Electronic Supplementary Material (ESI) for Analyst.

Surface plasmon resonance-based immunoassay for human fetuin A

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Previously developed covalent antibody immobilization procedure[1]

Surface cleaning of SPR Au chip (SIA kit) and APTES functionalization

The Au chip was cleaned with piranha etch [60 µL of H_2SO_4 (97.5%, v/v): 30 µL of H_2O_2 (30%, v/v)] for 2 min followed by extensive washing with UPW. The chip was then incubated with 100 µL of 2% (v/v) APTES for 1 h at room temperature (RT) in a fume hood followed by five washes with UPW.

EDC activation of anti-HFA antibody

Anti-HFA Ab (990 µL of 100 µg/ml in HBS) was incubated at RT for 15 min with 10 µL of cross-linking solution containing EDC (4 mg/mL) and sulfo-NHS (11 mg/mL) in 0.1 M MES buffer, pH 4.7. It leads to the activation of carboxyl groups on anti-HFA Ab with EDC.

Antibody immobilization procedure

Fifty microliters of EDC-activated Ab (100 µg/mL) was injected over all four flow cells of an APTES-functionalized Au chip at a flow rate of 10 µL/min and the baseline was allowed to stabilize. This leads to the covalent binding of EDC-activated anti-HFA Ab on the APTES-functionalized Au chip. Non-specific binding sites on the Ab-bound chip were then blocked by injecting 20 µL of 1% (w/v) BSA at 10 µL/min.

Covalent antibody immobilization procedure on CM5-dextran chip[1]

A CM5 dextran-functionalized Au chip was docked into the BIAcore 3000 and primed. The optimum pH of sodium acetate buffer for Ab immobilization, as determined by pre-concentration studies, was found to be 4.2. The CM5-dextran chip was activated by injecting a 50 µL cross-linking solution, containing 200 µg of EDC and 550 µg of sulfo-NHS in 0.1M MES buffer, pH 4.7, through all the flow cells at 10 µL/min. Thereafter, 50 µL of anti-HFA antibody (100 µg/mL) was injected in the four flow cells of an APTES-functionalized Au chip

at 10 µL/min and the baseline was allowed to stabilize. Non-specific binding sites on the chip were then blocked by consecutive injection of 20 µL of 1M ethanolamine hydrochloride, pH 8.5 and 20 µL of 1% (w/v) BSA at 10 µL/min. Ethanolamine hydrochloride blocked the unreacted ester groups in CM5-dextran matrix, while BSA blocked non-specific binding sites on the chip surface.

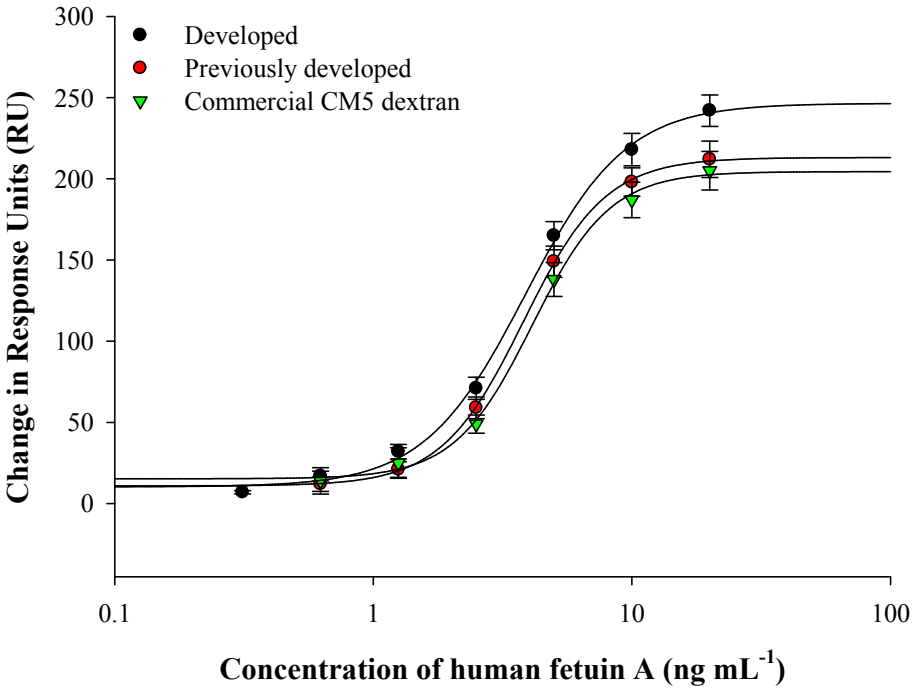

Fig. S1. Comparison of the developed SPR IA with our previously developed covalent and commercial carboxymethyl (CM5) dextran chip-based SPR IA formats. All experiments were done in triplicate with the error bars presented the standard deviation.

REFERENCES

(1) Vashist S. K.; Dixit, C. K.; MacCraith, B. D.; O'Kennedy, R. *Analyst* 2011, 136, 4431-4435.