Agilent AssayMAP

Agilent AssayMAP Bravo Platform

AUTOMATED PROTEIN AND PEPTIDE SAMPLE PREPARATION FOR MASS SPEC ANALYSIS

The Measure of Confidence

Agilent Technologies

GET FAST, ACCURATE, REPRODUCIBLE RESULTS

The Agilent AssayMAP platform is an easy to use yet flexible automation solution for preparing protein and peptide samples for LC/MS analysis.

With this platform you can simplify your most challenging workflow using a broad range of chemistries and laboratory-tested protocols that you can combine to meet your needs.

- Increase the precision of your LC/MS workflows by reducing the variability in your sample prep.
- Reduce tedious manual steps with walk-away automation so you can do more value-added work.
- Increase throughput without increasing variability or labor costs.

The AssayMAP platform is based on three key elements:

- Microchromatography cartridges with a wide range of standard chemistries
- The Bravo automated liquid handler equipped with proprietary, positive-displacement probe syringes
- Intuitive software designed for simplicity in an open access environment.

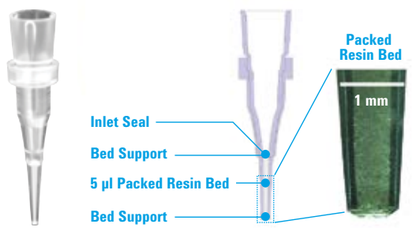
A SINGLE PLATFORM FOR MULTIPLE WORKFLOWS

With the AssayMAP platform, you can automate LC/MS sample preparation workflows that include one or more of the following applications: affinity purification from complex biological matrices, enzymatic digestion, reversed-phase peptide/protein cleanup, phosphopeptide enrichment, and peptide fractionation.

Diverse cartridge chemistries

AssayMAP cartridges incorporate a 5 µL packed bed of resin supported by membranes molded into the polypropylene cartridge, enabling chromatographic performance. Current cartridge options include:

- PA-W (protein A resin)
- PG-W (protein G resin)
- SA-W (streptavidin resin)
- RP-W (reversed-phase resin wide pore for proteins)
- RP-S (reversed-phase resin small pore for peptides)
- C18 (C18 resin)
- SCX (strong cation exchange resin)
- TiO2 (TiO2 resin)
- Fe(III)-NTA (Fe(III)-NTA resin)



Precise flow control syringes

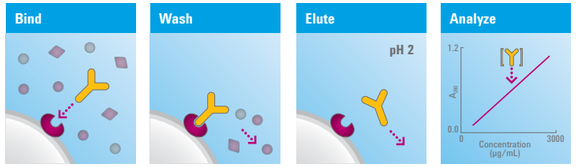
Liquid flow across the cartridge resin bed is controlled by near zero dead volume, positive displacement probe syringes. Flow rates can be set low enough to achieve quantitative binding or elution in a single pass.

The probe syringes are mounted in the special 96AM head used on the Bravo automated liquid handler. They can also mount pipette tips for standard liquid handling operations.

STREAMLINED SAMPLE PREPARATION FOR A GROWING RANGE OF APPLICATIONS

Quantitation purification

During the development and manufacturing of biotherapeutics, AssayMAP cartridges purify target proteins from complex samples such as cell culture supernatants, cell lysates, and serum. Purification is quantitative so you can easily determine the concentration of the target protein in the original sample and minimize the sample consumption. AssayMAP technology enables you to combine individual, often laborious, operations into one continuous, high-precision, high-throughput workflow. Resins conjugated to streptavidin, protein A, and protein G are available for easy adaptation of your existing purification workflows.

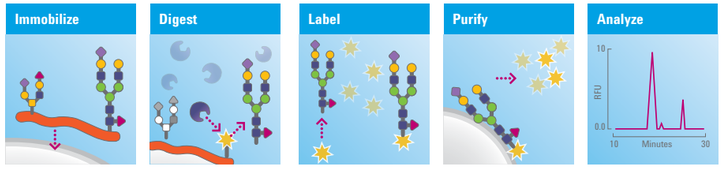


Purification of an antibody using protein A or protein G.

Affinity purification of an antigen using a biotinylated capture ligand immobilized on a streptavidin cartridge.

N-glycan analysis sample preparation

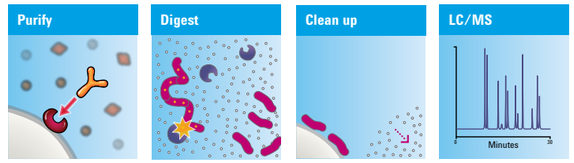
The AssayMAP automated method for N-glycan sample preparation, developed in collaboration with ProZyme,Inc, reduces N-Glycan sample prep from three days to four hours. The target glycoprotein is denatured, immobilized on a cartridge, and then digested with PNGase F that specifically releases N-glycans from the target protein. Next, the released glycans are chemically labeled with a fluorescent dye. A cleanup cartridge removes excess dye and reactants before HPLC, CE, or LC/MS analysis.



Glycan release and labeling using the N-glycan sample preparation workflow.

Peptide analysis

LC/MS is used to analyze proteins in a wide range of applications, which include characterizing post-translational modifications, quantifying biotherapeutics and biomarkers, and proteomics research. Preparing samples for these applications requires a complex series of steps: affinity purification of the analytical target, digestion with enzymes such as trypsin, and cleanup of the resulting peptide mixture. We have developed AssayMAP modules for each of these operations, so you can create combinations that meet the specific requirements of your end-to-end automated workflow.

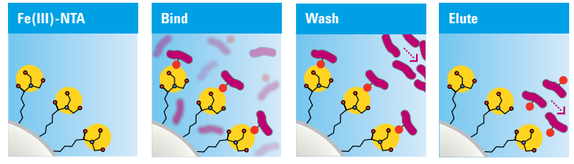


Purification, proteolysis, and desalting of a target protein using the peptide mapping workflow.

Phosphopeptide enrichment

Phosphorylation is a common post-translational modification that plays a significant role in a wide range of cellular processes. To characterize phophopeptides, you need a strategy to capture and enrich the phosphopepetides before you analyze them with LC/MS. The AssayMAP workflow gives you an automated solution that reproducibly enriches phosphopeptides using either the TiO_2 or Fe(III)-NTA resins. With AssayMAP, these technologies are easily accessible, whether you are a novice or an experienced user.

Enrichment and analysis of phosphopeptides with a TiO_2 workflow.



Enrichment and analysis of phosphopeptides with an Fe(III)-NTA workflow.

AUTOMATION MADE EASY

Intuitive, flexible interface

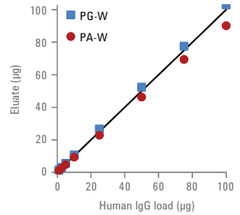
The AssayMAP platform includes a growing list of application-specific protocols in the application library. You can combine the applications in various sequences to address a wide variety of workflows. We designed the interface so that you can rapidly implement these applications without an onsite automation engineer. Harmonized design makes transitioning from one to another quick and easy. What's more, we created the AssayMAP protocols to enable seamless future integration with larger automation systems.

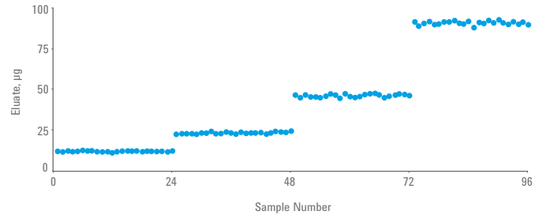
Ensure optimal performance

With the AssayMAP syringe test kit, you can simultaneously test the integrity of all 96 syringes in the AssayMAP Bravo head to confirm that the system is performing optimally and you are obtaining the quality results that you expect. The kit is simple to use and the test takes a short time to complete. A final report will identify any syringe that needs to be replaced. The AssayMAP syringe replacement kit provides all the tools you will need to quickly replace syringes and get your system back in operation.

Antibody purification

Antibody purification, recovery, and reproducibility were examined using Protein A (PA-W) and Protein G (PG-W) cartridges. Left panel: 1 – 100 µg of human IgG was serially diluted in cell culture supernatant (CCS) and then loaded onto PG-W and PA-W cartridges. Right panel: 24 CCS samples containing either 12.5, 25, 50, or 100 µg of human IgG was loaded onto PG-W cartridges. In both cases, recovery rates were greater than 90 % and %CVs were less than 5%.

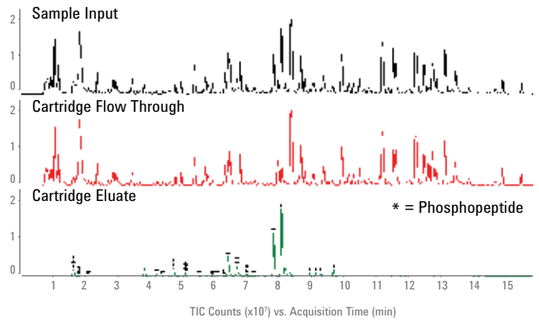




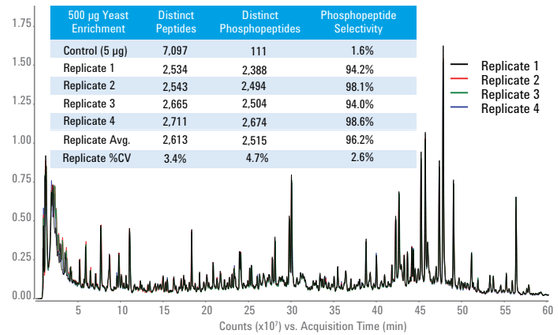
Sample No.	Load, µg	% Recovery	% CV
1-24	12.5	95.4	1.3
25 - 48	25	93.5	1.8
49 - 72	50	92.3	1.8
73 - 96	100	91.0	1.1

Phosphopeptide enrichment

Phosphopeptide enrichment and reproducibility were examined using Fe(III)-NTA cartridges. Left panel: Bovine α-Casein tryptic digests were loaded onto a Fe(III)-NTA. The sample input, flow through, and eluate were analyzed. Greater than 92% of the eluate TIC signal originated from phosphopeptides. Right panel: *S. cerevisiae* tryptic digests (n=4) were loaded onto Fe(III)-NTA cartridges and the elutes were analyzed. The average phosphopeptide selectivity was 96% and the average CV was 4.7%.



500 µg Yeast Enrichment	Distinct Peptides	Distinct Phosphopeptides	Phosphopeptide Selectivity
Control (5 µg)	7,097	111	1.6%
Replicate 1	2,534	2,388	94.2%
Replicate 2	2,543	2,494	98.1%
Replicate 3	2,665	2,504	94.0%
Replicate 4	2,711	2,674	98.6%
Replicate Avg.	2,613	2,515	96.2%
Replicate %CV	3.4%	4.7%	2.6%



Learn more
www.agilent.com/lifesciences/assaymap

Reach a local automation product specialist
1-866-428-9811

USA and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

Printed in the USA, December 7, 2015
5991-6273EN

Agilent Technologies