A New Platform for High Throughput Micro Chromatography Analysis

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Introduction

The advent of Quality by Design (QbD) has dramatically increased the experimentation required to develop a robust biopharmaceutical manufacturing process. Even relatively simple assays such as antibody detection have become a significant bottleneck for large designed experiments. Highly complex assays, such as glycan profile analysis, are equally important for process design space, but are well beyond current throughput capabilities.

The AssayMAP™ platform was designed to enable micro-scale analytical binding/lotel chromatography and enzymatic digestion to be performed in a high-throughput, parallel format compatible with microplate liquid handling.

AssayMAP™ Cartridge

AssayMAP™ utilizes disposable cartridges containing ~5 µL packed bed with any resin (particle size 20 – 100 µm). The bed is retained by inserting molded bed support films. Above the bed is an inlet seal and hub (used for robotic operation) and a sample cup (which can hold up to 200 µL).

In operation, the cartridges are loaded into racks stacked on the appropriate plates, and sample buffers and other reagents are pipetted into the cartridge cups. The cartridge/rack/plate is then spun in a standard microplate centrifuge to draw the liquid through the bed. With a 2 plate rotor, up to 320 samples can be processed simultaneously (up to 384 with a 4 plate rotor). Fewer samples can be processed by loading fewer cartridges in the rack.

Automation Approaches

A variety of different approaches may be used to perform AssayMAP™ protocols. Automated processes (such as the MAb Titer Assay) can be easily and effectively run manually with multi-channel pipettes. Throughputs of up to 384 samples per hour can be obtained in this way.

MAB Titer Assay

Antibody concentration or titer assays are a known bottleneck in cell culture process development. The current “gold standard” method – protein A affinity HPLC – is limited to at most 4 to 12 samples per run. Other methods are either complex to perform or require dedicated instrumentation. The AssayMAP MAB Titer assay was developed to utilize the same method and chemistry as the “gold standard” (i.e. protein A affinity chromatography), while delivering dramatically improved throughput without special instruments or equipment.

The MAB Titer assay protocol is illustrated below. The cartridges are first prepared by purging 200 µL buffer into the cup and spinning into the receiver plate at high g, which evacuates the resin and drives all the air out of the bed. The resin is then equilibrated with the sample and the sample is placed in the cup and spun at a low g to ensure complete binding. The cartridge is then washed to remove any medium and a sample withholding to an empty receiver plate. The bound, purified IgG is then eluted with 50 µL low pH buffer with a medium g to spin into a half-area microplate.

Readout of the plate is performed in a conventional plate reader. First the plates are read at 280 nm. Optionally a chromogenic protein assay reagent (such as Coomassie Blue) can be added for increased sensitivity and read at visible wavelengths (e.g. 590 and 450 nm).

Performance with Standards

The MAB Titer Assay cartridge functions by simultaneously and quantitatively binding the IgG from the sample (regardless of its volume) and eluting the bound IgG into a fixed final volume. The functional assay range is best expressed in terms of mass (µg) of IgG in the sample, and is ultimately determined by the binding capacity of the cartridge at high product concentration based on use of low volume, rapid washes. The mass load depends upon the volume sample which can vary from 5 – 100 µL and is related to the concentration by:

Concentration = Mass / Volume

This is illustrated in the data below. A 2X serial dilution of IgG was made from 5000 µg/mL down to 0.05 µg/mL, at 0.5 and 1 µL samples volumes were run on the cartridges. Readout was done using both OD 280 nm and Coomassie Blue reagent (using the ratio OD 590 nm/OD 280 nm). Plotting as µg/mL, the curves for the three sample volumes overlap. The OD 280 nm curve is highly linear up to just under 100 µg/mL, then flattens out, due to saturation of the binding capacity. The Coomassie Blue reagent is “10X more sensitive (with an LOD c1 µg), but becomes non-linear above ~15 µg/mL, due to saturation of the AGE binding.

The same data are shown below plotted vs. NgG concentration in µg/mL. If the full range of sample volumes and the two readout methods are utilized, the assay has an effective range of 10 – 10,000 µg/mL.

N-Glycan Profiling Assay

The precise pattern of glycosylation, set during cell culture, can critically affect the efficacy and pharmacokinetics of biopharmaceuticals, including antibodies. Glycan profile analysis is currently performed on HPLC, PAGE, or LC-MS, but such preparation is a major bottleneck. The target protein must first be purified from the sample. N-glycans are then specifically released by digestion with enzymes, prepared from the glycoprotein, and often, fluorochemically labeled and cleaned up for analysis. This sample prep is usually done manually, can take several days, and is cumbersome for large numbers of samples.

Prozyme, Inc. developed a new N-glycan preparation procedure (GlykoScre® Rapid Sample Preparation System, available under technology transfer) that has now been implemented on the AssayMAP platform. The procedure includes purification of antibody from crude samples using the MAB-Titer Assay described above. Using this high throughput approach, fluorochemically labeled N-glycans can be profiled in up to 384 samples in less than 3 hours and fully automated. The AssayMAP based protocol is summarized here:

Summary

AssayMAP provides a highly flexible platform which enables a wide range of analytical sample prep protocols involving binding/lotel chromatography and/or enzymatic digestion to be performed with high throughput on a broad range of automation platforms.