Supplementary Materials

An Immunoassay Utilizing DNA-Coated Cage Protein As a Signal Generator

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Materials

EZ-Link® NHS-PEG_{12}-Biotin was purchased from Thermo Scientific (Rockford, IL, USA) and streptavidin-HRP was from Abcam (Cambridge, UK). α-fetoprotein (AFP) and anti-AFP antibodies (capture: 2127435 and detection: 6127435) were purchased from Fitzgerald (Acton, MA, USA), human serum, bovine serum albumin (BSA), streptavidin and Tween 20 were from Sigma-Aldrich (St. Louis, MO, USA), Protector RNase Inhibitor (PRI) was from Roche (Mannheim, Germany), RNase H was from Takara (Japan), and TMB substrate solution and stop solution were from Gendepot (Barker, TX, USA). Acetylene-PEG4-maleimide was from Jena Bioscience. All compounds for DNA synthesis were from Glen Research.

Acetylene Modification of Cage Protein

Cage protein HSP16.5 was expressed and purified according to the literature.[1] Cage protein (100 μL, 4.8 mg/mL) with the cystein residue displayed at the outer surface was first treated with TCEP (1.2 μL, 0.5 M) and incubated 1 hour at room temperature. The reaction mixture was then purified by Zeba™ column (Pierce) and the concentration of the resulting protein was determined by a Bradford assay kit. To the protein pre-treated with tris(2-carboxyethyl)phosphine (100 μL, 3.75 mg/mL) was added acetylene-PEG4-maleimide in PBS buffer (30 μL, 12.5 mg/mL) and the mixture was incubated at 4 °C overnight. Finally, the reaction mixture was purified by Zeba™ column.

Preparation of DNA-coated cage protein

The 10-mer oligonucleotide 5'-N_3-AACCACAGTG-biotin was obtained by converting 5'-iodine- AACCACAGTG-biotin which was synthesized by using the standard protocol for synthesis of DNA oligonucleotides. The click reaction was carried out by the modified-protein (45 μL, 2.9 mg/mL), the azide-DNA (40 μL, 1 mM), Cu(I)/tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), sodium ascorbate, triethylammonium acetate buffer pH 7. The sample was mixed at 25 °C, 1000 rpm for 16 h in an Eppendorf thermomixer. The catalyst was removed by using the Amicon™ filter (MWCO = 10 K, Millipore, Ireland) (14000 × g, 10 min, 4 °C). The residue was washed with 1 × PBS (4 mL) twice and diluted to 100 μL by adding 1 × PBS. The concentration of protein was determined by the Bradford assay.

Biotinylation of the detection antibody

EZ-Link NHS-PEG_{12}-Biotin (0.4 μL, 20 mM) was mixed with dAb (15 μL, 6.2 mg/mL) and incubated for 30 min at room temperature. The biotinylated dAb was then purified using Zeba™ column (MWCO = 7K, Thermo Scientific).

ELISA

Each well of a transparent 96-well plate (Nunc, Denmark) was coated with the capture antibody (cAb, 5 μg/mL, 100 μL) by incubation 1 h at room temperature, followed by washes with PBS (3 × 300 μL). The cAb-coated wells were blocked with 200 μL of Blocking buffer (PBS containing 3% (w/v) BSA and 0.1% (v/v) Tween 20) by incubating for 2 h at room temperature and rinsed with PBST (PBS containing 0.05% (v/v) Tween 20). The solutions of varying concentrations of AFP (0, 0.625, 1.25, 2.5, 5.0, 10.0 ng/mL) in 100 μL Assay buffer (PBS containing 1% (w/v) BSA and 0.05% (v/v) Tween 20) was added to the wells and followed by incubation for 1 h at room temperature. The plate was then washed with the PBST (3 × 300 μL) before the addition of biotinylated-dAb (2 μg/mL) in Assay buffer (100 μL) and incubation for 1 h at room temperature. After rinsing with PBST (3 × 300 μL), streptavidin-HRP (2 μg/mL) in Assay buffer was added and incubated for 1 h at room temperature. The plate was further washed with PBST (3 × 300 μL) before adding 100 μL TMB substrate solution and followed by incubation for 10 minutes at room temperature. After addition of TMB stop solution (100 μL) for each well, optical density at 450 was immediately measured by means of a 96-well microplate reader (SpectraMax Plus, Molecular Device, Sunnyvale, CA, USA).

OLISA

Black 96-well microplates (Nunc, Denmark) were coated with 5 μg/mL cAb in PBS (100 μL/well) by incubation for 1 h at room temperature, followed by washes with PBS (3 × 300 μL), then blocked with Blocking buffer (200 μL) and incubated for 2 h at room temperature. After rinses with PBST, AFP solutions of varying concentrations (0, 0.0625, 1.25, 2.5, 5.0, 10.0 ng/mL) in 100 μL Assay buffer were added to the wells, followed by incubation for 1 h at room temperature. The plate was washed with PBST before addition of biotin-dAb conjugates (2 μg/mL, 100 μL) in Assay buffer and incubated for 1 h at room temperature. After rinsing with PBST (3 × 300 μL), the solution of Streptavidin (2 μg/mL, 100 μL) was added to the wells and incubated for 1 h at room temperature. After rising with PBST (3 × 300 μL), biotin-DNA solutions (biotin- AACCACAGTG, 200 nM, 100 μL) were added to the wells, followed by incubating for 1 h at room temperature, and the plate was rinsed with PBST (3 × 300 μL). Finally, the RNase H (100 μL) solution containing 200 nM F-RNA-Q probe (FAM-5′-CACUGUGGUU-3′-BHQ1), 0.4 U of PRI and 20 U of RNase H in RNase H buffer (40 mM Tris-HCl, 4 mM $MgCl_2$, 1 mM DTT, 0.003% BSA, pH 7.7) was added and incubated for 1 h at 37 ^{o}C. The fluorescence intensities were measured by Apliskan (Thermoscientific, Waltham, MA, USA) with the excitation/emission filter sets of 485/535 nm.

1. Bova, M. P.; Huang, Q.; Ding, L.; Horwitz, J. *J. Biol. Chem.* **2002**, *277*, 38468.