Supporting Material

Highly Sensitive Fluorescence-Linked Immunosorbent Assay for the Determination of Human IgG in Serum Using Quantum Dot Nanobeads and Magnetic Fe₃O₄ Nanospheres

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Table of Contents

Section-1 The hydrodynamic diameter and zeta potential of Fe₃O₄@Ab1IgG and QBs@Ab2IgG
Section-2 The infrared spectrum characterization of Fe₃O₄@Ab1IgG and QBs@Ab2IgG
Section-3 Selection of the best excitation wavelength
Section-4 The calculation method of coupling efficiency
Section-5 Optimization of Fe₃O₄@Ab1IgG preparation
Section-6 Optimization of QBs@Ab2IgG preparation
Section-7 The detection results of human serum by using FLISA and commercial ELISA kit
Section-1 The hydrodynamic diameter and zeta potential of Fe₃O₄@Ab1IgG and QBs@Ab2IgG

The formation of Fe₃O₄@Ab1IgG was characterized by analyzing the hydrodynamic diameter and zeta potential. As shown in Figure. S1a, the hydrodynamic diameters of Fe₃O₄@Ab1IgG (1409 nm) was significantly larger than Fe₃O₄ (354.5 nm), indicating that Ab1IgG was successfully coupled to the surface of Fe₃O₄. The Zeta potential measurement results of Fe₃O₄ and Fe₃O₄@Ab1IgG are shown in Figure. S1b. The Zeta potential of Fe₃O₄, Ab1IgG, and Fe₃O₄@Ab1IgG was -30.5 mV, -3.80 mV, and -28.5 mV, respectively. The activation of Fe₃O₄ surface and its coupling with antibody led to a decrease in potential, which further confirmed that Ab1IgG successfully conjugated to the surface of Fe₃O₄.

Similarly, as can be seen from Figure. S1c, the hydrodynamic diameters of QBs@Ab2IgG and QBs were 829.4 nm, 484.1 nm, respectively. The result indicates the antibodies were successfully immobilized on the surface of QBs. It was shown that the zeta potentials of QBs, Ab2IgG, and QBs@Ab2IgG were -30.8 mV, -0.513 mV, and -15.3 mV, respectively (Figure. S1d). It was also further confirms the formation of QBs@Ab2IgG.

![Image S1](image.png)

**Figure S1.** The hydrodynamic diameter (a) and zeta potential (b) of Fe₃O₄@Ab1IgG. The hydrodynamic diameter (c) and zeta potential (d) of QBs@Ab2IgG.

Section-2 The infrared spectrum characterization of Fe₃O₄@Ab1IgG and QBs@Ab2IgG

The infrared spectrum is showed in Figure. S2. Compared with Figure. S2b, Figure. S2a has another characteristic absorption peak which is associated with
protein. The same is true of comparing Figure. S2c with Figure. S2d. Obviously, these results are enough to prove that the corresponding antibodies have been successfully immobilized on the surface of Fe₃O₄ and QBs.

**Figure S2.** The infrared spectrum of Fe₃O₄@Ab1IgG (a), Fe₃O₄ (b), QBs@Ab2IgG (c), QBs (d).

**Section-3 Selection of the best excitation wavelength**

Under the same conditions, we measured the fluorescence intensity at the excitation wavelengths of 350nm (Figure.S3a), 360nm (Figure.S3b), 370nm (Figure.S3c) and 380nm (Figure.S3d), respectively. As can be seen from Figure.S3, when the excitation wavelength was at 350nm and 360nm, it may be the Raman scattering light that affected the fluorescence spectrum. When the excitation wavelength was at 370nm, the fluorescence intensity at 610nm was the highest and there was no background fluorescence effect, so the optimal excitation wavelength was selected at 370nm.
**Figure S3.** Selection of the best excitation wavelength. The fluorescence intensity under the excitation wavelength of 350nm (a), 360nm (b), 370nm (c) and 380nm (d).

Section-4 The calculation method of coupling efficiency

The coupling efficiency between antibody and magnetic Fe₃O₄ nanospheres or QBs was calculated according to the following formula. The realization of the washing step: magnetic Fe₃O₄ nanospheres were operated by magnetic separation and QBs was operated by centrifugation.

\[
\text{Coupling efficiency (\%)} = \frac{OD_{595\text{pre}} - OD_{595\text{post}} - OD_{595\text{(wash1)}} - OD_{595\text{(wash2)}}}{OD_{595\text{pre}}} \times 100\%
\]

Section-5 Optimization of Fe₃O₄@Ab₁IgG preparation

In addition to the reaction time and the amount of antibody, several other reaction conditions also affect the quality of the capture probe. Hence, the coupling efficiency of magnetic Fe₃O₄ nanospheres and Ab₁IgG at different concentrations of Sulf-NHS and EDC, different kinds of buffer solutions and different reaction temperatures were discussed respectively. As exhibited in Figure S4, when the amount of EDC was 1.0g, the amount of Sulf-NHS was 2.0g, the buffer solution was PBS, and the reaction time was 37°C, the coupling efficiency reached the maximum.
Figure S4. Optimization of Fe₃O₄@Ab₁IgG preparation: (a) The dosage of Sulf-NHS and EDC; (b) The effect of different immune reaction buffers; (c) The reaction temperature of Fe₃O₄ and Ab₁IgG.

Section-6 Optimization of QBs@Ab₂IgG preparation

In order to obtain a satisfactory fluorescence detection probe, we designed a three-factor, three-level orthogonal experiment to optimize the dosage of Ab₂IgG, Sulf-NHS, and EDC. From Table S1, it can be concluded that the amount of Sulf-NHS had the largest influence on the fluorescence intensity of QBs@Ab₂IgG, and the EDC was the smallest, and Ab₂IgG had the largest effect on the coupling efficiency, followed by Sulf-NHS. Therefore, the optimal dosage of Ab₂IgG, Sulf-NHS, and EDC were 30 µg, 20 mg, and 5 mg, respectively.

Table S1 The experiment results of orthogonal optimization

| ID | factor | index |
|----|--------|-------|
|    | A₁: Ab₂IgG(µg) | B₁: Sulf-NHS(mg) | C₁: EDC(mg) | coupling efficiency (%) | fluorescence intensity (I) |
| 1  | 10     | 10    | 5      | 14.6     | 10572    |
| 2  | 10     | 20    | 10     | 0        | 9833     |
| 3  | 10     | 40    | 20     | 0        | 8448     |
| 4  | 20     | 10    | 10     | 39.3     | 11071    |
| 5  | 20     | 20    | 20     | 45.4     | 13442    |
| 6  | 20     | 40    | 5      | 34.4     | 8410     |
| 7  | 30     | 10    | 20     | 64.2     | 9353     |
| 8  | 30     | 20    | 5      | 71.8     | 9628     |
| 9  | 30     | 40    | 10     | 60.6     | 10319    |
| 10 | 28853  | 30996 | 28610  |          |          |

 fluorescent intensity (I)  degree of influence: B>A>C
 R 3623 5726 2633
 I 14.6 118.1 120.8
 coupling efficiency (%)  degree of influence: A>B>C
 R 182.0 23.1 20.9

R: the amount of each factor in the experiment; I: the fluorescence intensity of QBs@Ab₂IgG; degree of influence: A>B>C; B>A>C.
Section-7 The detection results of human serum by using FLISA and commercial ELISA kit

After diluting the collected serum sample by 50000 times, the content of human IgG was detected by the method established in this study and the commercial kit, and the results are shown in Table S2.

**Table S2** Testing results of human serum by FLISA and commercial ELISA kit (n = 20)

| ID   | C<sub>IgG</sub> (ng·mL<sup>-1</sup>) | ID   | C<sub>IgG</sub> (ng·mL<sup>-1</sup>) |
|------|----------------|------|----------------|
|      | commercial ELISA kit | FLISA | commercial ELISA kit | FLISA |
| T120 | 19.56           | 16.06 | T229 | 21.99           | 20.15 |
| T122 | 23.22           | 32.58 | T230 | 17.13           | 16.44 |
| T124 | 19.95           | 26.49 | T232 | 19.83           | 19.75 |
| T125 | 19.85           | 18.18 | T242 | 19.78           | 18.14 |
| T126 | 17.95           | 17.43 | T243 | 18.24           | 18.53 |
| T127 | 19.12           | 21.01 | T245 | 19.19           | 19.92 |
| T131 | 23.08           | 25.46 | T246 | 19.95           | 20.52 |
| T133 | 21.46           | 25.29 | T254 | 20.10           | 19.67 |
| T217 | 21.47           | 19.04 | T257 | 22.58           | 21.43 |
| T225 | 19.39           | 23.06 | T259 | 21.87           | 19.49 |
| T228 | 17.36           | 25.50 | T263 | 22.96           | 24.44 |