Electronic Supplementary Information

Ternary covalent conjugate (antibody-gold nanoparticle-peroxidase) for signal enhancement in enzyme immunoassay

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Determination of HRP content in the Ab-GNPs-HRP conjugate

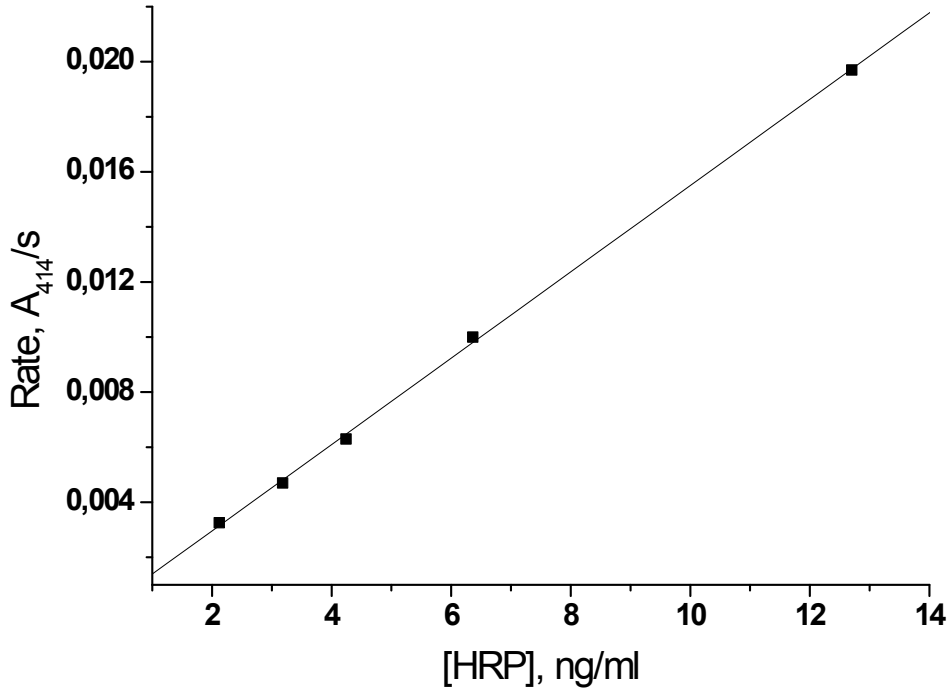

Fig. S1. Calibration curve for the determination of HRP content using ABTS as a substrate.

The concentration of GNPs calculated from the absorbance (A_{535}) in the Ab-GNPs-HRP solution was 1.46 x 10^{14} particles/L. The determination of rate of ABTS oxidation catalyzed by Ab-GNPs-HRP and use of the calibration curve for native HRP allowed calculating the HRP concentration in the Ab-GNPs-HRP solution equal to 3.09 x 10^{-9} moles/L. Therefore, each GNP of the conjugate contains covalently immobilized HRP whose activity is equivalent to that of 13 molecules of native HRP.

Determination of Ab IC4 content in the Ab IC4-GNPs-HRP conjugate

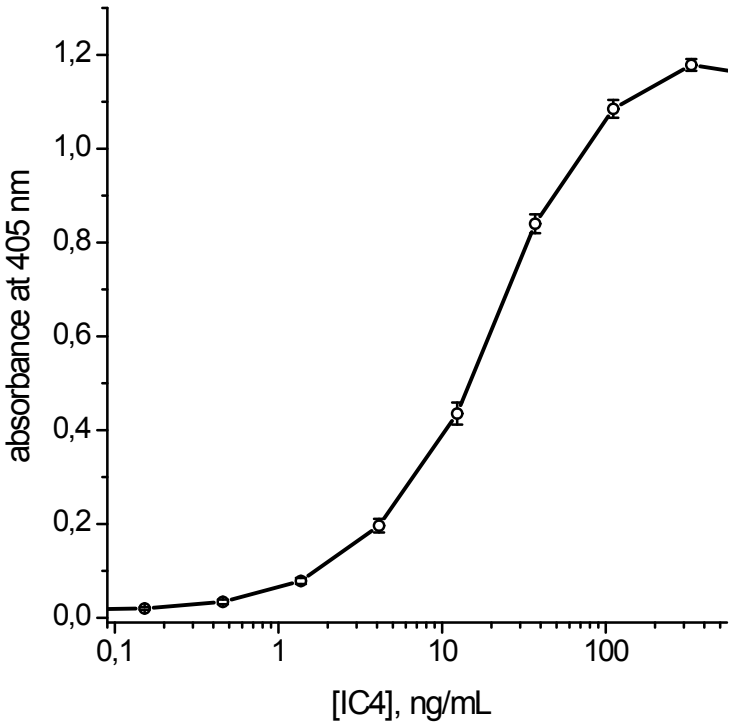

Fig. S2. Calibration curve for the determination of Ab IC4 content with sandwich ELISA.

ELISA allowed determining the Ab IC4 concentration in the Ab-GNPs-HRP solution equal to 1.86×10^{-10} moles/L. Therefore, each GNP of the conjugate contains covalently immobilized Ab IC4 whose activity is equivalent to that of 1 molecule of the intact antibody.