**DEVELOPMENT OF SENSITIVE ANTIGEN-DETECTION SYSTEM USING PHOTOACTIVATABLE ANTIBODY FC-BINDING PROTEIN CAPABLE OF INTRODUCING ORIENTED ANTIBODY**

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The photoactivatable antibody Fc-binding protein (PFcBP) allows oriented antibody (Ab) immobilization on various surfaces. Previously, we developed a system for producing the PFcBP containing photo-methionine (pMet) in *E. coli*, which can induce the covalent crosslinking to the Ab-Fc region, by engineering of methionyl-tRNA synthetase and FcBP derived from Protein G (1). In this study, we improved the photo-crosslinking efficiency by multipoint mutagenesis of PFcBP. and optimized the Ab immobilization process. The mutant PFcBP with 7-point substitutions showed the 25-30% enhanced photo-crosslinking efficiency as compared that with 4-point substitutions. The PFcBPs were immobilized onto the solid surfaces using the bifunctional crosslinkers with NHS and maleimide groups, and the Abs were then photo-crosslinked to the PFcBPs upon UV irradiation. The longer spacer arm length of the crosslinker was critical for immobilization of 1xPFcBP with a single Ab-Fc binding domain, but less critical for immobilization of 2xPFcBP with two domains. We also conjugated the PFcBP to the fluorescent beads, and subsequently photo-crosslinked detection Abs upon UV irradiation. Finally, we developed a cassette system capable of introducing capture and detection Abs with orientation onto the PFcBP immobilized chips and fluorescent beads, respectively, and demonstrated the effectiveness of the system in the detection of antigens in sera (Figure 1). We also first prepared the Ab-FcBP conjugates by direct photo-crosslinking of Abs and PFcBPs. After removing free PFcBP by gel filtration, the conjugates were immobilized onto the maleimide-activated surface. This process allowed more sensitive antigen detection than the sequential Ab-immobilization process.

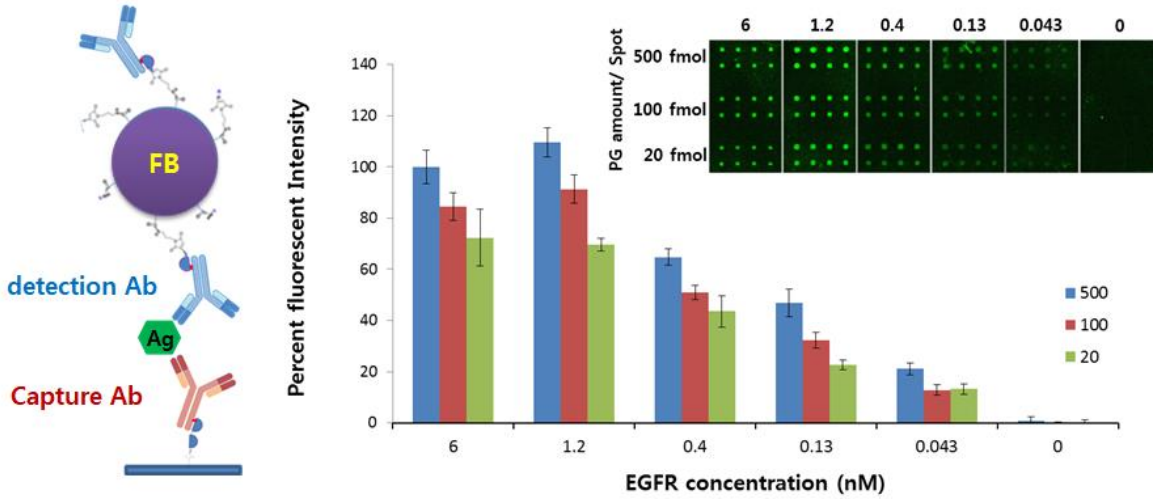


*Figure 1 – Antigen detection in sera using the cassette system. The capture antibody and detection antibody were photo-crosslinked to the PFcBP modified array chip and the fluorescent bead (FB), respectively. The antigen (EGFR) diluted in the human sera as indicated was applied onto the capture Ab-chip. The bound EGFR proteins were monitored using the detection-Ab modified FBs.*

(1) Lee Y, Jeong J, Lee G, Moon JH, Lee MK. (2016) Covalent and Oriented Surface Immobilization of Antibody using Photoactivatable Antibody Fc-Binding Protein Expressed in Escherichia coli. Anal Chem. 88(19), 9503-9.