Fluorescence Enhancement Effect by Metal Nanoparticles-immobilized Microplate

Shuyi SUN,* Kyohei MATSUI,* So TANABE,* Dung NGUYEN,* Takamasa KINOSHITA,* Yojiro YAMAMOTO,*,** and Hiroshi SHIIGI*†

*Department of Applied Chemistry, Osaka Prefecture University, 1-2 Gakuen, Naka, Sakai, Osaka 599-8570, Japan
**GreenChem. Inc., 19-19 Tsuruta, Nishi, Sakai, Osaka 593-8323, Japan
†To whom correspondence should be addressed.
E-mail: shii@chem.osakafu-u.ac.jp

In this reported work, we achieved high-throughput, highly sensitive fluorescent analysis using an enzyme-linked immunosorbent assay (ELISA) that employed a metallic nanoparticle (NP)-immobilized 96-well plate. The immobilization of metallic NPs on a 96-well plate effectively amplified fluorescent signals of the assay. The silver (Ag) NP-immobilized plate showed the best fluorescent enhancement effect of all plates immobilized by metal NPs. Our results demonstrate the potential of applying Ag NPs to enhance the efficiency of direct and indirect ELISA by the labeling of targets.

Keywords Microplate, silver nanoparticle, fluorescence enhancement, ELISA

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Fig. 1  (A) Absorption spectra and (B) digital photographs of (a) EDTA-covered Ag NP dispersion in the well and (b) the Ag NP-immobilized well after incubating for 12 h.

Fig. 2  (A) Relationship between the amount of Ag NPs immobilized on the well and the concentration of Ag NP dispersion.  (B) Dependence of the fluorescence intensity of fluorescein at 535 nm on the amount of immobilized Ag NPs ($N = 10$).

Fig. 3  (A) Digital photographs of 96-well plate immobilized Ag, Au, and Pd NPs.  (B) Fluorescence enhancement efficiency ($I/I_o$) of Ag, Au, and Pd NP-immobilized wells. $I$ and $I_o$ represent the fluorescence intensity of the fluorescein molecule in a NP-immobilized well and the unmodified well, respectively.
We applied the one-step immobilization method for gold (Au) and palladium (Pd) NPs (see Supporting Information). Both kinds of NPs showed a negative zeta potential (Au NPs: $-31.2\ \text{mV}$, Pd NPs: $-34.4\ \text{mV}$) owing to negatively charged citrate molecules surrounding the NPs, since two carboxylic groups of citrate were dissociated at pH 4.0. They were uniformly immobilized on the well owing to electrostatic interactions. The insides of the wells were stained reddish purple and black, respectively (Fig. 3). This was due to the LSPR inherent in metal NPs. LSPR is strongly dependent not only on the metal species, but also on the size, shape, aggregation state, and local environment. Given these equal conditions on the wells, metal species-based LSPRs appeared at different wavelengths, and thus were deeply involved in the fluorescence enhancement. The fluorescence intensity of each well ($I$) was measured after incubation of the fluorescein aqueous solution ($0.10\ \text{mM}, 40\ \mu\text{L}$) for 12 h, and removal of the supernatant. The fluorescence enhancement was confirmed for the Au NP-immobilized well ($I/I_o = 2.4$) and Pd NP-immobilized well ($I/I_o = 1.3$) compared with that for the unmodified well ($I_o = -213$). In addition, it was found that immobilized NPs of the same metal that had a larger diameter led to greater fluorescence enhancement. We found that the 96-well plate immobilizing 30 nm Ag NPs ($4.2\ \mu\text{g cm}^{-2}$) resulted in a largest fluorescence enhancement for fluorescein ($I/I_o = 22$).

The Ag NP-immobilized 96-well plates have been established to enhance the fluorescent signal. Applying this plate to an ELISA is expected to improve the sensitivity of the assay. Furthermore, the labeling of various target species based on the specific binding of antibodies, aptamers, and molecularly imprinted polymers allows for a high throughput and sensitive detection using the proposed procedure.

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**Supporting Information**

Further information is available regarding the experimental details. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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