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Development of plate-based sialic acid assays to support clone screening and early Stage upstream process development

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DEVELOPMENT OF PLATE-BASED SIALIC ACID ASSAYS TO SUPPORT CLONE SCREENING AND EARLY STAGE UPSTREAM PROCESS DEVELOPMENT

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Sialic acid, a post-translational modification, is an important product quality attribute of glycoproteins. The levels of sialic acid may impact solubility of the final product and the half-life (product clearance). In addition, the N-glycolylneuraminic acid (NGNA) form of sialic acid may elicit an immunogenic response in humans. In an effort to have a more high-throughput evaluation of these product quality attributes, two plate based assays were developed.

Specifically, a direct NGNA ELISA was developed using a polyclonal chicken anti-NGNA antibody. This assay was implemented as one of the clone screening tools to eliminate clones that may produce more of the immunogenic sialic acid. In addition, the assay was developed using both purified materials and harvest cell culture fluid (HCCF), thereby eliminating the need for downstream processing and enabling high-throughput of samples. The results of the assay were verified using the more traditional Dionex method.

The second assay developed was a fluorescence based plate assay to measure total sialic acid levels on our protein of interest. During early stage process development, different culture conditions resulted in varying levels of total sialic acid on the final product. This assay was used to monitor the impact of changes in media/feed components and operation parameters on total sialic acid levels. Although results of this plate based assay from purified material gave the same ranking as those from the Dionex method, assays performed with HCCF gave much higher background values. Therefore, a high-throughput resin plate method of purification to provide suitable and representative samples was established. The purity of these one step purified materials were > 80% and the assay results were in line with the traditional methods.

Using both of the assays developed ensured we established a cell line and upstream process that produced a protein with the desired quality of sialic acid content, while minimizing the potentially immunogenic NGNA.