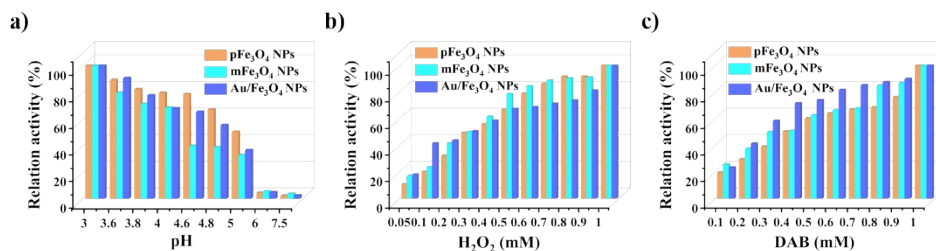
Fig. S2. Dependency of pFe$_3$O$_4$, mFe$_3$O$_4$ and Au/Fe$_3$O$_4$ NPs catalytic activity on (a) pH, (b) H$_2$O$_2$, (c) DAB, and the optimize parameters are pH 3.0, H$_2$O$_2$ concentration 1.0 mM, and DAB concentration 1.0 mM.

As shown in Figure S2, the catalytic activity of the three particles reached the maximum when the pH value was 3.0, and the catalytic activity decreased continuously until alkaline condition was applied. The catalytic activity of the particles was inhibited when pH > 5, probably due to the decrease of the binding ability of DAB with the nanozyme and the decomposition of H$_2$O$_2$ in neutral and alkaline conditions, leading to the hydroxyl radical (HO·) reduced greatly and then the reduced reactivity. At the same time, when pH > 5, decomposition of accelerates, the effective concentration of the reaction decreases, and. Therefore, pH 3.0 was selected in the reaction system.

The presence of H$_2$O$_2$ is essential for the catalytic activity of nanoparticles in peroxide catalytic experiment. As shown in the Figure S2(b), with the increase of concentration of H$_2$O$_2$, the catalytic activity increased and reached to the highest activity when the concentration is 1.0 mM, indicating the material we prepared had high catalytic stability. The effect of substrate DAB concentration on catalytic performance shows in Figure S2(c), with the increase of substrate concentration, the relative reactivity increased and reaches the maximum when DAB concentration was 1.0 mM. Therefore, the optimal reaction conditions selected in this experiment are as follows:

1 pH value is 3.0, [H_2O_2] 1.0 mM, and [DAB] 1.0 mM.

2 **2. The stability of nanozyme**

3 Magnetic nanoparticles were stored in cold storage at 4 °C for one month, which

4 had little effect on the catalytic performance of the materials. After one month, the

5 catalytic activities of the three magnetic materials still remained above 90%, as shown

6 in Figure S3.

7 These results indicate that pFe_3O_4, mFe_3O_4 and Au/Fe_3O_4 NPs have good

8 reproducibility and stability, and have a broader application prospect in the field of
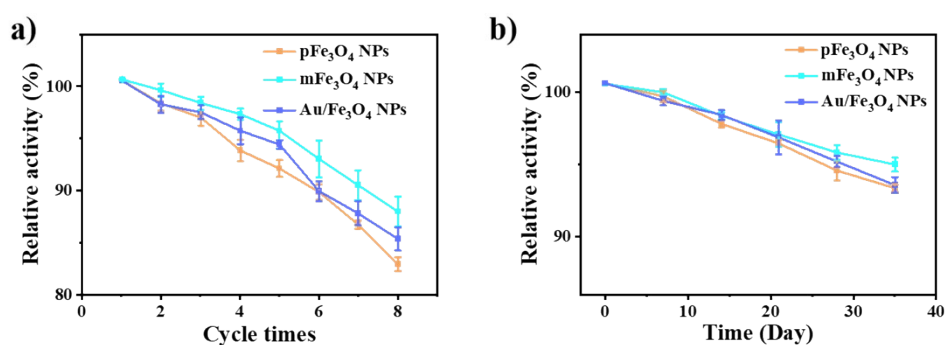

9 bioanalysis and medicine.

10 **Fig. S3.** The stability test of pFe_3O_4, mFe_3O_4 and Au/Fe_3O_4 nanoparticles by storing these

11 materials for one month at 4 °C.

12 **3. The parameters in the synthesis of nano-probes**

13 The weight ratio of EDC:NHS, and the amount of antibodies were optimized

14 during the preparation of probes. As shown in Figure S4(a), when 1 mg of Au/Fe_3O_4

15 NPs as labeled material and the amount of β-hCG antibody was fixed as 100 μg. The

16 mass of the EDC was fixed at 0.0050 g, with the ratio of NHS:EDC increase from 0.54,

17 0.77, 1 to 1.5, the coupling rate increased from 53.8% to 96.87 %, and then a little bit

1 decreases when the ratio beyond 1.6. The maximum coupling rate was obtained when

2 the ratio of NHS:EDC is 1.5, while the particles tend to form irreversible aggregations

3 due to the excessive ester groups instead of carboxylate groups on the surface of

4 nanoparticle, leading to the weaken of electrostatic repulsion between nanoparticle and

5 the instability of nanoparticles. Therefore, the optimal mass ratio of NHS:EDC is 1,

6 coupling rate is 95.7%. Moreover, the coupling time effected significantly on the

7 coupling rate, as shown in Figure S4(b), the optimal time was 3 hours and the coupling

8 rate was 94.6%.
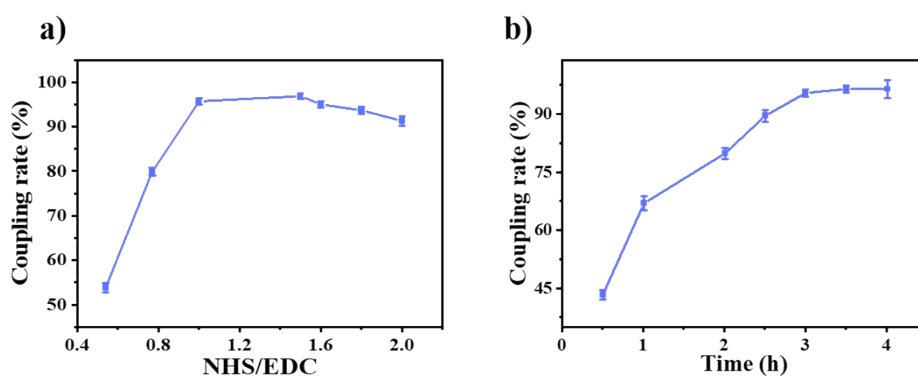
9 **Fig. S4.** The effect of NHS:EDC ratio on the coupling rate (a) and the coupling time effect the

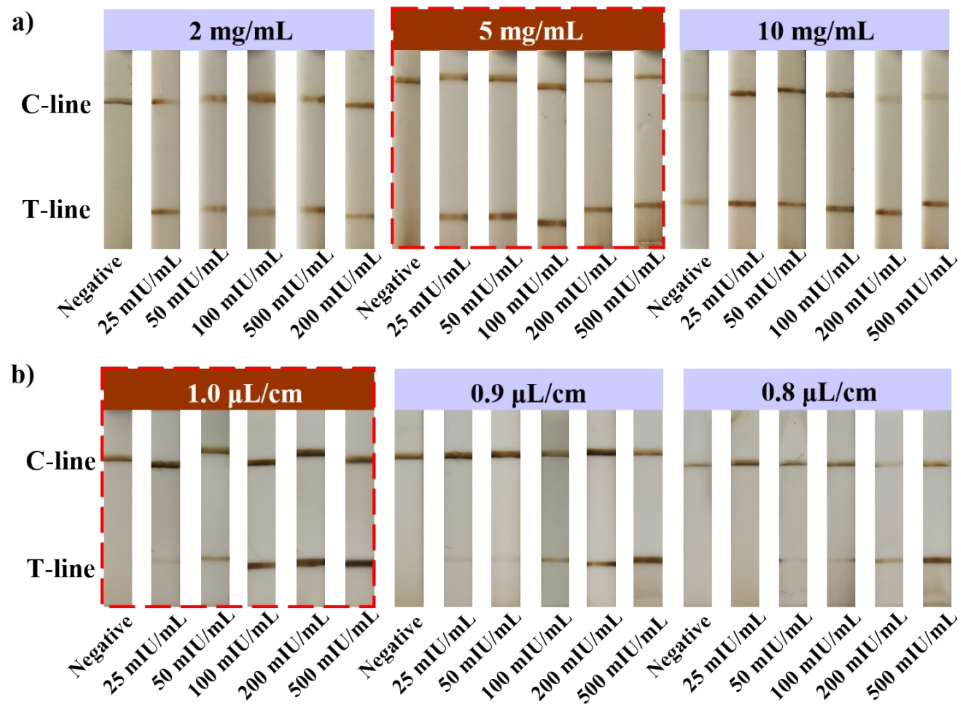10 coupling rate (b)

11 **4. The optimization of detection**
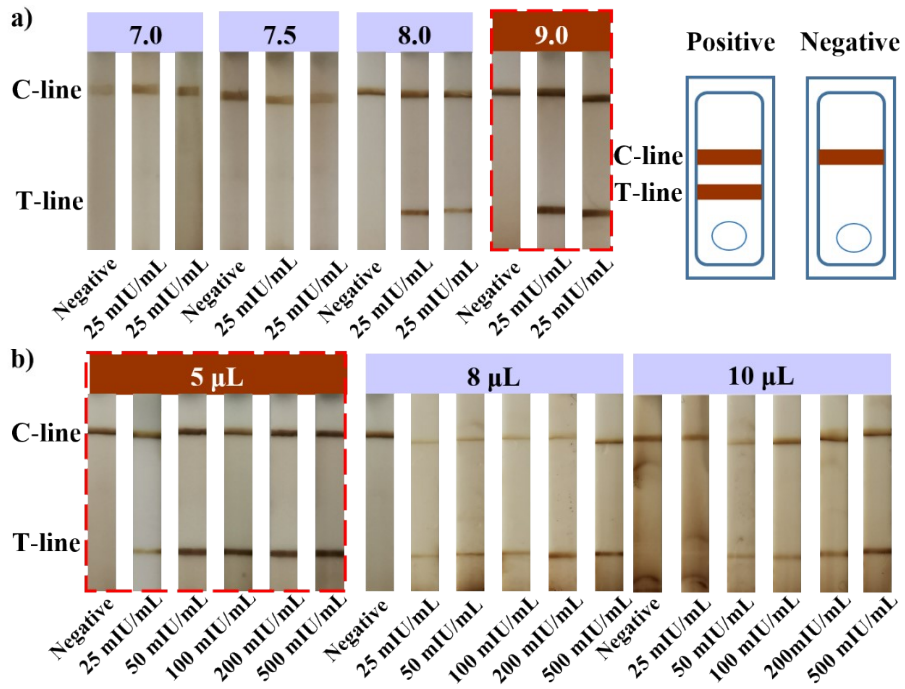
Fig. S5. The optimization of parameters in the ICTs' assembly. (a) pH of the coupling buffer, (b) the amount of Au/Fe$_3$O$_4$-mAb applied on the conjugate pad.
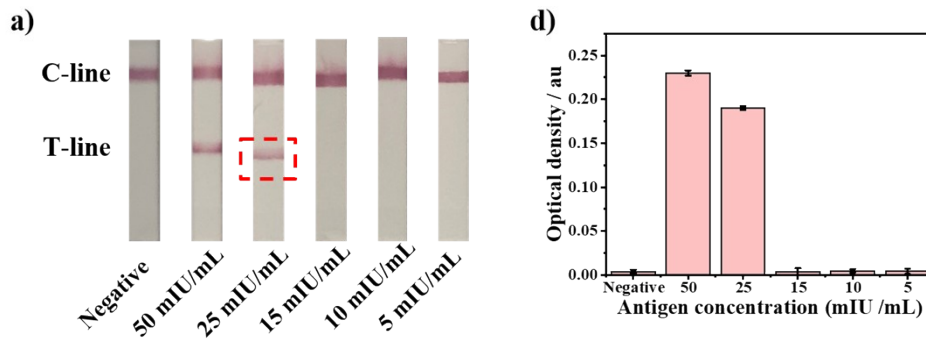

Fig. S6. The optimization of parameters in the ICTs' assembly. (a) the concentration of capture antibody, (b) the dispensed rate of antibody on T-line and C-line.

5. Sensitivity detection

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2   **Fig. S7.** Commercial strip testing results. (a) Various concentration of hCG sample. (b) The

3   corresponding optical intensity on T-lines analysis by Image J.