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Supporting Information

**Fluorescent Microsphere Immunochromatographic Assays for
Detecting Bone Alkaline Phosphatase Based on Biolayer
Interferometry-selected antibody**

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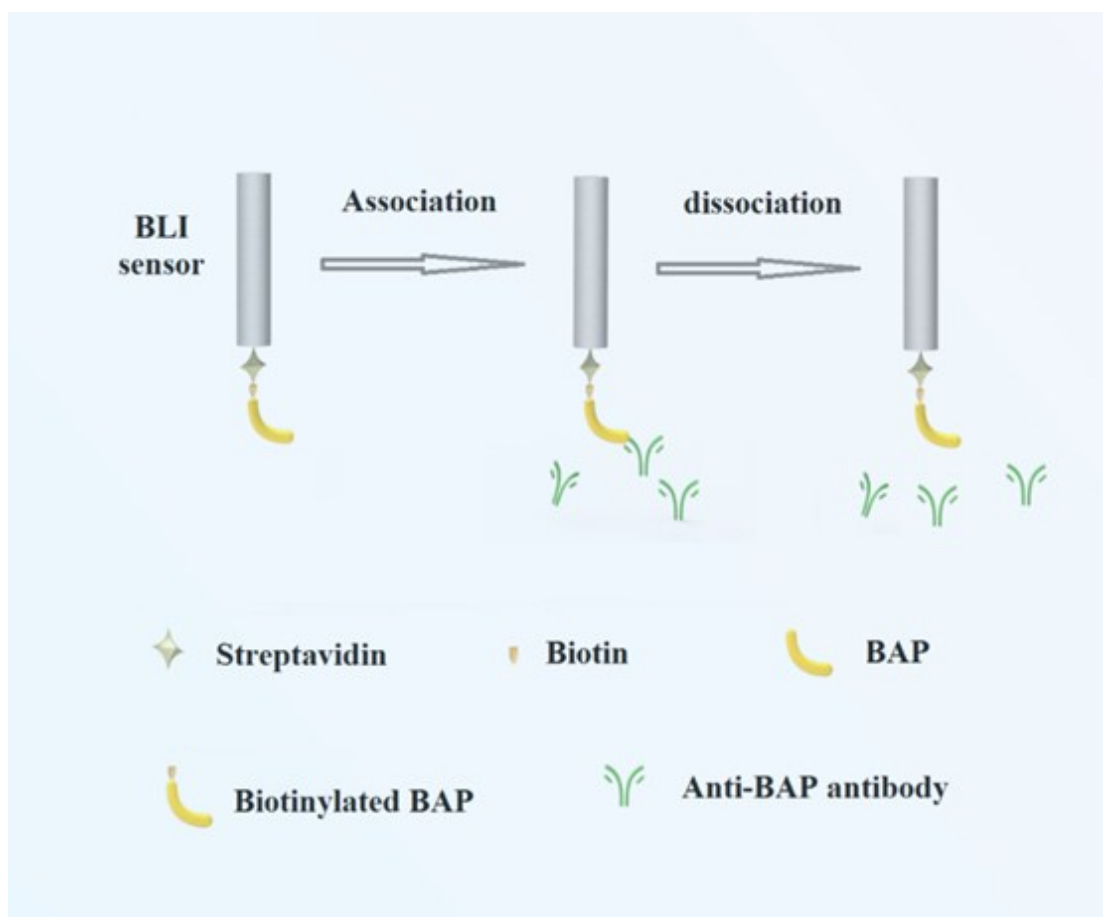
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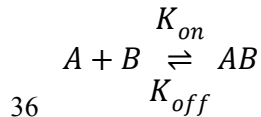
27 Fig. S1 Schematic representative of the BLI system steps.

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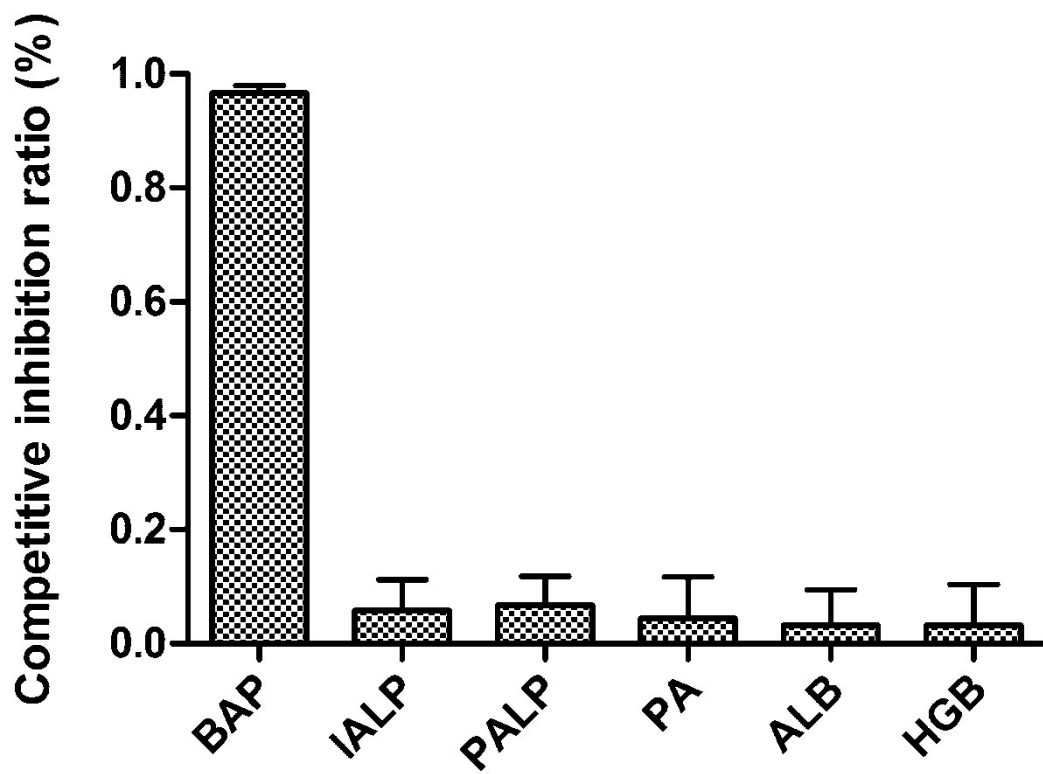
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31 The relationship between K_{on} , K_{off} , K_D , and 1:1 binding model
32 The association constant (on-rate, K_{on}), dissociation constant (off-rate, K_{off}), and
33 equilibrium dissociation constant (K_{off}/K_{on} , K_D) were calculated by the built-in BLItz
34 software as a 1:1 binding model. The 1:1 binding model used to describe the
35 interaction between BAP and anti-BAP mAb was represented by the equation below:

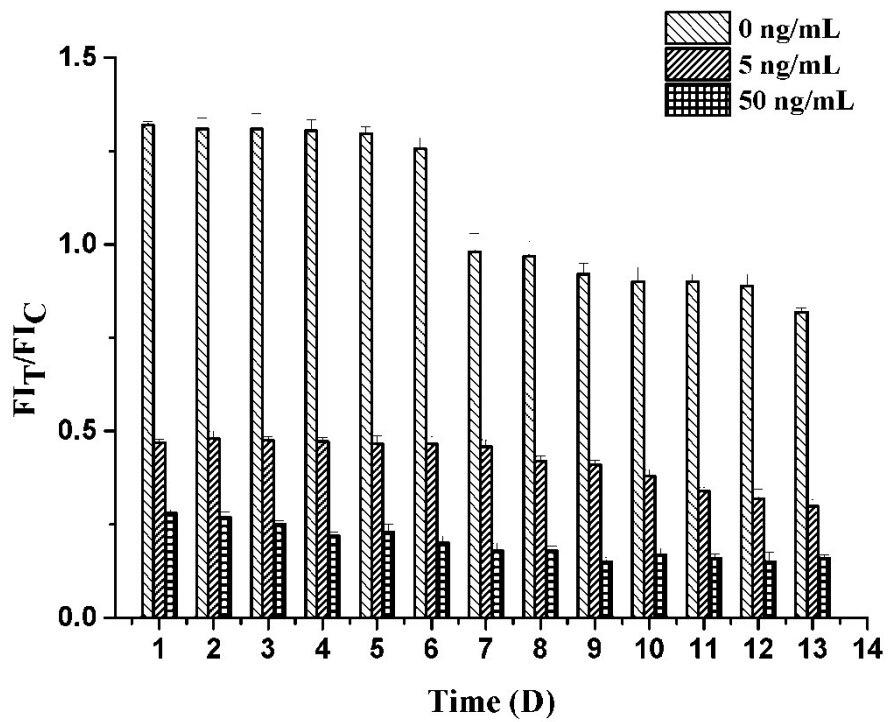


37 Where A represents the BAP immobilized on the surface of the sensor, and B is
38 the anti-BAP mAb in PBS buffer. This 1:1 binding model assumes that one BAP
39 molecule interacted with one anti-BAP mAb molecule. The K_{on} represents the number
40 of AB complexes formed per second in a 1 molar solution of A and B. the term K_{on} is
41 expressed in $m^{-1}s^{-1}$. The K_{off} represents the decay of AB complexes per second. The
42 term K_{off} is expressed in s^{-1} . K_D was calculated as K_{on} divided by the K_{off} , which
43 measures how tightly the BAP binds to its anti-BAP mAb.



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45 Fig. S2. Specificity analysis of the FM-LFIA. The concentration of all samples was
46 500 ng/mL. Data were obtained from three replicates.



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48 Fig. S3. Stability analysis of the FM-LFIA. Three spiked concentrations of BAP were

49 0, 5 and 50 ng/mL. Data were obtained from three replicates.