Electronic Supplementary Information

Single Nanoparticle Analysis for Homogeneous Immunoassay

of CA19-9 for Serological Evaluation

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| Samples | ECL(U/mL) | Present method(U/mL) | Relative deviation (%) |
|---------|-----------|----------------------|------------------------|
| 1 | 3.71 | 3.80 | 2.4 |
| 2 | 8.5 | 9.43 | 10.9 |
| 3 | 11.69 | 11.61 | -0.7 |
| 4 | 12.19 | 11.23 | -7.9 |
| 5 | 13.11 | 13.22 | 0.8 |
| 6 | 20.38 | 21.16 | 3.8 |
| 7 | 22.69 | 22.59 | -0.4 |
| 8 | 22.84 | 22.15 | -3 |
| 9 | 27.92 | 30.20 | 8.2 |
| 10 | 28.67 | 29.89 | 4.2 |
| 11 | 34.01 | 31.02 | -8.8 |
| 12 | 34.39 | 33.99 | -1.2 |
| 13 | 36.06 | 38.45 | 6.6 |
| 14 | 143.8 | 126.70 | -11.9 |
| 15 | 375.1 | 360.96 | -3.8 |
| 16 | 377.5 | 386.62 | 2.4 |
| 17 | 915.8 | 977.52 | 6.7 |
| 18 | 1000 | 1013.80 | 1.4 |

Table S1 Comparison of the results of two different methods forhuman serum samples detection.

| | Serum 1 | Serum 2 | Serum 3 | Serum 4 |
|--------------------------|------------------|----------------|----------------|------------------|
| #1 (U mL ⁻¹) | 21.16 ± 0.14 | 22.58 ± 0.65 | 23.37 ± 1.51 | 33.47 ± 0.30 |
| #2 (U mL ⁻¹) | 18.36 ± 0.47 | 22.20 ± 0.79 | 22.64 ± 0.39 | 35.62 ± 0.83 |

Table S2 Reproducibility analysis results for CA19-9 detection.

Fig. S1 Dynamic light scattering (DLS) determination of AuNPs with different diameters.



Fig. S1 Diameters of different AuNPs determined by DLS

Fig. S2 UV-vis spectrometry of AuNPs with different diameters.



Fig. S2 UV-vis spectrometry of AuNPs with different diameters. A) 39.06 nm, B) 43.05 nm, C) 54.50 nm and D) 62.23nm.



Fig. S3 UV-vis spectrometry of AuNPs modified with different amounts of antibodies.

Fig. S3 Optimization of two kinds of antibody amount on AuNPs.

Fig. S4 Optimization of immunoreaction time.



Fig. S4 Immunoreaction time optimization.

Fig. S5 Reproducibility of the immunoassay for CA19-9 detection.

Reproducibility was assessed by testing four human serum samples with different batches of probes. The CA19-9 values of four samples measured by electrochemiluminescence (ECL) were 20.38, 22.69, 22.84 and 34.39 U mL⁻¹, respectively. The results of each sample were based on three measurements. CA19-9 results were calculated from the calibration curve at each time. As shown in Fig. S5, the coefficient of variation (CV) across different batches were all less than 10.0%.



Fig. S5 Comparison of results obtained from different batches of probes for CA19-9 detection.