A New Platform for High Throughput Micro Chromatography Analysis

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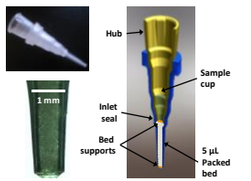
Introduction

The advent of Quality by Design (QbD) has dramatically increased the experimentation required to develop a robust biotherapeutic manufacturing process. Even relatively simple assays such as antibody concentration 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.

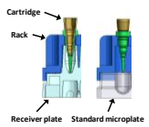
The AssayMAP™ platform was designed to enable microliter-scale analytical bind/elute chromatography and enzymatic digestion to be performed in a high-throughput, parallel format compatible with microplate liquid handling.

AssayMAP™ Cartridge



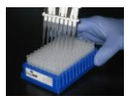
AssayMAP utilizes disposable cartridges containing a 5 µL packed bed with any resin (particle size 20 – 100 µm). The bed is retained by insert-molded bed support filters.. Above the bed is an inlet seal and hub (used for robotic operation) and a sample cup (which can hold up to 200 µL).

The cartridges are used in special molded racks which stack on either a standard microplate to collect eluted product for analysis or a special "receiver plate", which keeps the cartridge tip immersed in liquid to prevent the bed from drying out during centrifugation.

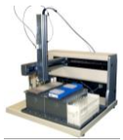


In operation, cartridges are loaded into racks stacked on the appropriate plates, and sample, buffers and other reagents are pipetted into the cartridge cups. The cartridge/rack/plate is then spun in a standard microplate centrifuge to drive the liquid through the bed. With a 2-plate rotor, up to 192 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



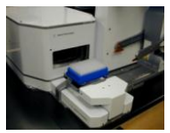
A variety of different approaches may be used to perform AssayMAP protocols. Simple protocols (such as the MAb Titer Assay) can be easily and effectively run manually with multi-channel pipets. Throughputs of up to 384 samples per hour can be obtained in this way.



Complex protocols (such as the N-Glycan Profiling Assay) can be efficiently performed using a single channel automated liquid handler, such as the Gilson GX271 shown here. This low cost approach provides a significant improvement in reliability and precision over manual liquid handling methods.

AssayMAP protocols can also be fully automated for applications requiring very high throughput and/or full "walk-away" capability. These photos show an assay being performed at Agilent Automation Solutions, using the Bravo liquid handler, BenchCel plate stacker/handler and VSpin robotic centrifuge.

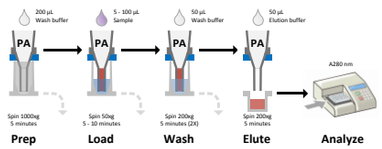




MAb Titer Assay

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/hour. 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 pipetting 200 µL buffer into the cup and spinning into the receiver plate at high x g, which rewets the resin and drives all the air out of the bed. The receiver plate is emptied and the sample is placed in the cup and spun at a low x g to insure complete binding. The cartridge is then washed using two medium x g spins with 50 µL buffer into an empty receiver plate. The bound, purified IgG is then eluted with 50 µL low pH buffer with a medium x g spin into a half-area microplate.



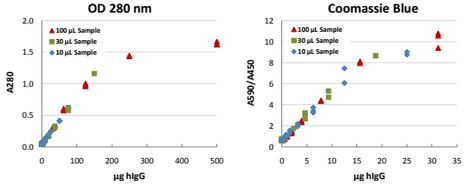
Readout of the plate is performed in a conventional plate reader. First the plates are read at 280 nm. Optionally a colorimetric protein assay reagent (such as Coomassie Blue) can be added for increased sensitivity and read at visible wavelengths (e.g. 590 and 450 nm).

Performance with Standards

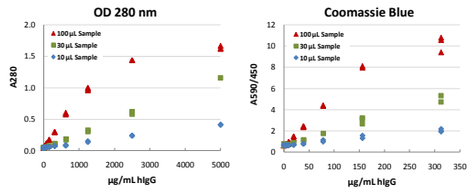
The MAb Titer Assay cartridge functions by selectively and quantitatively binding the IgG from the sample (irrespective of its volume) and eluting the bound IgG into a fixed final volume for readout. The functional assay range is best expressed in terms of mass (µg) of IgG in the sample, and is ultimately determined by the binding capacity of the cartridge at high product concentration and the sensitivity of the readout method at low concentration. The mass loaded depends upon the sample volume (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 hIgG was made from 5000 µg/mL downward, and 10, 30 and 100 µL samples of each standard were run on the cartridges. Readout was done using both OD 280 nm and with Coomassie Blue reagent (using the ratio OD 590 nm/OD 450 nm). Plotted as µg hIgG, the curves for the three sample volumes overlap. The OD 280 nm curve is highly linear up to just under 100 µg hIgG, then flattens out, due to saturation of the cartridge binding capacity. The Coomassie Blue readout is ~10X more sensitive (with an LOQ <1 µg), but becomes non-linear above ~15 µg hIgG, due to saturation of the dye binding.



The same data are shown below plotted vs. hIgG 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.



Sensitivity & Matrix Effects

The low end sensitivity of the assay is determined by binding and elution of non-IgG protein in the sample. These results show the Coomassie Blue results for runs with 25 µL samples of two cell culture media and 10,000 µg/mL fish gelatin (an ELISA blocker), compared with 0 and 1.56 µg/mL hIgG in buffer.

Sample	A590/450	µg IgG from Standard Curve
Buffer Blank	0.644	0.00
1.56 µg hIgG in buffer	0.734	1.56
Culture Medium A	0.700	0.02
Culture Medium B	0.646	0.00
250 µg fish gelatin in buffer	0.786	1.70

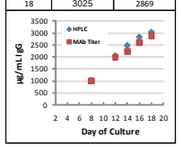
Monitoring Cell Culture Optimization

A primary application for the MAb Titer assay is monitoring product IgG concentration during cell culture process optimization. Many biopharmaceutical companies are finding this assay to be a bottleneck as they develop technologies for high throughput cell culture development to optimize state-of-the-art high titer production processes and to meet the needs of QbD.

The results shown below are from a set of bioreactor runs under three different operating conditions. The product was a monoclonal antibody produced in mammalian cells transfected using the GPEx® technology at Catalent Pharma Solutions (Middleton, WI). Periodic samples were taken from the bioreactor from 4 – 18 days. Samples were analyzed using the standard affinity HPLC method at Catalent, and analyzed by the MAb Titer assay (25 µL sample, readout by OD280) at BioSystem Development. Samples and HPLC data were provided by Ian Collins and John Otto of Catalent Pharma Solutions.

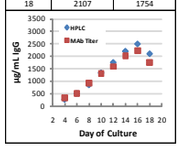
Condition 1

Day	HPLC	MAb Titer
4		
6		
8	1029	1015
10		
12	2046	1983
14	2473	2242
16	2823	2610
18	3025	2869



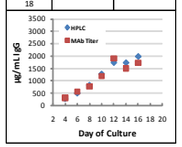
Condition 2

Day	HPLC	MAb Titer
4	277	337
6	518	511
8	861	925
10	1343	1314
12	1762	1587
14	2211	2014
16	2501	2233
18	2107	1754



Condition 3

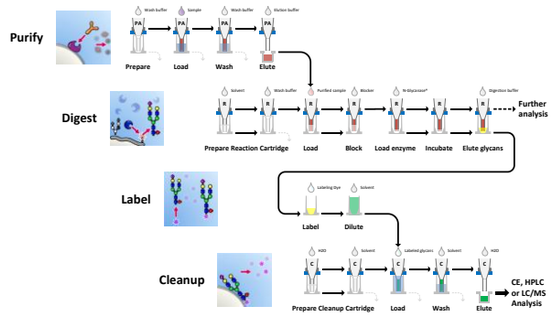
Day	HPLC	MAb Titer
4	267	318
6	491	569
8	815	770
10	1266	1194
12	1728	1906
14	1721	1513
16	1973	1727
18		



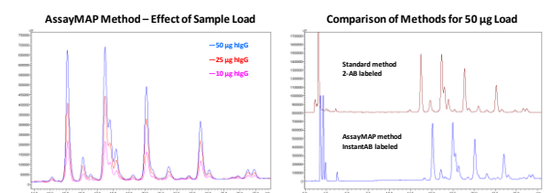
N-Glycan Profiling Assay

The precise pattern of glycosylation, set during cell culture, can critically affect the efficacy and pharmacokinetics of biotherapeutics, including antibodies. Glycan profile analysis is currently performed by CE, HPLC or LC/MS, but sample preparation is a major bottleneck. The target protein must first be purified from the sample. N-glycans are then specifically released by digestion with enzyme, separated from the glycoprotein, and often fluorescently 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. has developed a new N-glycan preparation procedure (GlykoScreen™ 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, fluorescently labeled N-glycans can be produced on up to 192 samples in less than 3 hours and fully automated. The AssayMAP-based protocol is summarized here:



The HPLC chromatograms below show the results of running this protocol on 10, 25 and 50 µg samples of hIgG standard, and labeling with GlykoScreen™ InstantAB™. For comparison, N-glycans released using standard methods from 50 µg of hIgG using N-Glycanase® PNGase F were labeled with 2-AB (GKK-404 Signal™ 2-AB Labeling Kit). The prepared glycans were analyzed by HPLC using a GlykoSep™ N-Plus Column run with a single binary gradient of ammonium formate buffer and acetonitrile. The glycan recovery appears to be linear with sample load and the conventional and AssayMAP protocols show very comparable glycan profiles.



Summary

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