Aggregate Content of NIST mAb RM 8671 by the Proteometer-L Kit - Comparison With NIST Values

NOVILYTIC

BACKGROUND AND INTRODUCTION

Novilytic's Proteometer-L Kit compares titer and aggregate content of various human or humanized monoclonal antibody (mAb) samples directly in the clarified fermentation broth (CFB) of cultured mAb-expressing cells. Its unique attributes make it a valuable tool at various mAb development and production stages. In the early stages of product development, circumvention of Protein A purification prior to analyses saves both time and labor. The product provides highly reproducible data, allowing it to be utilized for process monitoring and control throughout the therapeutic discovery, development and production process. Data and graphs illustrative of these two attributes of Novilytic's Proteometer-L Kit is provided below.

PRINCIPLE

The Proteometer-L Kit employs Novilytic's proprietary Mobile Affinity Sorbent Chromatography (MASC™) technology, whereby specific fluorescence coding of the analyte and size-based separation of sample components occur concurrently in the Proteometer-L reactor[1]. The Proteometer-L Kit provides specificity for the detection of the mAb analyte using a fluorescent molecular recognition agent. This agent binds specifically to the human Fc region of monoclonal antibodies, bispecifics, and fusion proteins. The Proteometer-L Kit may therefore be used for the determination of relative aggregate content and titer of these molecules directly in CFB of both CHO and HEK-293 cell cultures.

Aggregate content, whether measured using the Proteometer-L Kit or by traditional methods such as size exclusion chromatography of purified mAbs coupled with UV absorbance detection at A280 nm (SEC-UV) is not an absolute value. Both methods yield a relative value for the following reasons:

- On a molar basis, the aggregate and monomer mAb species may differ from one another in their fluorescence response (Proteometer-L) and in their A280nm absorbance (SEC-UV).
- The nature and composition of the aggregate species determines their fluorescence intensity per mole (Proteometer-L) as well as their A280nm molar extinction coefficient (SEC-UV).

This application note demonstrates the excellent reproducibility of the Proteometer-L assay. Additionally, it compares the aggregate content of NIST mAb RM 8671 (NmAb) formulated in CFB of CHO cells, as measured by the Proteometer-L Kit, with the aggregate content reported by NIST using two different methods for the same purified material. Although the results are comparable, they are not identical for the reasons stated above.

RESULTS

NIST mAb (NmAb, RM 8671, Lot no. 14HB-D-002) was formulated in the CFB from CHO cells at a concentration of 1 mg/mL[1]. The HPLC system was set up with a Protometer-L Kit, and injections of NmAb in CHO CFB were conducted as specified. An overlay of representative chromatograms obtained by injecting NmAb across a range of 0.5 -16 µg is shown in Figure 1. Despite the presence of host cell proteins in the CFB sample matrix, no interference from these components is observed. As a result, the aggregate content can be determined without any sample cleanup prior to analysis. The inset in Figure 1 shows that excellent linearity is obtained by the Proteometer-L assay with a coefficient of determination (R^2) value of

0.9999. The data shown in Figure 2 illustrates the relative aggregate content of NIST mAb as measured by 76 different Proteometer-L Kits. Each bar is the average of 5 injections of NmAb (4 µg). The high degree of repeatability is demonstrated with a CV of 6.67% across these different Proteometer-L Kits. Table 1 compares the relative aggregate content results from Proteometer-L with size heterogeneity values reported by NIST using orthogonal methods.

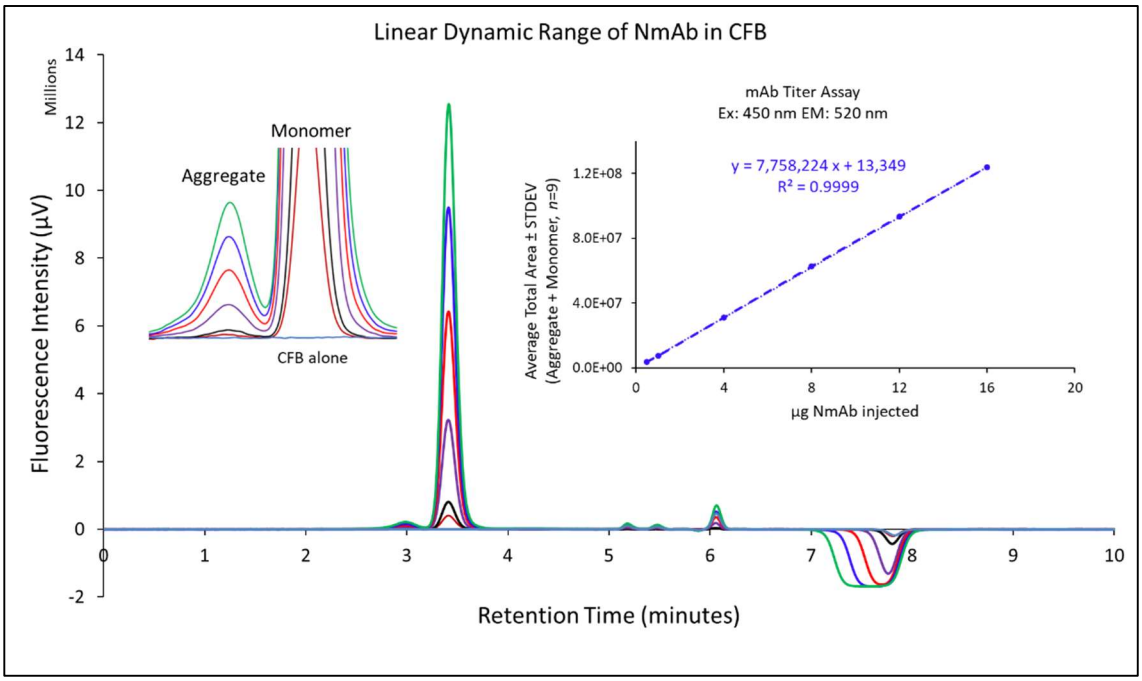

Figure 1. Linear dynamic range of NmAb in CFB by Proteometer-L Kit

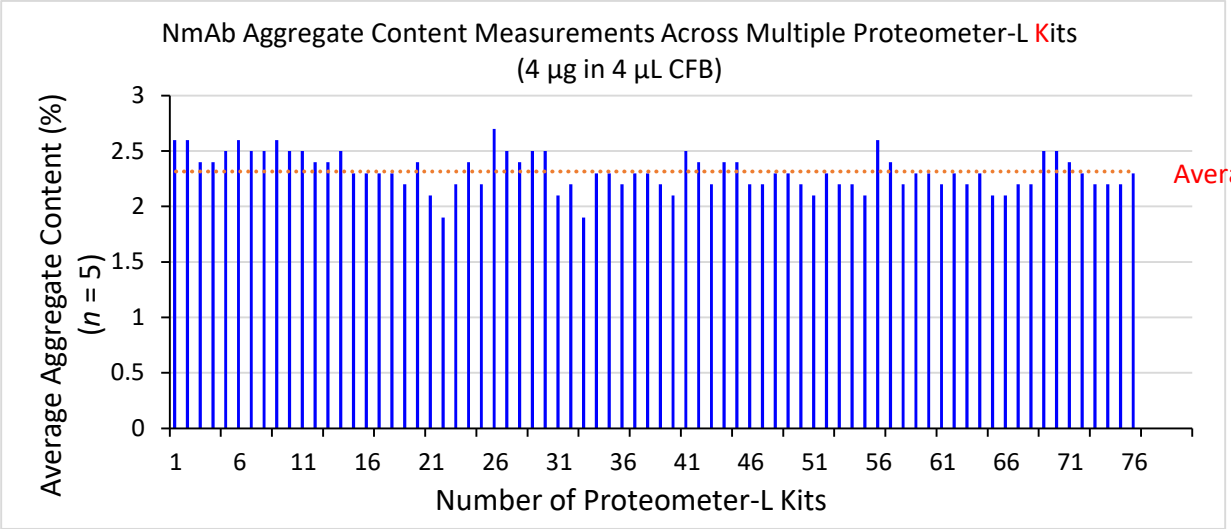

Figure 2. Proteometer-L repeatability and size heterogeneity of NmAb in CFB by Proteometer-L across 76 kits

Method	Novilytic	NIST	
	Proteometer-L	Qualified SEC-UV	Non-reducing CE-SDS
Sample Matrix	CHO CFB	L-His Buffer	L-His Buffer
Avg. Monomeric Purity (%)	**97.68**	**96.63**	**98.47**
Avg. Aggregate Content (%)	2.32	3.17	1.53 (calculated)

Table 1. Comparison of size heterogeneity of data of NmAb

CONCLUSIONS

The data in Table 1 shows that the aggregate content of NIST mAb RM 8671 (NmAb) formulated in CFB from Chinese hamster ovary (CHO) cells, as measured by the Proteometer-L Kit, is comparable but not identical to the aggregate content reported by NIST using two orthogonal methods for the same purified material.

Reproducibility and sensitivity of the Proteometer-L assay are two of its salient features. Conventional or standard SEC-UV aggregate analyses of mAbs require considerably larger amounts of mAb per analysis. For example, the USP standard HPLC method requires 200 µg[2]. Additionally, the values reported by NIST using SEC-UV (Table 1) were obtained by analysis of 60 µg of pure NmAb[3]. The Proteometer-L method aggregate values reported in Table 1, Figure 2 only required 4 µg.

REFERENCES

1. Narsimhan ML, Kim J, Morris NA, Bower MA, Gunawardena HP, Bowen E, Regnier FE. Mobile Affinity Selection Chromatography Analysis of Therapeutic Monoclonal Antibodies. Anal Chem. 2023. ***95:***16115-22. DOI: 10.1021/acs.analchem.3c02180
2. Aggregation Analysis Using SE-HPLC and SE-UHPLC Methods *in* USP General Chapter Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies <129> https://www.usp.org/sites/default/files/mAb/USP_AggregationAnalysis_ApplicationNote_FINAL.pdf
3. Tarlov MJ, Choquette SJ. *In* Report of Investigation Reference Material 8671, NIST mAb, Humanized IgG1k Monoclonal Antibody. 2016. pp 1-16.