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Fast and efficient phosphopeptide enrichment using TiO2 coated magnetic beads and its application to Drosophila Kc167 phosphoproteomic analysis

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OVERVIEW
We have demonstrated an easy and fast TiO2 magnetic beads (Merck KGaA, Germany) protocol to be highly sensitive and selective for phosphopeptide enrichment by tests using both standard peptide mixtures as well as complex real sample. This method is directly compatible with phosphopeptide sequencing and quantitation by LC-MS/MS.

RESULTS

Results from phosphopeptide standard mixtures:
The described phosphopeptide enrichment method using TiO2 magnetic beads has been tested with standard mixtures including P02700 (asphage), self-oligomers in protein mix and a control native protein digest with 50 nM phosphoprotein to non-phosphoprotein ratio.

Specificity

We have demonstrated a fast and selective phosphopeptide enrichment method using TiO2 magnetic beads (Merck KGaA) from tests with various standard mixtures and Drosophila Kc167 cell lysate. The addition of DHB (Glycolic acid) in loading condition is not needed to obtain a high phosphopeptide specificity. With this work flow, hundreds of phosphoproteins sites can be identified from a single enrichment and single LC-MS run using low amount of Kc167 cell lysate (around 50 ug without any pre-fractionation procedure). A specificity of more than 90% can be achieved. This protocol can easily be automated for large scale phosphorylation analysis.

CONCLUSIONS
We acknowledge the support from Merck KGaA and Biotech Research & Innovation Center for collaboration on this project.

REFERENCES

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