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EasyPep Sample Preparation Technology for Rapid and Efficient Mass Spectrometry-based Proteomics

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ABSTRACT

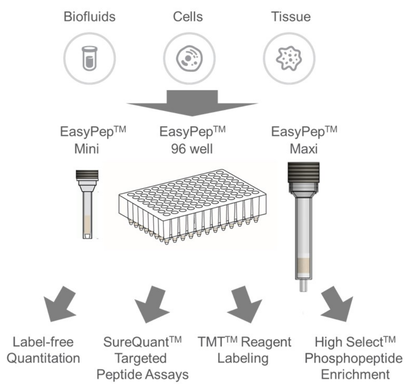
Advances in mass spectrometry (MS) instrumentation have enabled routine analysis of complex protein samples. However, protein sample preparation for mass spectrometry still largely lacks cohesive standardization, which generally leads to inconsistent and irreproducible analyses. We have expanded our recently introduced Thermo Scientific™ EasyPep™ sample preparation technology, which simplifies and standardizes proteomic sample preparation to enable efficient and reproducible preparation of cell, tissue and plasma samples by introducing two new formats – a large-scale (Maxi) sample preparation kit to streamline the analysis of protein post-translational modifications and a 96-well plate format kit to enable high throughput and automation. Here, we describe sample type-specific examples to highlight the unique features of each format for different sample types and applications.

INTRODUCTION

Sample preparation is a crucial step in the proteomics workflow which greatly affects protein/peptide identification rates. However, the sample preparation methods are not standardized with many protocols taking 8-24 hours in addition to suffering from low peptide yields, low reproducibility and poor digestion. So, we developed simplified, easy to use EasyPep Mini sample preparation kit for processing 10-100µg protein samples from cells, tissues, plasma and serum in less than 3 hours. Recently, we expanded our EasyPep technology by introducing two new formats (Figure 1) - a large scale (Maxi) sample preparation kit optimized for 0.5-2 mg of protein sample amounts to streamline analysis of post-translational modifications and a 96-well format kit optimized for 10-100µg of protein sample amounts to enable high throughput and automation.

Three operation modes including centrifugation, vacuum, positive pressure have been evaluated and optimized for 96-well EasyPep sample preparation filter plate. Both centrifugation and vacuum modes were assessed and optimized for larger Maxi column format. We have also successfully adapted the EasyPep 96 sample preparation method to a fully automated workflow using a Hamilton™ STARlet™ automated liquid handling system. Here, were compared the three EasyPep formats (Mini, Maxi, 96 well) with different sample types including human cell lines, human serum/plasma, yeast, *E. coli*, fresh tissues and formalin-fixed, paraffin-embedded (FFPE) tissues. In addition, we assessed the compatibility of EasyPep MS sample preparation chemistry with phosphopeptide enrichment using Fe-NTA resins and TMT™ labeling before or after digestion. Overall, our EasyPep sample preparation technology has been shown to enable rapid and efficient processing of different samples, scales and throughput for mass spectrometry-based proteomics.

Figure 1. EasyPep Mini, 96well and Maxi formats



MATERIALS AND METHODS

Sample Preparation

HeLa S3 cells were grown in sMEM media supplemented with 10% FBS, 1X Glutamax and 1% Pen/Strep. HEK293 and A549 cells were grown in MEM supplemented with 10% FBS and 1% Pen/Strep. Human Plasma was obtained from BioIVT. Mouse tissues were obtained from Pelfreeze. Yeast BY4741 WT Parental strain, MAT A obtained from GE Healthcare was grown in YPD broth. BL21-Gold (DE3) *E.coli* competent cells obtained from Agilent Technologies were grown in LB broth. Protein lysates were prepared from several cell and tissue types, including FFPE samples, as well as plasma samples, using our standardized EasyPep sample preparation procedures in replicates. *E.coli* pellets were lysed using lysozyme and EasyPep Lysis buffer. Yeast cell pellets were lysed using glass beads with our optimized protocol. Protein concentration was measured using Thermo Scientific™ Pierce™ Rapid Gold BCA Assay kit. Peptide concentration was determined using Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay kit prior to LC-MS Analysis. IMAC enrichment was performed using Thermo Scientific™ High-Select™ Fe-NTA phosphopeptide enrichment kit. The samples were labeled with Thermo Scientific™ TMT™ reagents according to the manufactures protocol before sample clean up.

LC-MS Analysis

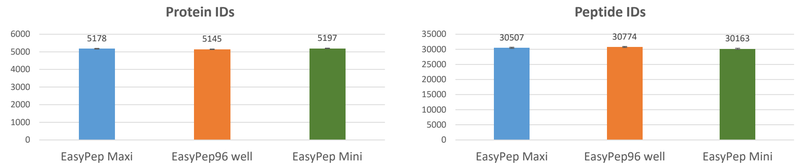
Triplicate protein digest samples (1µg per injection) were separated using a Thermo Scientific™ Dionex™ UltiMate™ 3000 Nano LC system using a 50 cm C18 Thermo Scientific™ EASY-Spray™ column with an acetonitrile gradient from 3% to 28% over 85 min, 28% to 45% over 30 min, at a flow rate of 300nL/min on a Thermo Scientifc™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer.

Data Analysis

LC-MS data were analyzed using the SEQUEST® HT search engine in Thermo Scientific™ Proteome Discoverer™ 2.4 software using static carbamidomethyl (C), dynamic oxidation (M), TMT6plex (K, N-term), Phospho (S, T, Y) and deamidation (N, Q) modifications. Data were searched against the Uniprot human protein database, *E.coli* or yeast and results were filtered using a 1% protein FDR threshold.

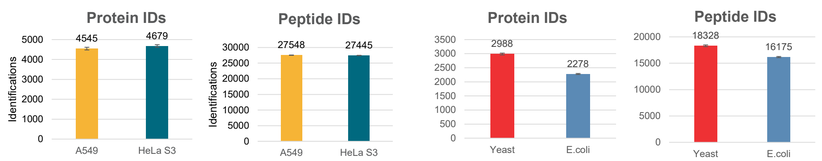
RESULTS

Figure 2. Efficient sample preparation with EasyPep technology for larger sample amounts and throughput



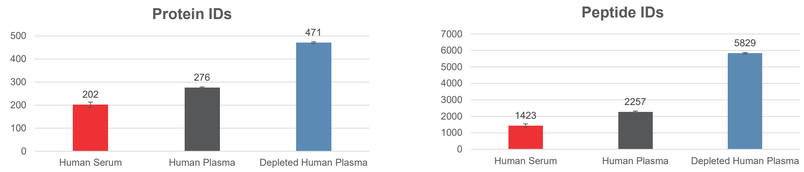
HeLaS3 cell pellets were lysed with the lysis buffer, reduced, alkylated and digested using a Trypsin/Lys-C protease mix followed by the detergent removal using peptide mini or maxi clean-up columns or 96well filter plate. Protein digest (1µg) was analyzed by LC-MS and processed as described in the methods. The results in the figure show that all the three formats yielded identical protein and peptide identifications in less than 4 hours workflow.

Figure 3. Compatibility with different sample types



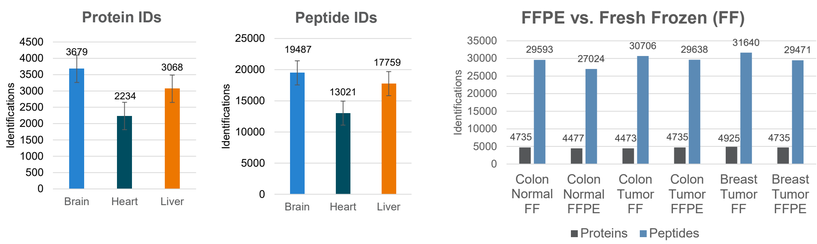
HeLa S3 and A549 cell pellets were lysed, reduced/alkylated and digested using a Trypsin/Lys-C protease mix followed by the peptide clean-up procedure. Yeast and *E.coli* (BL21) cell pellets were lysed as mentioned in the methods and processed with EasyPep workflow. Protein digest (1µg) was analyzed by LC-MS and analyzed as described in the methods. The results demonstrated that our workflow is compatible with the various sample types yielding high protein/peptide identification rates.

Figure 4. Sample Preparation with Human Plasma and Human Serum



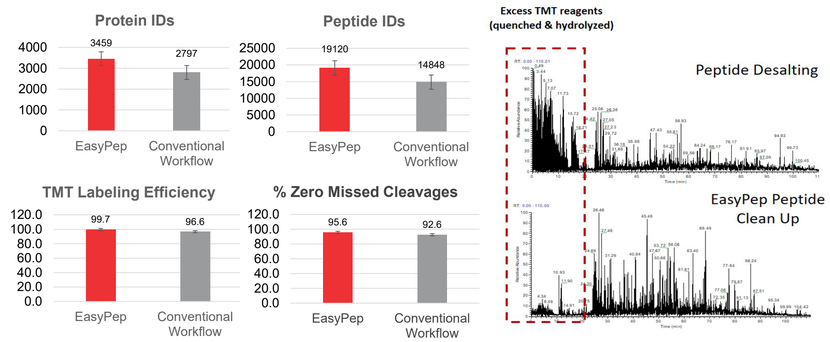
Human Serum and Human Plasma Digest was prepared using EasyPep workflow. Human Plasma was depleted using High-Select Top14 Abundant protein depletion mini spin columns before processing with EasyPep technology. Protein digest (1µg) was analyzed by LC-MS and analyzed as described in the methods. The results demonstrate that our workflow is compatible with both non-depleted and depleted human plasma and human serum.

Figure 5. Compatibility with various tissue types



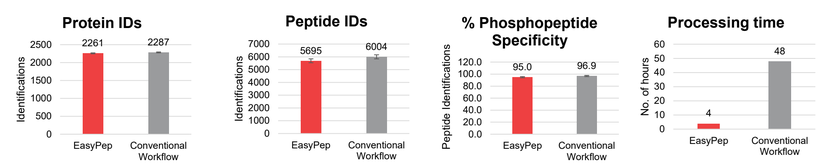
Mouse tissues (Brain, Heart and Liver) were lysed, reduced/alkylated and digested using a Trypsin/Lys-C protease mix followed by the peptide clean-up procedure. Paraffin removal was carried out using xylene and sequential ethanol washes. The optimized extraction protocol and EasyPep MS sample prep kits (Mini and 96 well plate) were used to prepare the digest from FFPE sections and fresh frozen (FF) normal and tumor breast and colon tissues.

Figure 6. TMT labeling and excess TMT tag removal with EasyPep Peptide Clean Up



HeLa cell pellets were lysed, reduced, alkylated and enzymatically digested. The samples were labeled with TMT reagents after digestion and cleaned-up using the peptide cleanup procedure. As shown in the figure, our EasyPep workflow is compatible with TMT labeling reagents and yielded 10-20% higher protein and peptide IDs with lower missed cleavages and labeling efficiency of >99%. The chromatograms in the figure shows that our optimized wash buffers in the EasyPep workflow enable efficient removal of excess TMT reagents along with removing detergents and interfering buffer salts from the samples while maintaining high, unbiased peptide yields and improved peptide identifications..

Figure 7. Compatibility with downstream phospho-enrichment workflows



Nocodazole-arrested HeLa S3 cell pellets were processed using EasyPep Maxi kits and conventional workflow before IMAC enrichment. The number of protein/peptide identifications with EasyPep workflow was not significantly different than conventional workflow. The results demonstrate that an efficient sample preparation can be performed in 3-5 hours using our EasyPep workflow with 95% phosphopeptide specificity.

CONCLUSIONS

- Our EasyPep sample preparation technology enables rapid and efficient processing of different samples, scales and throughput for mass-spectrometry based proteomics.
- Peptide clean-up using a 96-well filter plate, Maxi kit or Mini kit shows identical peptide/protein identification rates and reproducibility (CVs <1%) for HeLa cell pellets and plasma samples.
- Our standardized workflow is compatible with several sample types including cell lines and mouse tissues, plasma and serum, yeast, bacteria, FFPE with high reproducibility (CVs <5%) and low missed cleavages (<90%).
- Our sample preparation chemistry is directly compatible with isobaric labeling reagents such as TMT for the relative protein quantification and generates protein digests compatible with downstream phosphopeptide enrichment.

TRADEMARKS/LICENSING

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