**Automated High-throughput Phosphopeptide Enrichment Using TiO₂ Dispersive Pipette Extraction**

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**INTRODUCTION**

Phosphorylation events are key signal transduction mechanisms within the eukaryotic cells and further understanding such signalling pathways can elicit crucial molecular signatures of various human diseases (1-3). The global deep profiling of phosphoproteins has been successfully optimized in the procedures of enrichment, mass spectrometry, and data analysis (4-5). However, phosphopeptide enrichment is labile under both extremes (6-9). For instance, the pH range of 2-5 is preferred for phosphopeptides, which makes the isolation challenging (10). The presented method uses automated liquid handling systems, such as liquid handling robots, to isolate phosphopeptides from complex mixtures and try to phosphorylation events.

**MATERIALS AND METHODS**

HER2K17 cells were treated with 5 mM HCl for 15 minutes and stained with cold PBS. The cells were lysed with 8 M Urea buffer containing a protease and phosphatase inhibitor cocktail. 10 mM dithiothreitol was added to reduce at 56 °C for 30 minutes, then 25 mM iodoacetamide was added for alkylation for 30 minutes in the dark followed by overnight tryptic digestion at 37 °C. For automatic buffer containing a protease and phosphatase inhibitor cocktail. 10 mM dithiothreitol was added to reduce at 56 °C for 30 minutes, then 25 mM iodoacetamide was added for alkylation for 30 minutes in the dark followed by overnight tryptic digestion at 37 °C. For automatic sample processing, we developed a method for the YML-Omics four steps and other high-throughput robotic systems (Figure 2).

**RESULTS**

We established a selected reaction monitoring (SRM) method for TiO₂ triple quadrupole mass spectrometry with optimal conditions. For global phosphoprotein identification, we used a Q Exactive HF mass spectrometer coupled with Ultimate 3000 nano–ESI–MS system.

**CONCLUSIONS**

Descriptive pipelines for the enrichment of phosphopeptides using an automated liquid handling system increases reproducibility and specificity for high-throughput approaches. IMCSTips containing TiO₂ showed nearly 100% recovery of the phosphoproteome standards even in the presence of alpha and beta casein peptides. For the global phosphoproteome, 1,250 phosphopeptides with 98.9% specificity were enriched from HER2K17 cell lysates. The enrichment process involved minimal hands-on time and the entire process was executed on the robotic system without additional manipulation. The ability to automate such complex sample preparations would be highly useful on the sample, and the key signal of the cellular phosphorylation event is a limited number of biological replicas will be checked with less time and effort.

**REFERENCES**


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**REFERENCES**

1. Phosphorylated amino acid.