Electronic Supplementary Information for Figs. S1 and S2

Nominal effective immunoreaction volume of magnetic beads at single bead level

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Principle of EDC-NHS method

The main strategy for coupling proteins to carboxylate particles is by means of an aqueous, carbodiimide-mediated process with EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) either in a single-step coupling reaction or in a two-step reaction which employs N-hydroxysuccinimide (NHS) or sulfo-NHS.[1] During this reaction, the carboxylate particles are activated with the water-soluble carbodiimide EDC to create an intermediate ester at first. This ester is reactive directly with amines on proteins in single-step reaction. In the two-step reaction, combining with NHS or sulfo-NHS will result in the formation of other intermediates, such as NHS ester or sulfo-ester, which is more stable in aqueous solution than the one formed with EDC. Therefore, the protein yield of the two-step reaction is higher than that using EDC alone. Moreover, by forming the secondary ester, the spare EDC can be removed from the particles before adding protein. Due to the presence of both

amines and carboxylates on most proteins, it prevents carbodiimide-mediated protein polymerization effectively.[1]

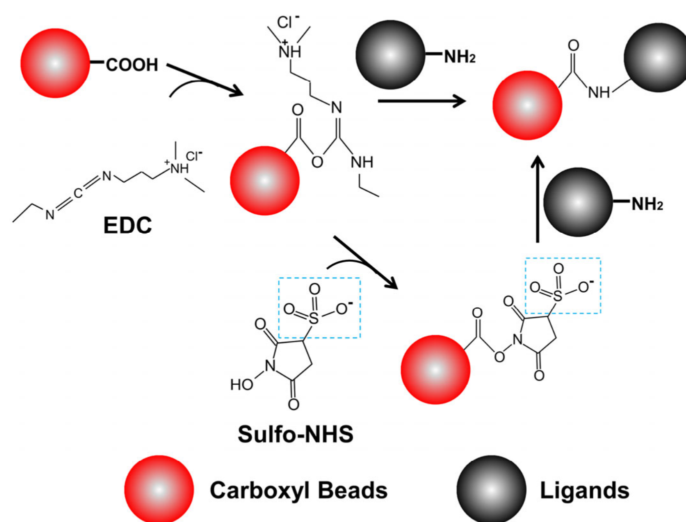

Fig. S1 Principle of EDC-NHS method for antibody modification of magnetic beads

Two-step EDC-NHS protocol [2]

In preparation, EDC (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) was dissolved in cold MES (25 mM, pH 6) (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) to a concentration of 50 mg mL^{-1}. Similarly, 50 mg mL^{-1} NHS (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) was prepared with the same operation.

In the first step, Dynabeads (Thermo Fisher Scientific Inc., Waltham, MA USA) were washed twice with 25 mM MES for ten minutes with good mixing. Then, 50 μL EDC and 50 μL NHS were added into the tube and the solution was incubated at room temperature for 30 minutes with slow tilt rotation. After incubation, the tube was placed on MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Promega Biotech Co., Ltd., WI, USA) for 2 minutes and the supernatant was removed. Next, the Dynabeads were washed twice with 300 μL MES and the

supernatant was removed by magnetic separation. Here, the Dynabeads were activated by EDC and NHS and they were ready for coating with protein ligands.[1]

In the following step, protein ligands were dissolved in 100 μL MES (25mM, pH 6). Then the solution was added to the tube containing activated Dynabeads as well mixed. Next, the tube was incubated at room temperature for at least 30 minutes with slow tilt rotation. After incubation, the Dynabeads were washed twice with 1 % PBST to remove the spare protein ligands. In the last step, non-specific adsorption sites on Dynabeads surface were blocked by PBS buffer (10 mM, pH 7.4) containing 2 % BSA (Sangon, Shanghai, China) and the system was incubated at room temperature for 2 hours with slight stirring. After blocking, the Dynabeads were washed three times with 1 % PBST. Finally, the Dynabeads were resuspended in PBS buffer (10 mM, pH 7.4) containing 2 % BSA and stored at 4 ºC for further study.

Preparation of IMB probes

In this study, Type One IMBs were Dynabeads® MyOne™ Carboxylic Acid (CAS: 65011) with the diameter of 1 μm and Type Two IMBs were Dynabeads® M-270 Carboxylic Acid (CAS:14305D) with the diameter of 2.7 μm. Both types of Dynabeads were coated with anti-PSA primary antibody using EDC-NHS method. The dose of antibody coated was followed the instructions from manufacturer. For Type One IMBs, they were labeled with 50 μg antibody per milligramme Dynabeads (7×10^{8} beads approximately). Hence, each bead was coupled with roughly 7.14×10^{-8} μg antibody. The working concentration of Type One IMBs was 1 mg mL^{-1}, which

was equivalent to 7×10^{8} beads mL^{-1}. The Type Two IMBs were coupled with 20 μg antibody per milligramme Dynabeads (6.67×10^{7} beads roughly). Thus, each bead was coated with around 3×10^{-7} μg antibody. The working concentration of Type Two IMBs was also 1 mg mL^{-1}, which was nearly 6.67×10^{7} beads mL^{-1}.

Principle of IMBs-based ELISA for PSA detection

PSA, also known as gamma-seminoprotein or kallikrein-3 (KLK3), is a glycoprotein enzyme encoded in humans by the KLK3 gene and its molecular weight is 34 KD.[3] It is universal that PSA is detected by sandwich enzyme linked immunosorbentassay (ELISA).

In IMBs-based ELISA, magnetic beads are employed as solid phase carriers and their surface have already been modified by anti-PSA primary antibody in preparation. In immunoreaction, PSA will be captured by IMBs specifically by forming antigen-antibody conjugates. Furthermore, as shown in figure S2, these conjugates will combine with the secondary antibody to form ternary complexes like “sandwiches”. Non-combined PSA and secondary antibody will be removed by magnetic separation. Because the secondary antibody was labeled with horseradish peroxidase (HRP), chromogenic reaction will be carried out when tetramethylbenzidine (TMB) was added as substrate.[4]

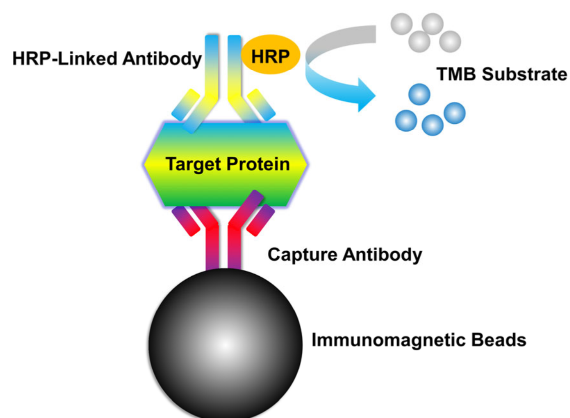

Fig. S2 Principle of IMB-based ELISA assay for PSA antigen detection

References

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