An Automated Sample Preparation Solution for Mass Spectrometry-based Proteomics

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ABSTRACT

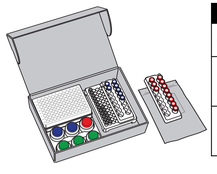
Purpose: We developed an automated sample preparation solution that simplifies and standardizes mass spectrometry (MS) sample preparation. The new automated sample preparation platform is an intuitive turnkey system (instrument, software, reagents) that enables standardized, hands-off operation and provides robust workflows for label-free proteomics and TMT applications.

Methods: The automated platform enables hands-off protein reduction and alkylation, digestion, TMT labeling, pooling and cleanup, and peptide concentration measurement, providing several workflows and companion reagents to perform several LFQ, TMT11plex, and TMTpro16plex workflows at different sample scales. It guides the researchers across the whole workflow using an embedded user interface to easily place reagents and samples and recover processed samples. A software ecosystem enables researchers to be assisted during the design of their experiments according to the biological changes. The prepared samples are ready to be injected into the external LC-MS system with known concentrations. The system was used in conjunction with built-for-purpose reagent sets.

Results: The automated system can process up to 36 samples containing 10-100 µg of initial protein within 4-6 hours at a time. The final peptides have a minimum missed cleavage rate of less than 10%, complete cysteine reduction/alkylation reactions, and most importantly, can be readily analyzed by LC-MS with a known concentration. The sample preparing process is robust and reproducible with less than 10% CV between technical replicates. This novel automated sample preparation platform for proteomics samples standardizes and simplifies MS sample preparation for proteomics applications having the potential to increase 10X the productivity in proteomics laboratories.

INTRODUCTION

Unlike genomic and transcriptomic fields, MS-based proteomics lacks standardized reagents and methods for sample preparation. Current methods are tedious, time-consuming, highly variable, unsuitable for processing large numbers of samples, and tend to negatively affect the robustness of the LC-MS instrumentation. Therefore, we developed a novel automated sample preparation platform that standardizes and simplifies MS sample preparation for proteomics applications.



Automated MS Sample Prep Kit	
Label-Free	Label-Free MS SP kit – 36 reactions (10-100µg)
	Label-Free MS SP kit – 16 reactions (10-100µg)
TMT11plex	TMT11plex kit, 3 X 11 reactions (10-25µg)
	TMT11plex kit, 1 X 11 reactions (25-100µg)
	TMT11plex kit, 3 X 11 reactions (25-100µg)
TMTpro 16plex	TMTpro 16plex kit, 2 X 16 reactions (10-25µg)
	TMTpro 16plex kit, 1 X 16 reactions (25-100µg)
	TMTpro 16plex kit, 2 X 16 reactions (25-100µg)

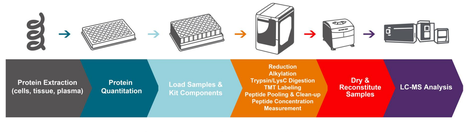


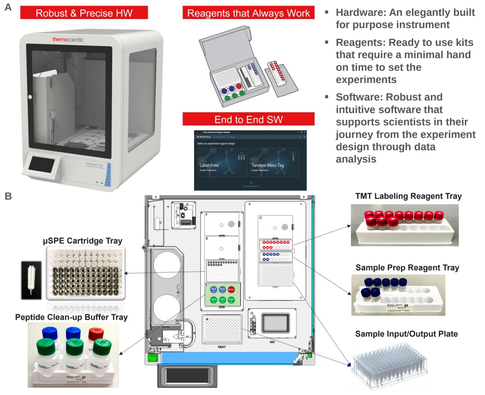
Figure 1. Supporting kits and workflows of the automated sample preparation system

MATERIALS AND METHODS

Various samples including mammalian cells, plasma, tissue, purified protein, and bacteria were prepared on the AccelerOme automated sample preparation system based on a liquid handling robotic station that executes different steps including mixing, heating/cooling, reactions, sample clean-up, and peptide concentration measurement. After preparing samples on the instrument, dry and reconstitute the samples that are ready to be injected into the external LC-MS system. The system was used in conjunction with Thermo Scientific™ AccelerOme™ reagent sets. Both label-free and isobaric labeling (TMT11plex and TMTpro 16plex)-based protein quantification were evaluated. A nanoLC (75-µm i.d., 120-min gradient) and a Thermo Scientific™ Q Exactive™ Plus mass spectrometer were used for sample analysis. Generated data were analyzed using the Thermo Scientific™ Proteome Discoverer™ (PD) 2.4 and 2.5.

RESULTS

The AccelerOme Automated Sample Preparation Ecosystem



- **Hardware: An elegantly built for purpose instrument**
- **Reagents: Ready to use kits that require a minimal hand on time to set the experiments**
- **Software: Robust and intuitive software that supports scientists in their journey from the experiment design through data analysis**

Figure 2. AccelerOme System (A) The intuitive and turnkey AcclerOme ecosystem (instrument + reagents+ software) provides robust and reproducible samples for MS proteomics analysis. (B) The layout design of the instrument deck and the ready to use kit components enables ease of use and minimal user interactions.

Gold Standard Benchmark of AccelerOme Automated vs. EasyPep Manual

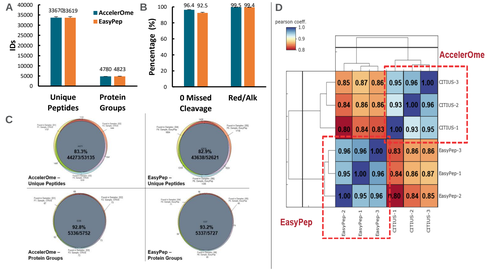


Figure 3. Benchmark of AccelerOme automated sample preparation method against EasyPep manual kit for processing 50 µg HeLa cell lysate. (A) Comparable peptide and protein IDs between the automated and manual methods; (B) Improved digestion efficiency of the automated method (96% vs. 93% zero peptide missed cleavages) and complete cysteine reduction and alkylation (99%) with both methods; (C) Overlap of identified peptides (83%) and proteins (93%) among three replicates demonstrate high identification reproducibility using both methods; (D) Pairwise correlations of peptide abundances (without normalization) among three replicates show high quantification reproducibility of both methods.

On-system peptide quantitation

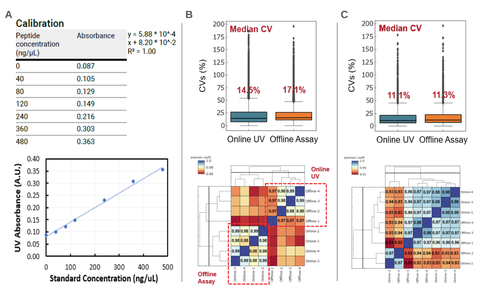


Figure 4. Quantitative proteomics analysis using the on-system UV peptide quantitation vs. the offline colorimetric peptide assay. (A) Calibration curve generated by the system for peptide measurement. (B-C) CVs and pairwise correlations of peptide abundances (without normalization) among three replicates using the HeLa cell lysate (100 µg) and plasma (100 µg) samples, respectively, indicating slightly higher proteomics quantification reproducibility using the on-system UV peptide quantitation approach compared to the conventional colorimetric peptide assay.

Sample Compatibility: Various Mammalian Cells

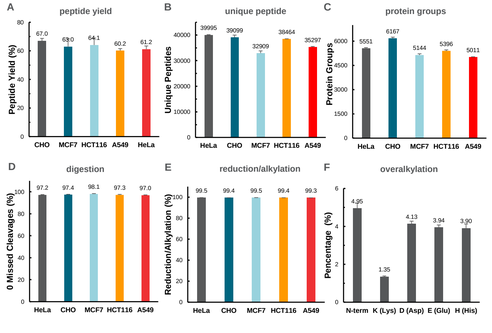


Figure 5. Performance of the automated system for processing various mammalian cell lines including CHO, MCF7, HCT116, A549, and HeLa. Excellent performance in terms of (A) peptide yields (>60%), (B) unique peptides (32,000-40,000), (C) protein groups (5,000-6,200), (D) digestion efficiency (≥97% 0 peptide missed cleavages), (E) cysteine reduction and alkylation efficiency (>99%), and (F) overalkylation for non-cysteine residues (<5%) was achieved. The input sample mass for all the mammalian cell lysates was 50 µg and data were analyzed using PD2.5.

Sample Compatibility: Tissue, Plasma, Purified Protein, Bacteria

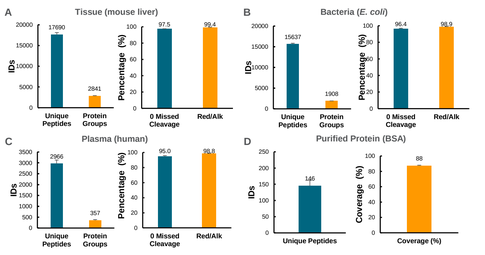
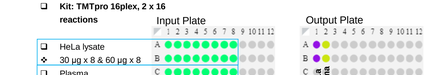


Figure 6. Performance of the automated system for processing samples including (A) tissue, (B) bacteria, (C) plasma, and (D) purified protein. Different protein input samples (50 µg) processed Reproducible protein identifications, high digestion efficiency (≥95% 0 peptide missed cleavages), complete cysteine reduction/alkylation (≥99%), and/or high sequence coverage (e.g., 88%) could be achieved for complex and/or single protein samples.

TMT-Labeling MS Sample Preparation for Multiplex Proteomics



Output Table			
Position	Batch	Est.Volume (µL)	Est.Peptide Mass (µg)
A1	1	300	72.50
B1	1	300	72.85
A2	2	300	108.71
B2	2	300	109.69

Figure 7. Experimental design and output peptide quantitation for TMT-based proteomics. HeLa cell lysate and plasma samples were used for TMTpro labeling proteomics sample preparation with 30 µg for 8 tags and 60 µg for another 8 tags. For each batch, two replicate, pooled final peptide samples with known concentrations/masses in the output plate were generated for multiplex proteomic analysis.

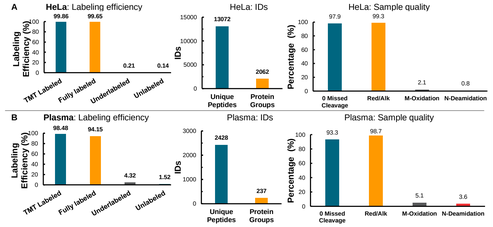


Figure 8. Performance of the TMT-labeled sample processing for (A) HeLa and (B) plasma samples in terms of labeling efficiencies, IDs, and sample qualities. High labeling efficiency (>99% for HaLa and >98% for plasma), high digestion efficiency (>94% 0 peptide missed cleavages), complete cysteine reduction/alkylation (≥99%) and minimal undesired M-oxidation and N-deamidation modifications (≤5%) were achieved. Data were analyzed using PD2.5.

Evaluation of TMT-labelling-based quantitative proteomics

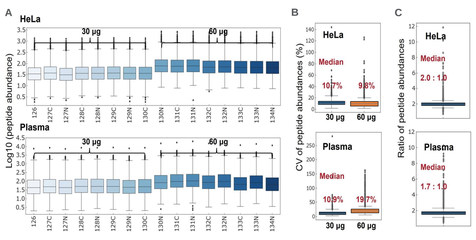


Figure 9. TMT quantification reproducibility and accuracy for HeLa and plasma samples. (A) Distribution of peptide abundances across all TMT channels with different input protein masses without normalization. (B) CVs of peptide abundances within the same input protein amounts show good quantification reproducibility (10-20% CVs) for both HeLa and plasma samples. (C) Ratios of peptide abundances between the 60 µg and the 30 µg input protein masses, indicating good quantification accuracy (theoretical ratio: 2:1).

CONCLUSIONS

We developed an automated sample preparation solution (instrument + reagents + software) that enables standardized, hands-off operation and provides robust workflows for label-free proteomics and TMT applications.

- High sample quality: equal/better peptide/protein IDs than the gold standard method; complete C reduction/alkylation (≥99%), high digestion efficiency (≥90% 0 peptide missed cleavages), undesired modifications (<10%), high TMT labeling efficiency (≥99%), high peptide yield (>50%), and undetectable polymer/detergent contamination.
- Reproducible identification and quantification for both label-free and TMT labeling workflows.
- Compatibility and flexibility in processing various samples (cells, tissues, plasma, purified proteins, microorganisms, etc.) and input amounts (10-100 µg) with known final peptide concentration for MS analysis.
- High throughput of processing up to 36 samples at one time within 4-6 hours (<10 min/sample).
- Easy to use with minimal user interaction with method development and sample processing.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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