AGILENT ASSAYMAP SOLUTION FOR PEPTIDE SAMPLE PREP BEFORE MASS SPEC ANALYSIS

Transforming digestion, cleanup, and fractionation workflows to enable previously unachievable precision and throughput

Highlights

- Total workflow precision
- Increased throughput and push-button operation
- Flexible platform that supports discovery through validation
- Frees skilled labor from repetitive motion tasks

"Using the combination of extremely consistent, parallelized digestion with automated reversed-phase clean-up via AssayMAP at a scale that is appropriate for ultrasensitive proteomics applications has enabled us to contemplate collaborative studies of previously unheard of scales and throughputs."

Dr. Jake Jaffe,
Assistant Director Proteomics Platform,
The Broad Institute, Cambridge, MA, USA

Simplify your sample preparation workflows

AssayMAP Peptide Sample Prep Solution, from Agilent Technologies, is an automated solution for protein digestion, peptide cleanup, and fractionation. It is based on the powerful combination of miniaturized, packed bed chromatography, the state-of-the-art Agilent Bravo Automated Liquid Handling Platform, and a simple, applications-based user interface that creates an open access environment for both novices and experienced users and simplifies the most challenging sample preparation workflows.

With the AssayMAP Sample Prep Solution, 8–384 protein samples may be digested in parallel. The samples are desalted using reversed-phase chromatography and optionally simplified in fractions using cation exchange chromatography. This streamlined solution dramatically reduces hands on time while improving reproducibility and throughput.

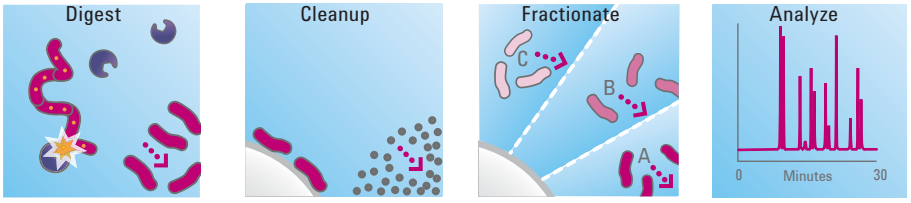

Figure 1. Peptide sample preparation using AssayMAP in-solution digestion, reversed-phase cleanup and fractionation.

Method comparison for processing 4 x 96 well plates

	AssayMAP Digestion & Cleanup	Manual Digestion & Vacuum Cleanup
Reproducibility	< 5% CVs	User-dependent
Hands-on time	~1.8 hours - general skill level	~ 5 hours - high skill level
Manual pipetting steps	1	48
Time to results	10–22 hours	13–25 hours
Elution volume	10 µL	~100 µL

Precise, automated trypsin digestion and cleanup

AssayMAP Peptide Sample Prep Solution was used to digest 64 replicates each of 2 sample types: BSA in urea and guanidine HCL. The samples were cleaned using AssayMAP reversed-phase cartridges and analyzed using an Agilent Advance BioPeptide column, Agilent 1290 Infinity LC and an Agilent 6550 iFunnel Q-TOF mass spectrometer. The experiment was repeated on day two to examine reproducibility. %CV was determined for 25 peptides within each sample as shown in Table 1. Three different % CV bins are shown, illustrating the contribution to the total average %CV. To further showcase the reproducibility, peak areas for representative peptides are shown in Figure 2.

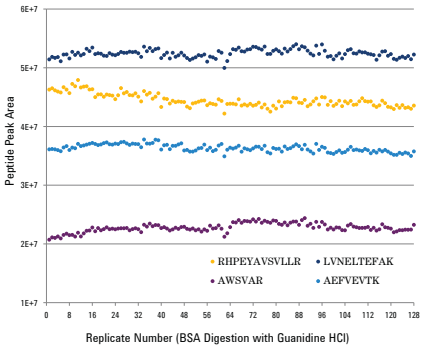

Figure 2. Scatter plots showing peak area of 4 peptides over 2 days.

25 peptides	Urea (n = 64, 62)	
	Day 1	Day 2
Avg Peak Area %CV	3.3	3.7
Peptides with %CV <5	23	21
Peptides with 5 > %CV <10	2	3
Peptides with %CV >10	0	1

Guanidine HCl (n = 64, 64)	
Day 1	Day 2
2.3	2.6
25	23
0	1
0	1

Table 1. %CVs by day with different % CV bins.

Consistent recovery across a wide mass load range

Serial dilutions of a BSA digest were loaded onto AssayMAP C18 cartridges, in triplicate. Samples were eluted, lyophilized, reconstituted and 5 µL (1.25 µg) were analyzed by LC/MS. Signals from 25 peptides were extracted, normalized to the 75 µg load signal and binned based upon their hydrophobicity index (HI). The graphs show representative peptides from each bin (HI<22.5, 22.5<HI<36, HI > 36) and reveal strong, consistent peptide recovery performance across the mass load range for peptides of low-to-moderate HI values. As expected, very hydrophobic peptides are a challenge to recover at low mass loads.

Powerful peptide isolation by fractionation

See our ASMS 2013 poster for information on how the AssayMAP SXC cartridge can be used to isolate unique peptide sequences in step-wise elution fractions.

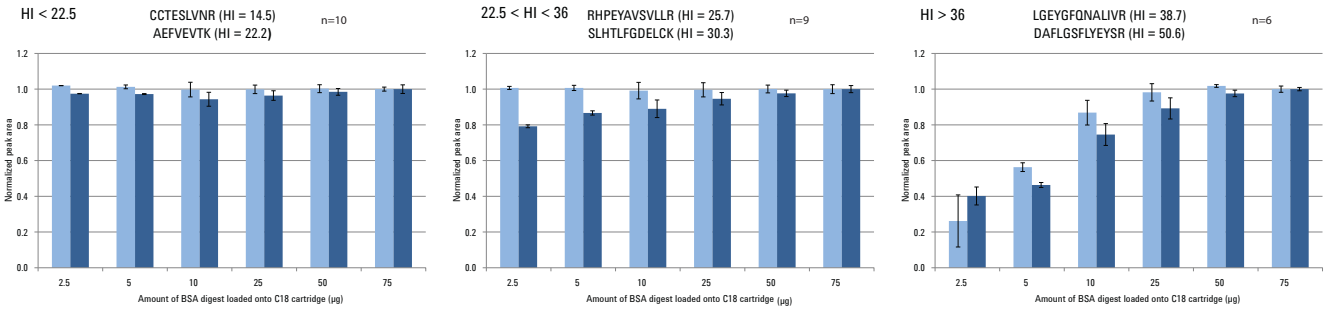

Figure 3. Representative peptide recovery based on hydrophobicity index (HI).

Call your Agilent Product specialists to find out how the AssayMAP Peptide Sample Prep solution can work for you.
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