Continuous Monitoring of IgG using Immobilized Fluorescent Reporters

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Abstract

In the manufacture of therapeutic monoclonal antibodies (mAbs), the clarified cell culture fluid is typically loaded onto an initial protein A affinity capture column. Imperfect mass transfer and loading to maximum capacity can risk antibody breakthrough and loss of valuable product, but conservative underloading wastes expensive protein A resin. In addition, the effects of column fouling and ligand degradation require the frequent optimization of IgG loading to avoid wastage. Therefore, continuous real-time monitoring of IgG flowthrough is of great interest. We previously developed a fluorescence-based monitoring technology that allows mix-and-read mAb detection in cell culture fluid. Here we report the use of reporters immobilized on CNBr-activated Sepharose 4B resin for continuous detection of IgG in column breakthrough. The column effluent is continuously contacted with immobilized fluorescein-labeled Fc-binding ligands to produce an immediately detectable change in fluorescence intensity. The technology allows rapid and reliable monitoring of IgG in a flowing stream of clarified cell culture fluid emerging from a Protein A column, without prior sample preparation. We observed a significant change in fluorescence intensity at 0.5 g/L human IgG, sufficient to detect a 5% breakthrough of a 10 g/L load, within 2 minutes at a flow rate of 0.5 mL/min.

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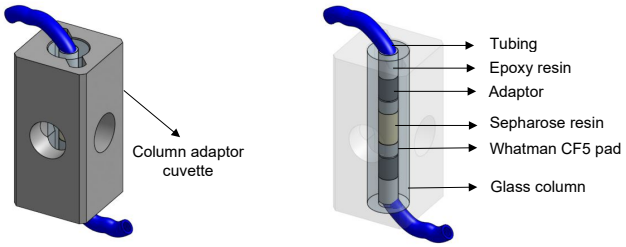
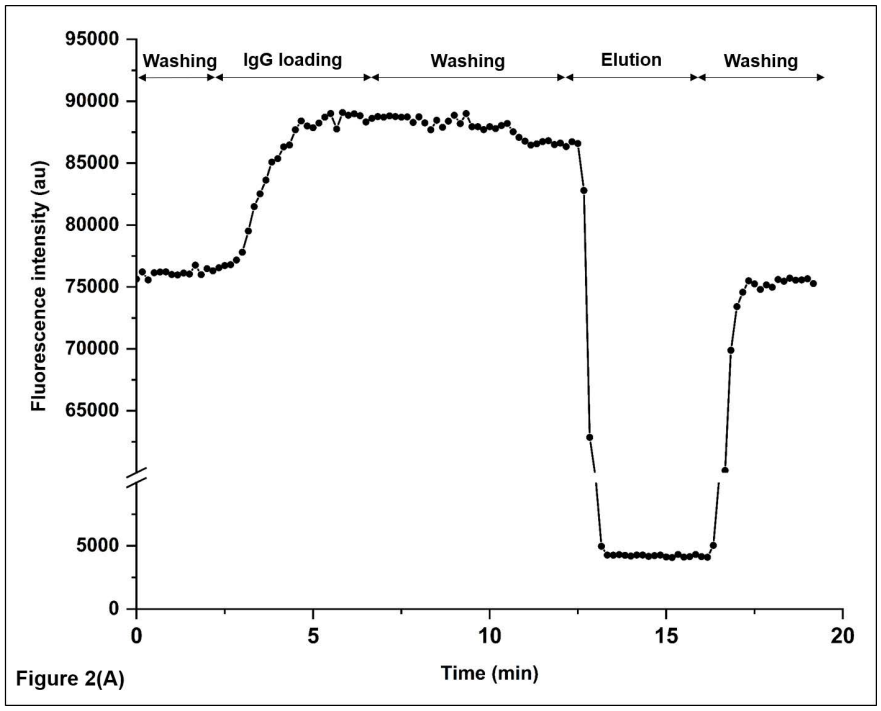


Figure 1.



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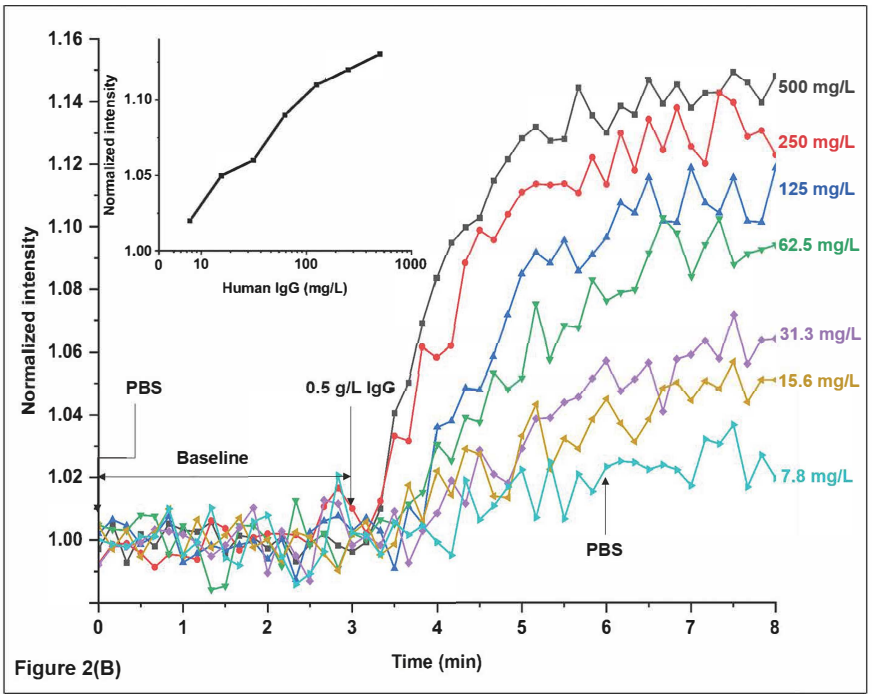

Figure 2(B)

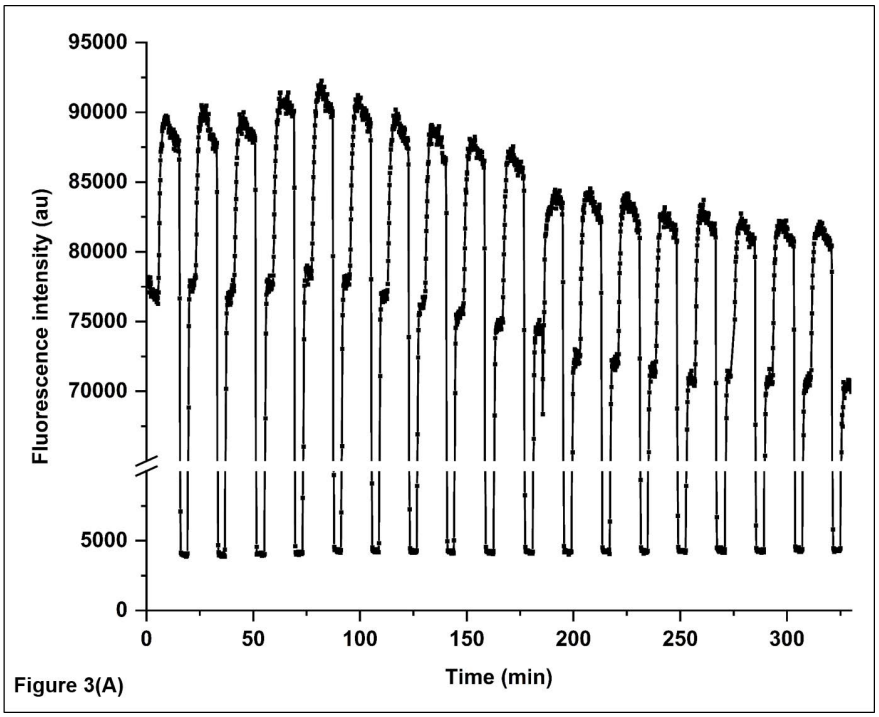

Figure 3(A)

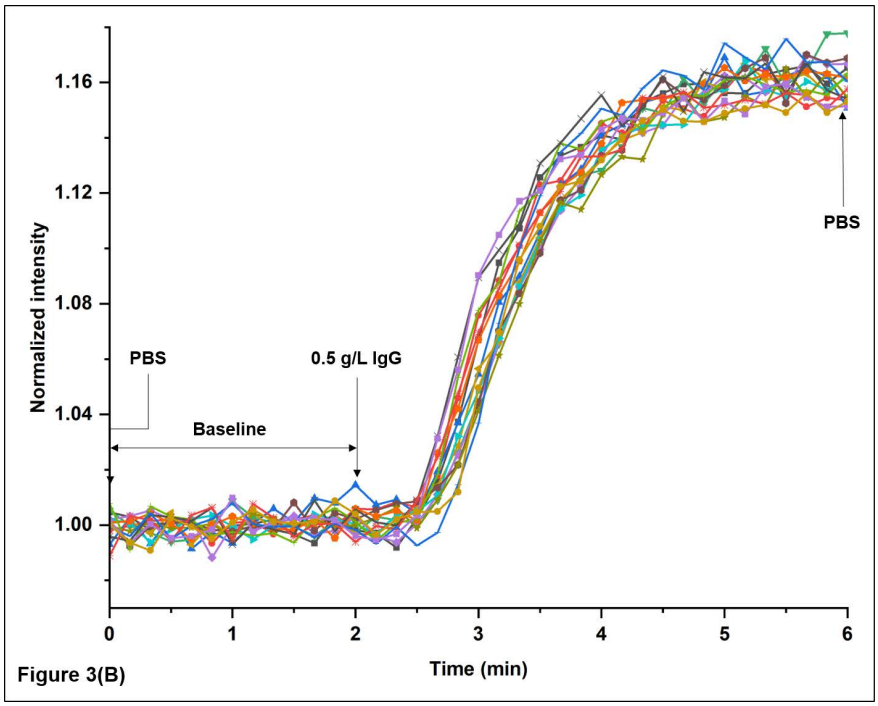

Figure 3(B)

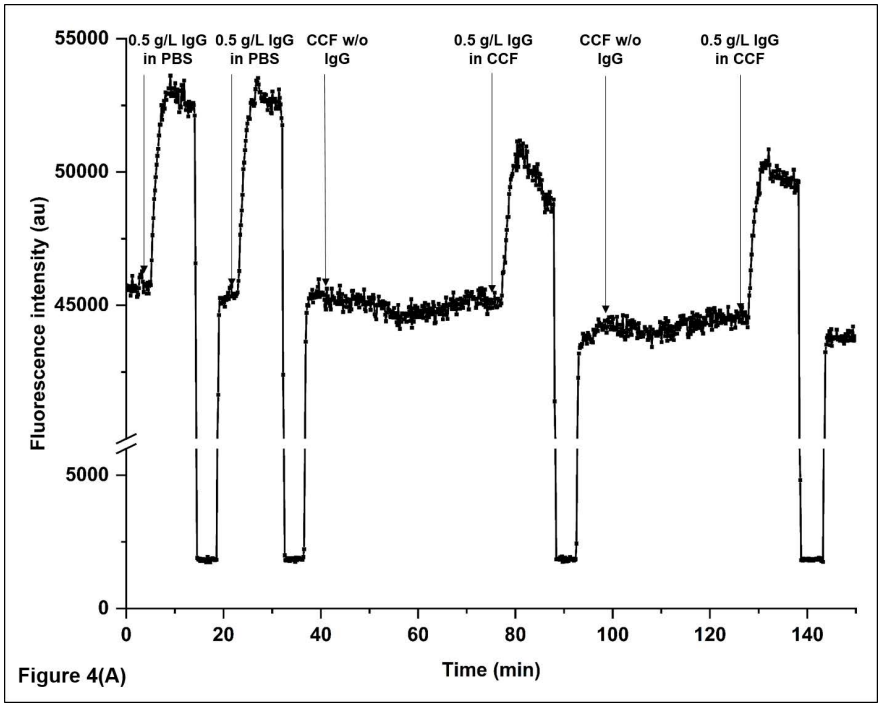

Figure 4(A)

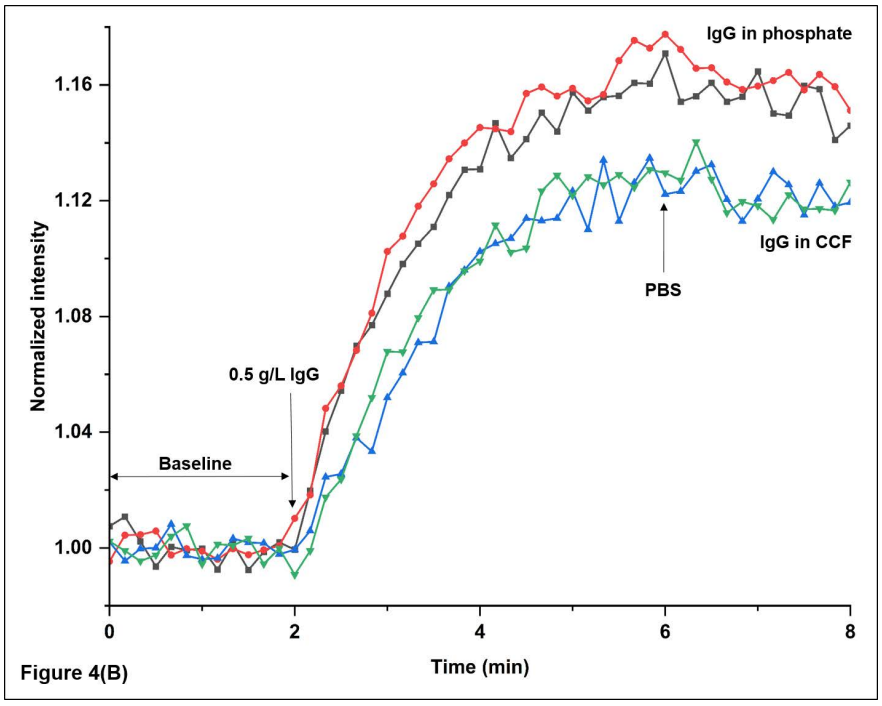

Figure 4(B)