Supporting Information for

Portable and Quantitative Detection of Protein Biomarkers and Small Molecular Toxins Using Antibodies and Ubiquitous Personal Glucose Meters

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Materials

Amine-modified magnetic beads (MBs) and epoxyl MB-antibody conjugation kit (Dynabeads M-270) were purchased from Invitrogen Inc. (Carlsbad, CA). Streptavidin-coated MBs (1 μm in diameter) and Amicon centrifugal filters were purchased from Bangs Laboratories Inc. (Fishers, IN) and Millipore Inc. (Billerica, MA), respectively. EZ-Link™ NHS-PEG4-Biotin, Sulfo-NHS, sulfosuccinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (Sulfo-SMCC) and streptavidin were obtained from Pierce Inc. (Rockford, IL). Mouse monoclonal anti-human PSA antibody (ab403), mouse monoclonal anti-ochratoxin A antibody (ab23965) and biotinylated goat polyclonal secondary antibody to mouse IgG (ab6788) were purchased from Abcam Inc. (Cambridge, MA). Biotinylated goat anti-human Kallikrein 3 IgG antibody (BAF1344) was from R&D System (Minneapolis, MN). Grade VII invertase from baker's yeast (S. cerevisiae), biotin, ochrotoxin A (OTA), tris(2-carboxyethyl)phosphine (TCEP), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), bovine serum albumin (BSA) and other chemicals for buffers and solvents were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

The commercial personal glucose meter used in this study was an *ACCU-CHEK Avia* glucose meter, and the test of a solution by the meter was simply conducted by contacting the solution with the strip loaded on the glucose meter.

The following oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA):
Amine-DNA:
5′-TTTTTAGCATCGGACA-NH_2-3′
Thiol-DNA:
5′-TGTCCGTAGCTAAAAAAAAAAAA-SH-3′

Buffers used in this study:
Buffer A: 0.2 M NaCl, 0.1 M sodium phosphate buffer, pH 7.3, 0.05% Tween-20
Buffer B: Phosphate buffered saline (PBS, pH 7.0, 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.8 mM KH_2PO_4), 0.025% Tween-20, 1 g/L BSA
Buffer C: Phosphate buffered saline (PBS, pH 7.0, 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.8 mM KH_2PO_4), 0.025% Tween-20

Conjugation chemistry:

(1) Biotin-invertase conjugation:

To 1 mL 20 mg/mL invertase in Buffer A without Tween-20, about 5 mg EZ-Link NHS-PEG4-Biotin was added and the mixture was well mixed at room temperature for 4 h. Then, the biotin-invertase conjugate was purified by Amicon-100K for 8 times using Buffer A without Tween-20.

(2) Biotin conjugation of amine-modified MBs:

A portion of 1 mL 2 mg/mL Amin-modified MBs was washed by 1 mL Buffer A without Tween-20 for three times, and the solid residue after magnetic separation was dispersed in 0.5 mL Buffer A without Tween-20 by vortexing. After that, 5 mg EZ-Link NHS-PEG4-Biotin was added to the MB solution and kept on a roller overnight. The biotinylated MBs were then washed by 1 mL Buffer A for 8 times to remove the un-reacted biotin reagents, and dispersed in 2 mL Buffer A. The as prepared 2 mL 1 mg/mL biotin-conjugated MBs were buffer exchanged to Buffer A twice, and then dispersed in 2 mL Buffer A. BSA was added to the solution to achieve a final concentration of 1 mg/mL and the mixture was well mixed for 1 h at room temperature to block non-specific binding sites. After that, the MBs were separated from the mixture by a magnet. The MBs were further washed by Buffer A once and then dispersed in 2 mL Buffer A.

(3) Anti-PSA conjugation of epoxy-modified MBs:

The antibody conjugation was following the protocol provided by the Invitrogen expoxy MB-antibody conjugation kit. Solutions C1, C2, HB, LB and SB were provided by the conjugation kit and the component information was not provided. A portion of 5 mg Dynabeads M-270 Epoxy magnetic beads was washed by 1 mL C1 solution and with supernatant removed by a magnet. Then, the solid residue was mixed with 50 μg mouse monoclonal anti-human PSA antibody (ab403) and dispersed in a 0.5 mL mixture solution of C1 and C2 (v/v = 1:1) by vortexing. The solution was put on a roller at room temperature for 1 day. After that, the supernatant of the solution was removed by a magnet, and the solid residue was dispersed in 0.8 mL solution HB by vortexing. This washing step was then repeated twice using 0.8 mL solution LB and SB instead of HB, respectively. Later, the solid residue was dispersed in 0.8 mL solution SB by vortexing and kept on a roller for 15 min before the removal of supernatant by a magnet. Finally, the solid residue was dispersed in 0.5 mL solution SB by vortexing and stored at 4 ^{o}C.

(4) DNA-OTA conjugation:

A mixture of 1 mg OTA, 2 mg EDC and 2 mg sulfo-NHS was dissolved in 50 μL anhydrous DMF and the turbid solution was kept on a roller at room temperature for 2 h. After that, to the solution, 10 μL of 1M $NaHCO_3$-Na_2CO_3 buffer (pH 8.7) and 40 μL of 0.5 mM Amine-DNA in water were added and mixed by vortexing. The resulting solution was kept on a roller for 12 h. Then, the DNA-OTA conjugate produced in the solution was purified by Amicon-10K using water by 8 times to remove all the small molecule reagents. The final water solution was characterized by MALD-TOF and 20% PAGE (Figure S1), and the results suggested the nearly complete transformation of the DNA-NH2 to DNA-OTA conjugate. The concentration of DNA in the final solution was determined by UV-Vis spectra.

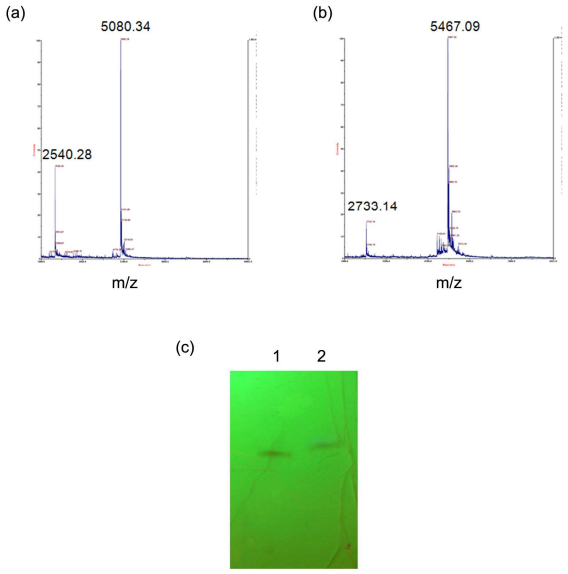

Figure S1. (a) MALDI-TOF spectra of Amine-DNA: m/z detected: 5080.3; calculated: 5082.4. (b) MALDI-TOF spectra of OTA-DNA: m/z detected: 5467.1; calculated: 5468.2. (c) 20% PAGE of Amine-DNA (1) and OTA-DNA (2) on a green fluorescent silica gel plate under UV light. The dark bands are DNA due to the absorbance of UV light and decrease of silica fluorescence by DNA. The lower mobility of OTA-DNA compared to Amine-DNA is because the OTA-DNA conjugate has a larger molecular weight.

(5) DNA-invertase conjugation:

To 30 μL of 1 mM Thiol-DNA in Millipore water, 2 μL of 1 M sodium phosphate buffer at pH 5.5 and 2 μL of 30 mM TCEP in Millipore water were added and mixed. This mixture was kept at room temperature for 1 hour and then purified by Amicon-10K using Buffer A without Tween-20 by 8 times. For invertase conjugation, 400 μL of 20 mg/mL invertase in Buffer A without Tween-20 was mixed with 1 mg of sulfo-SMCC. After vortexing for 5 minutes, the solution was placed on a shaker for 1 hour at room temperature. The mixture was then centrifuged and the insoluble excess sulfo-SMCC was removed. The clear solution was then purified by Amicon-100K using Buffer A without Tween-20 by 8 times. The purified solution of sulfo-SMCC-activated invertase was mixed with the above solution of activated Thiol-DNA. The resulting solution was kept at room temperature for 48 hours. To remove unreacted Thiol-DNA, the solution was purified by Amicon-100K for 8 times using Buffer A without Tween-20.

(6) MB-anti-OTA conjugation:

A portion of 2 mg/mL streptavidin-coated magnetic beads was washed by Buffer C three times and dispersed in 0.5 mL Buffer C. The solution was then added with 50 μL 2 mg/mL biotinylated goat polyclonal secondary antibody to mouse IgG (ab6788) and well mixed by vortexing. After being on a roller for 2 h, the supernatant of the solution was removed by a magnet. To the solid residue, 50 μg/mL mouse monoclonal anti-OTA antibody (ab23965) in Buffer C was added and the solution was put on a roller for 2 h. Finally, the magnetic beads were washed by Buffer C for 6 times, dispersed in 1 mL Buffer C, and stored at 4 ^{o}C.

Procedures for the detection of targets using PGMs:

(1) Streptavidin detection using biotin-conjugated MBs and biotin-invertase conjugate via the sandwich assay

A portion of 50 μL 1 mg/mL biotin-conjugated MBs in Buffer A was separated by a magnet and the solid MBs left after removing supernatant were used as the sensor reagent for each test. When doing one test, a 100 μL streptavidin sample of a certain concentration in Buffer A was added to the MBs and the mixture was mixed for 30 min at room temperature on a roller. After washing the MBs residue by 3 times using Buffer A containing 1 mg/mL BSA to remove unbound streptavidin and block non-specific binding sites, 100 μL 1 mg/mL biotin-invertase conjugate in Buffer A was added and the mixture was mixed for 30 min at room temperature on a roller. After washing the MBs residue by 5 times using Buffer A, 50 μL 0.5 M sucrose in Buffer A was added to the MBs residue and then mixed for 2 h on a roller at room temperature. A portion of 5 μL of the final solution was tested by a PGM.

(2) PSA detection using anti-PSA antibody-conjugated MBs, biotinylated anti-PSA antibody, streptavidin and biotin-invertase conjugate via the sandwich assay

The PSA antibody-conjugated MBs were buffer-changed to Buffer B to reach a final concentration of 3 mg/mL. Each 50 μL of this solution was then used for one assay. After separated by a magnet and with supernatant removed, the MB residue was dispersed in a 50 μL PSA solution of a certain concentration in Buffer B or 25% human serum in Buffer B and then kept on a roller for 1 h at room temperature. Then, the MBs were separated and the supernatant was removed. The solid residue was added 50 μL 1 mg/L BAF1344 antibody in Buffer B, followed by mixing at room temperature for 30 min. The MBs were further separated, dispersed in 50 μL 2 μM streptavidin in Buffer B, and then mixed and left on a roller for 30 min. Later, the MBs were separated again and dispersed in 50 μL 0.6 mg/mL (~4 μM) biotin-invertase conjugate in Buffer B. After mixing for 30 min at room temperature on a roller, the MBs were separated from the supernatant and washed by Buffer B for 4 times. Finally, a 50 μL solution of 0.5 M sucrose in Buffer B was added to the MBs, and 5 μL of the solution was tested by a PGM after 3 h (Buffer B) or 7 h (25% human serum) for the production of detectable amount of glucose for signal readout in the PGM. We used 30 min for the binding between antigens and antibodies because we found this time scale was sufficient and also comparable to published immunoassays.[11,42-45] In serum samples, because of the effect of high concentrations of serum proteins and other components, the binding efficiency of PSA onto MBs was lower and the formation of sandwich complex was harder, so the loading of invertase-conjugates on MBs under the same condition was lower than the assay in Buffer B. Therefore, we used a longer time for glucose production by invertase-catalyzed hydrolysis of sucrose for the assays in 25% human serum (7h) than in Buffer B (3h).

(3) Biotin detection using streptavidin-coated MBs and biotin-invertase conjugate via the competitive assay

A portion of 50 μL 1 mg/mL streptavidin-conjugated MBs in Buffer A containing 1 g/L BSA was separated by a magnet and the solid MBs left were used as the sensor for each test. When doing one test, a 50 μL biotin sample of a certain concentration in Buffer A was mixed with 50 μL 150 mg/L (~1 μM) biotin-invertase conjugate, and then added to the MBs and the mixture was mixed for 15 min at room temperature on a roller. After washing the MBs residue by 5 times using Buffer A, a 50 μL solution of 0.5 M sucrose in Buffer A was added to the MBs residue and then mixed for 30 min on a roller at room temperature. A portion of 5 μL of the final solution was tested by a PGM.

(4) OTA detection suing anti-OTA antibody-conjugated MBs, DNA-OTA conjugate and DNA-invertase conjugate via the competitive assay

A portion of 50 μL 1 mg/mL OTA antibody-conjugated MBs in Buffer C was separated by a magnet and the solid MBs left were used as the sensor for each test. When doing one test, a 50 μL OTA sample of a certain concentration in Buffer C was mixed with 50 μL 250 nM DNA-OTA conjugate, and then added to the MBs and the mixture was mixed for 20 min at room temperature on a roller. After washing the MB residue by 5 times using Buffer B, a 50 μL solution of 0.5 g/L (3 μM) DNA-invertase conjugate in Buffer C was added and the solution was kept on a roller for 20 min. Then, the supernatant was removed by a magnet and a 50 μL solution of 0.5 M sucrose in Buffer C was added to the MB residue. Finally, 5 μL of the solution was tested by a PGM after 2 h.