Feasibility of GlykoPrep™ Sample Preparation for Glycoanalysis on the AssayMAP® Bravo® Liquid Handling Workstation

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Abstract

Until recently, sample preparation for glycan analysis has been a manual, complex and time-consuming process that usually limits analysis to only the most critical decision points. To address the growing need for increased throughput of N-Glycan profiling, we present feasibility studies using the GlykoPrep Sample Preparation Platform (GlykoPrep) on the Agilent Technologies AssayMAP Bravo Workstation, encompassing the Bravo Liquid Handling System with the 96AM Syringe Head (AssayMAP Bravo, Figure 1) and compare throughput, time to results and data quality for two orthogonal analytical methods.

Methods

Increases in cell culture expression levels have the potential to significantly reduce post-translational modification (PTM) analysis, eliminating the therapeutic protein, which may affect its efficacy. N-Glycan profiling, using a wide array of analytical methods, is the core method for monitoring changes in N-glycosylation, and demands for this type of characterization are increasing. Manual sample preparation for N-Glycan profiling is complex and tedious, and can become a major bottleneck and source of error, precluding its use in screening applications, such as clone selection and cell culture optimization.

The GlykoPrep N-Glycan Sample Preparation Kit (GlykoPrep Kit) has significantly improved throughput and data quality, but until now has been limited to a manual, spin format. GlykoPrep incorporates AssayMAP high-throughput, microchromatography Cartridges, which have been specifically designed for the AssayMAP Bravo, capable of chromatographic separations, chemical reactions and enzymatic digestions. Figure 2 shows the modules and their individual steps for the GlykoPrep Kit, incorporating the essential functional steps of the current manual, in-solution method, to generate N-Glycans suitable for analysis by a number of analytical systems. This poster presents preliminary results for the GlykoPrep Kit on AssayMAP Bravo using human IgG (hIgG), with the goal of high-throughput, walkaway sample preparation with equivalent or better data quality to the manual GlykoPrep Kit. In addition, if AssayMAP Bravo can handle complex a process as GlykoPrep, then other simpler protocols may also be readily achievable.

Results

Bravo™ labeling performed on the Bravo yielded consistent results for hIgG. The overlaid and normalized CE/LIF chromatograms in Figure 7A represent replicate samples of hIgG processed simultaneously on the Bravo. A rapid (13-minute) CE/LIF method was used, and with this method several of the N-Glycans co-fractionated. Examples are G2, G1F, G1F and G1B and G2B. Replicate profiles are normalized to demonstrate sample-to-sample relative abundance. The relative abundance of the N-Glycans is normalized by percent peak area (Figure 7, right panel).

Discussion

Our results demonstrate that the complex procedures represented by N-Glycan analysis using the GlykoPrep Kit can be effectively automated on the AssayMAP Bravo. Reducing sample preparation time reduces overall time to results and, when 96 samples are processed simultaneously, increases throughput. The excellent reproducibility of the AssayMAP Bravo also reduces the variability generated in the manual, multi-step sample preparation process. Sample preparation can be completed in ~2 hours, allowing preparation of hundreds of samples during a work day. Data quality for relative N-Glycan profiling was improved over the manual GlykoPrep Kit using InstantAB labeling. Furthermore, the AssayMAP Bravo was committed to commercializing GlykoPrep on the AssayMAP Bravo.

Conclusion

The AssayMAP Bravo demonstrated improved throughput, faster time to results for sample preparation, and improved data quality using two orthogonal analytical methods. Agilent is committed to commercializing GlykoPrep on the AssayMAP Bravo.

http://www.prozyme.com/GlykoPrep

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Figure 1 - AssayMAP® Bravo® Workstation

Figure 2 - GlykoPrep™ Modules and Individual Steps of N-Glycan Sample Preparation

Figure 3 - AssayMAP® Cartridges

Figure 4 - Probe Syringe Liquid Handling

Figure 5 - InstantAB™

Figure 6 - GlykoPrep N-Glycan Sample Preparation: AssayMAP® Bravo® (Green) vs. Manual Spin Format (Blue) % Peak Areas for hIgG (see Table 3 for results)

Figure 7 - APTS