Proteometer-L Kit for Titer and Aggregate Analysis of mAbs Produced in HEK-293 Cells

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BACKGROUND AND INTRODUCTION

Human Embryonic Kidney 293 (HEK-293) cells are commonly used to produce recombinant proteins for diagnostics, research, and therapeutic applications[1,2]. HEK-293 cells can be grown in suspension culture using a chemically defined medium (serum-free) to high densities. Features that make HEK2-293 cells a popular choice for recombinant protein production include high transfectivity, ease of transformation, rapid and reproducible protein production, high protein yield, and human post translational modifications. Monoclonal antibodies (mAbs) as well as human Fc-tagged antigens and receptors are examples of proteins produced in HEK-293 cells[3].

By circumventing the need for Protein A purification in titer and aggregate quality management, scientists can reduce assay time and labor. In this application note, we demonstrate how the Proteometer-L kit can effectively monitor titer and relative aggregate content of human or humanized antibodies directly in clarified fermentation broth (CFB) obtained from HEK-293 cell cultures. NIST Monoclonal Antibody Reference Material RM8671 (NmAb), a widely recognized mAb standard, was used for establishing the Proteometer-L Kit performance.

PRINCIPLE

The Proteometer-L Kit uses an Fc domain-coding, fluorescently labeled molecular recognition agent to detect monomers and aggregates of human or humanized mAbs proteoforms[4]. Un-coded sample components are invisible to the fluorescence detector, thus providing cleaner and more easily calculated results. Furthermore, fractionation of the sample removes interference, if any, due to non-specific binding of the molecular recognition agent to matrix components. The requirement for Protein A purification prior to analysis is therefore eliminated.

RESULTS

Clarified fermentation broth was obtained from a 5-day culture of untransfected HEK-293 cells (88.4% viability) grown in a well-defined serum-free medium under standard conditions for recombinant mAb protein production (Leinco Technologies, MO). NmAb was formulated at a concentration of 1 mg/mL in HEK-293 CFB for analysis and analyzed using the Proteometer-L Kit. Shown in Figure 1 is the overlay of chromatograms obtained by injecting various amounts of NmAb. As seen in Figure 1 inset, no significant

interference by HEK-293 CFB is observed. The standard curve for NmAb quantitation exhibited excellent linearity between 0.5 and 16 µg (Figure 2). The relative aggregate content determined by the Proteometer-L assay was 2.4% (CV = 1.4; *n* = 10).

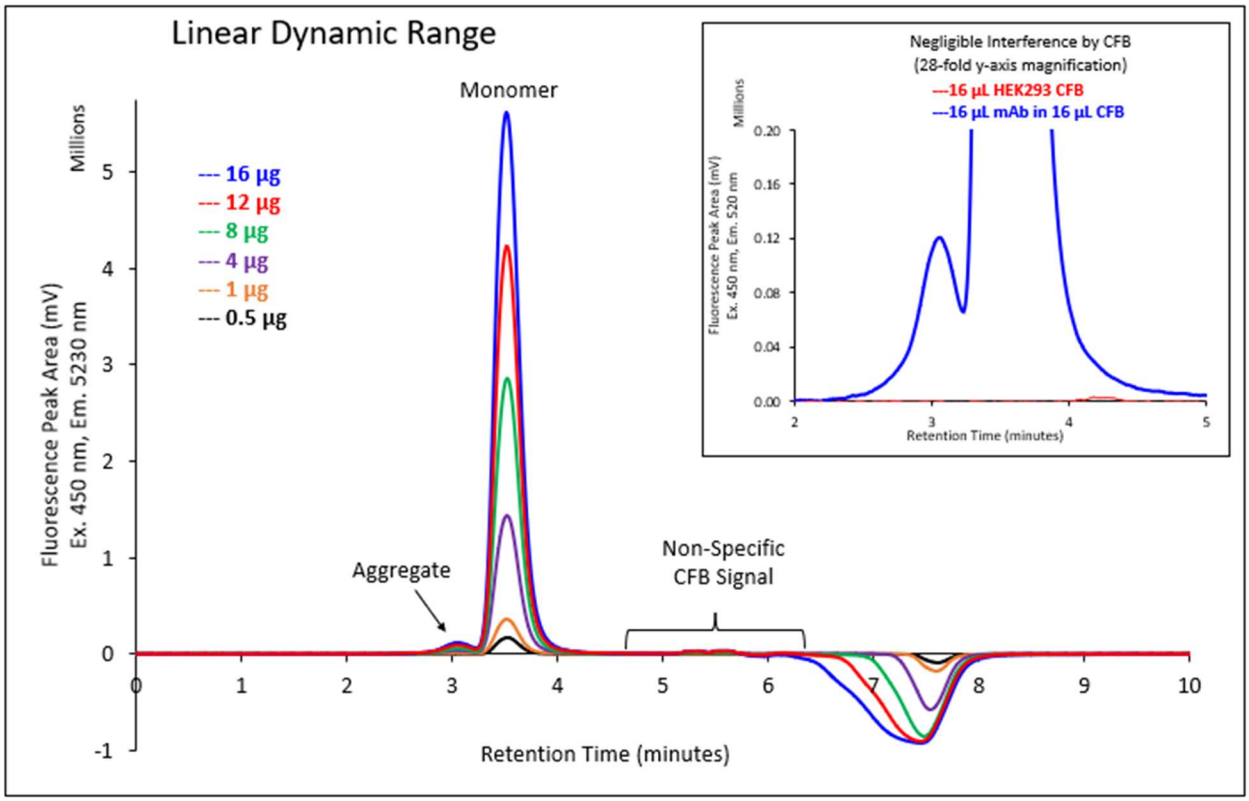

Fig. 1. Overlaid chromatograms showing Proteometer-L analysis of NmAb in HEK-293 CFB

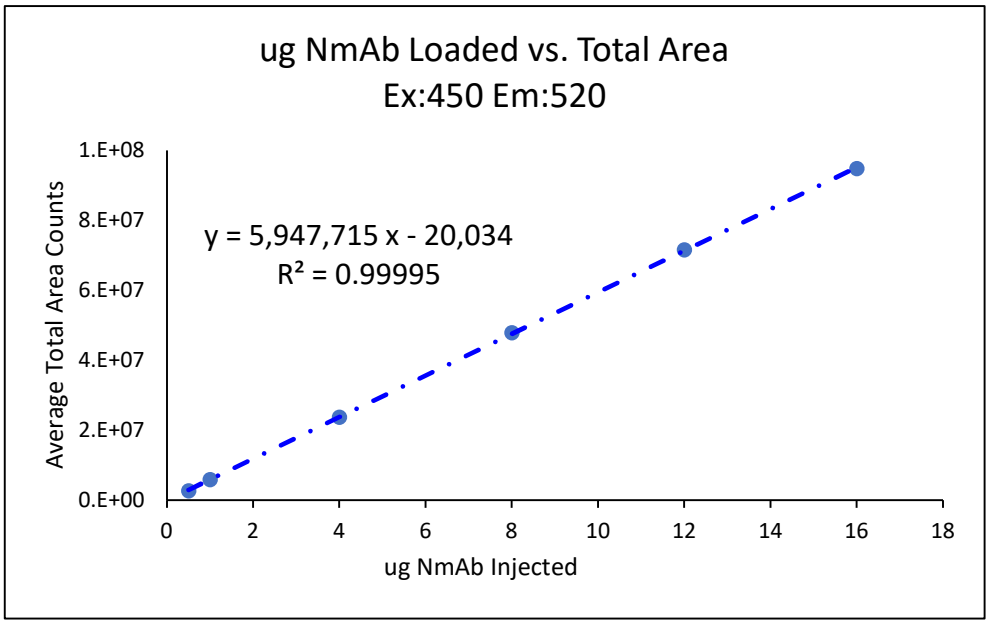

Fig. 2. Standard curve for NmAb quantification by Proteometer-L Kit

CONCLUSIONS

The specificity of the molecular recognition agent enables the Proteometer-L Kit to effectively monitor titer and relative aggregate content of human or humanized mAbs directly in CFB obtained from HEK-293 cell cultures. The components of HEK-293 CFB do not significantly interfere with the mAb signal. The results obtained are very similar to those observed with CHO cells[4].

REFERENCES

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