Rapid, High-Throughput, High Sensitivity Determinations of MAb Concentration for Cell Line Screening and Process Optimization

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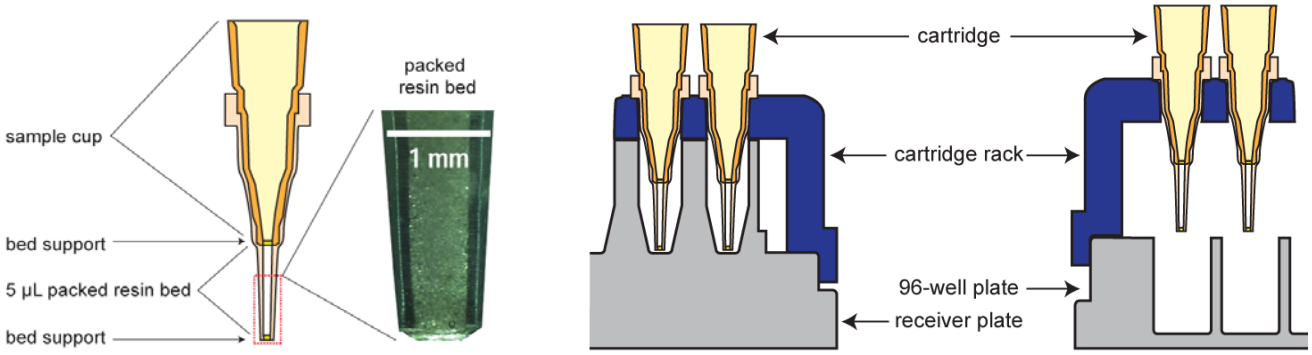
Introduction

Determination of MAb concentrations or titers in cell culture supernatant samples is a critical tool for all stages of biopharmaceutical development. In cell line screening and selection, the actual titer levels are low and sample volumes are limited. ELISA is the most widely used screening method, but it is cumbersome to perform, challenging to automate and has high variability.

For cell culture process optimization, high sensitivity is not required, but high analytical precision is a must. Since the advent of Quality by Design (QbD), the experimentation required to develop a robust cell culture process has increased dramatically. The current "gold standard" method – protein A affinity HPLC – is reliable, selective, sensitive and precise, but throughput is limited to at most 4 – 12 samples/hour. Other methods are either complex to perform or require dedicated instrumentation. The AssayMAP® MAb Titer assay was developed to deliver dramatically improved throughput for both of these applications while utilizing standard laboratory equipment.

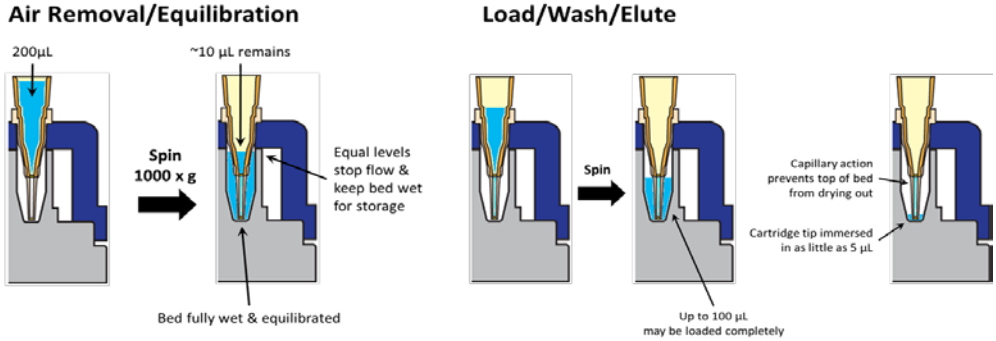
AssayMAP® Cartridge Technology

The AssayMAP disposable cartridges contain a 5 µL bed packed with affinity resins, retained by insert-molded support filters. Above the bed is a 200 µL sample cup. The cartridge functions as a spin column, with centrifugal force used to drive liquids from the sample cup through the bed and out the tip.



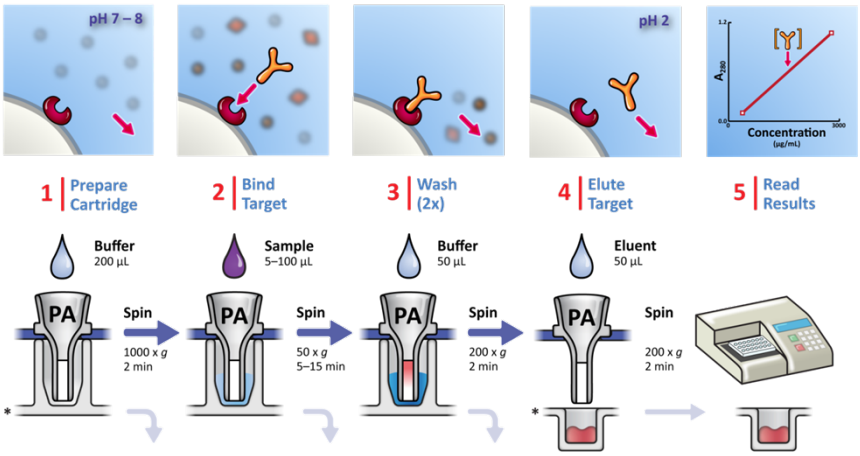
The cartridges are used in a molded rack which can hold up to 96 units. The rack conforms to the ANSI/SBS 96-well microplate standard, and can be mounted on any standard microplate to collect the output of the cartridges.

In order to obtain high analytical precision it is necessary for the cartridges to provide fully quantitative binding and elution, which is impossible with pipet tip columns and similar devices. Rapid mass transport resins and optimized residence times contribute to quantitative results. It is also critical to keep air bubbles out of the bed. A special receiver plate (patent pending) designed to work with the cartridge and rack enables high speed centrifugation to clear air from the bed and keeps the outlet tip immersed in liquid through low-speed centrifugation loading, wash and elution, which prevents drying of the bed.

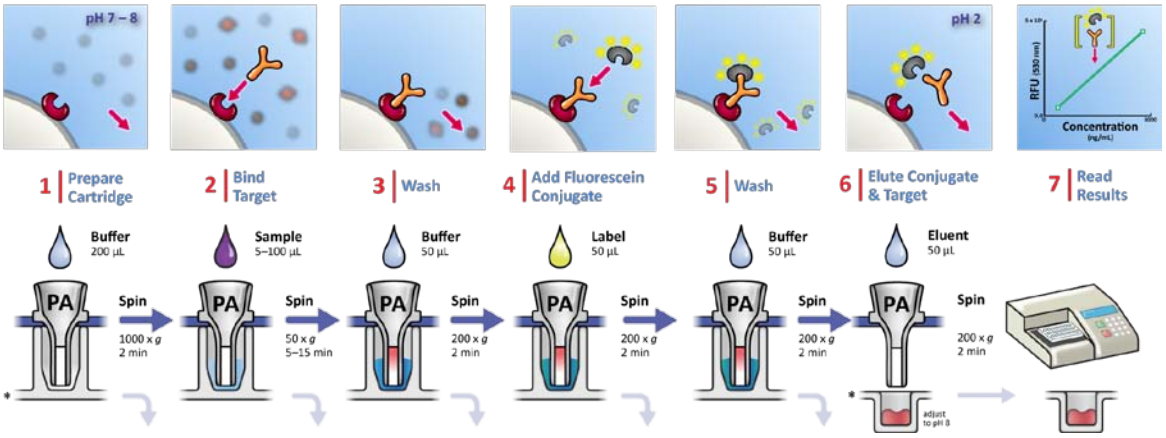


AssayMAP MAb Titer Assay Methods

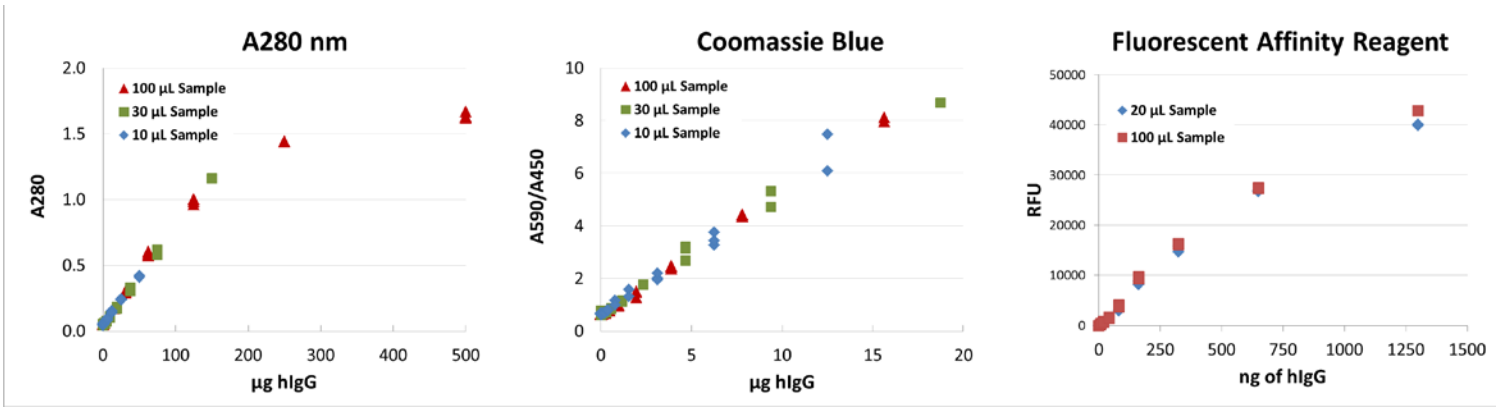
For many MAb titer assays, cartridges are packed with POROS® MabCapture A (Life Technologies, Carlsbad, CA) protein A resin. The simplest protocol (below) is similar to affinity HPLC, except that multiple samples (up to 4 racks or 384 at a time) are run in parallel. Purified IgG is eluted into a microplate and read in a plate reader at 280 nm. Optionally a colorimetric reagent (such as Coomassie Blue) can be added for increased sensitivity.



An alternative protocol increases the sensitivity into the range required for cell line screening. A fluorescently-labeled reagent that selectively binds hIgG but does not interact with the immobilized ligand is added. Both the bound MAb and labeled reagent are eluted and read in a fluorescence plate reader.



Standard curves for hIgG for the different protocols and detection methods:

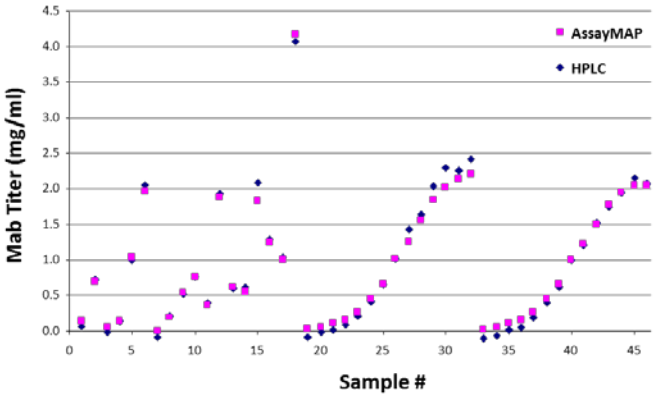


The cartridge selectively binds all of the target in the sample and elutes it into a fixed volume for detection. Thus the signal is a function of the mass of target loaded in the sample, not the concentration. The upper limit is determined by the binding capacity of the cartridge and the lower limit by the sensitivity of detection and matrix background signal. The sample concentration equals the measured target mass divided by the sample volume. The ranges for different sample volumes with the three methods are shown here:

		A280nm	Coomassie Blue	Fluorescent Ligand
Detection Limit	*µg*	5	0.5	0.005
Capacity Limit	*µg*	100	20	1.5
10 µL Range	*µg/mL*	500 - 10000	50 - 2000	0.5 - 150
25 µL Range	*µg/mL*	200 - 4000	20 - 800	0.2 - 60
100 µL Range	*µg/mL*	50 - 1000	5 - 200	0.05 - 15

Results with Cell Culture Samples

These data show a comparison of AssayMAP results with protein A HPLC for CHO-derived MAb cell culture process samples, including two daily bioreactor growth curves. The AssayMAP MAb Titer Assays were run using A280nm detection on a Tecan EVO liquid handling system equipped with an Agilent VSpin robotic centrifuge.



This table shows the results of running the same cell culture reference sample on both the AssayMAP system and the affinity HPLC system. Both the average determined values and analytical precision are quite comparable.

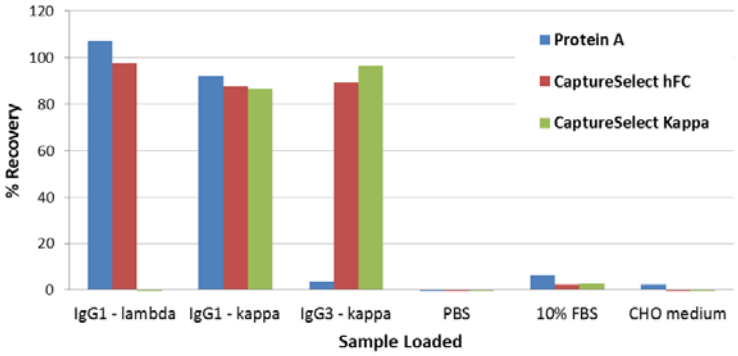
		n	Average	%CV
AssayMAP				
	Intra-Assay	8	1.82	1.24
	Inter-Assay	26	1.82	2.77
Affinity HPLC		65	1.87	1.14

Sample Matrix Effects

The low end sensitivity of the AssayMAP MAb Titer Assay is, in part, determined by the signal arising from non-IgG proteins in the sample due to non-specific interactions. These results show the Coomassie Blue detection for runs with 25 µL samples of two typical cell culture media, compared with 0 and 1.56 µg/mL hIgG in buffer. The non-specific signal is well below the limit of quantitation for Coomassie Blue, which is 0.5 µg.

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

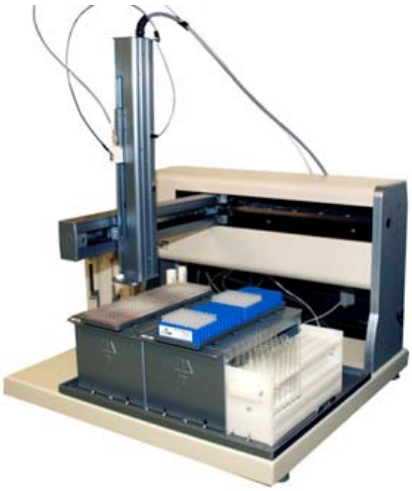
Alternative Affinity Ligands



In some analytical applications protein A may not be ideal as an affinity ligand. Protein A does not bind to IgG_3, for example, and will bind bovine IgG from screening media containing fetal bovine serum. CaptureSelect® affinity ligands (BAC B.V., Naarden, The Netherlands) are created by a proprietary technology based on Camelid-derived single domain antibody fragments. CaptureSelect ligands specific for human Fc (all subclasses) and kappa were immobilized on POROS resin and packed into AssayMAP cartridges. These cartridges were compared with AssayMAP MAb Titer (protein A) cartridges for binding of human IgG_3 and IgG1 with Kappa light chains,IgG_1- with, lambda light chains, as well as buffer,10% FBS and serum free cell culture media. The data below show the % of the sample load recovered from a 20 µg sample.

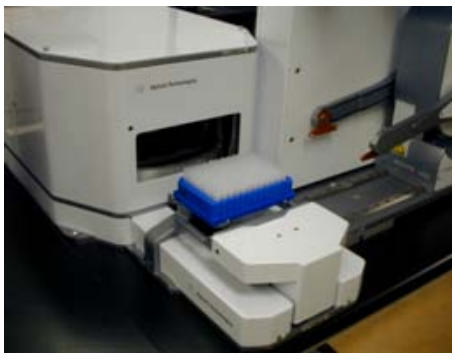
Automation Approaches

A variety of different approaches may be used to automate the MAb Titer assay. Even in manual mode, the assay typically requires 15 - 30 minutes to complete. If a 4-plate centrifuge is used, the throughput of the assay in manual mode can exceed 384 samples per hour.



The next level of automation is to use an automated liquid handling system (such as the Gilson GX271 single channel system shown here) for pipetting, with manual transfer of the cartridge racks to the centrifuge. This eliminates the most tedious element of manual methods. Automated liquid handling is also very helpful when the sample reformatting or dilutions of standards are required.

The MAb Titer assay can also be fully automated. These photos show the MAb Titer assay being run at Agilent Automation Solutions (setup and programming by Manuel Gomez of Agilent, Santa Clara, CA), using the Bravo liquid handler, BenchCel plate stacker/handler and VSpin robotic centrifuge.



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