Supporting Information

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

Miao Liu, a Li-Feng Zeng, b Ya-Jie Yang, a Li-Ming Hu, a and Wei-Hua Lai *a

*Corresponding authors:

Dr. Wei-Hua Lai

State Key Laboratory of Food Science and Technology, Nanchang University
Address: 235 Nanjing East Road, Nanchang 330047, PR China
Tel.: +86 13879178802; fax: +86 791 88333708.
E-mail address: talktolaiwh@163.com

a State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

b Department of Clinical Laboratory, Jiangxi Provincial People’s Hospital, Nanchang 330006, China
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![Schematic diagram of BLI system steps](image-url)

Fig. S1 Schematic representative of the BLI system steps.
The relationship between $K_{\text{on}}$, $K_{\text{off}}$, $K_D$, and 1:1 binding model.

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

$$A + B \overset{K_{\text{on}}}{\underset{K_{\text{off}}}{\rightleftarrows}} AB$$

Where $A$ represents the BAP immobilized on the surface of the sensor, and $B$ is the anti-BAP mAb in PBS buffer. This 1:1 binding model assumes that one BAP molecule interacted with one anti-BAP mAb molecule. The $K_{\text{on}}$ represents the number of AB complexes formed per second in a 1 molar solution of A and B. The term $K_{\text{on}}$ is expressed in $\text{m}^{-1}\text{s}^{-1}$. The $K_{\text{off}}$ represents the decay of AB complexes per second. The term $K_{\text{off}}$ is expressed in $\text{s}^{-1}$. $K_D$ was calculated as $K_{\text{on}}$ divided by the $K_{\text{off}}$, which measures how tightly the BAP binds to its anti-BAP mAb.
Fig. S2. Specificity analysis of the FM-LFIA. The concentration of all samples was 500 ng/mL. Data were obtained from three replicates.
Fig. S3. Stability analysis of the FM-LFIA. Three spiked concentrations of BAP were 0, 5 and 50 ng/mL. Data were obtained from three replicates.