Adapting EasyPep™ MS Sample Preparation for 96-well Automated Liquid Handling Systems

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ABSTRACT

Advances in mass spectrometry (MS) instrumentation have enabled routine analysis of complex protein samples. However, sample preparation methods are not standardized with many protocols taking 6-24 hours in addition to suffering from low peptide yields, poor digestion efficiency and low reproducibility. Here, we describe a simplified sample prep kit containing pre-formulated reagents and a standardized protocol that can be used to efficiently process 100 to 200 samples per day. We compared this to a previous method (Figure 1) and showed reduced handling time, better peptide yield, and improved reproducibility. To improve our workflow, we introduced an automated liquid handling system to enable 96-well plate processing, with protein yield, digestion efficiency, and reproducibility compared to a manual system (Figure 2). Our new protocol utilizes a flow-through digestion system, a robotic liquid handling system, and a standardizing workflow to ensure improved results. Our optimized kit reduces sample preparation time from 6-24 hours to approximately 3-4 hours, making it more efficient for large-scale sample preparation. The new protocol was validated using a human plasma protein sample and demonstrated improved peptide yield and digestion efficiency.

INTRODUCTION

Sample preparation is a crucial step in the proteomics workflow that impacts protein and peptide identification rates. Conventional workflows typically involve multiple steps, including lysis, denaturing buffers, and protease digestion, which can be time-consuming. We developed a new protocol, EasyPep, which reduces the time to prepare samples from 6-24 hours to approximately 3-4 hours. Our protocol utilizes a flow-through digestion system and automated liquid handling, leading to improved peptide yield and digestion efficiency. We have validated this protocol using a human plasma protein sample and demonstrated improved peptide identification rates.

RESULTS

Figure 3: Efficient sample preparation in less time with higher identification rates

Figure 4: Compatibility with different cell types

Figure 5: Assessing Workflow Scalability

Figure 6: Assessing Workflow Reproducibility

Figure 7: Protein/Peptide Idents and enzymatic digestion efficiency for various enzymes

Figure 8: TMT labeling with EasyPep and Conventional workflow

Figure 9: 96-well filter plate for EasyPep sample prep automation

Figure 10: Sample Preparation of Human Plasma using Different Methods

MATERIALS AND METHODS

Sample Preparation

HeLa S3 cell pellets were lyzed and reduced using a Trypsin/Lys-C protease mix followed by the detergent removal using the mixed-mode peptide clean-up column. Protein digest (tryptic) was analyzed by LC-MS and processed as described in the methods. The results demonstrate that our standardized workflow enables efficient 3-hour sample preparation with higher protein/peptide identification rates as compared to conventional workflow, which used a 2.5-day protocol.

CONCLUSIONS

Our new kit provides a superior method in terms of time saved, peptide/protein identification rates, and reproducibility compared to previous proteomics methods and greatly simplifies proteome sample preparation for protein identification and quantification. Our standardized workflow is compatible with several sample types including cell lines and mouse tissues, purified proteins, plasma and serum with high reproducibility (CVs < 5%) and low missed cleavages (<60%). Our sample preparation chemistry is compatible with isotopic labeling reagents such as Tandem Mass Tags (TMT) for the relative protein quantification. Peptide clean-up using a 96-well filter plate shows equal or better peptide/protein identification rates and reproducibility (CVs < 1%) compared to manual spin column for HeLa cell pellets and plasma samples.

TRADEMARKS/LICENSING

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