Guide to clean up and desalting for export

Phosphopeptides peak may be detected with a lactic acid peak caused by the residual lactic acid in an elute. When using on-line desalting system by column switching, a lactic acid peak may appear. We recommend the following protocol as an example to remove lactic acid from enriched phosphopeptide elute before using MS or LC-MS.

Solution B

It contains 100% lactic acid.
Lactic acid is used as an inhibitor of non-specific peptides adsorbing form crude sample such as a cell lysate to titanium dioxide.

Fig.1 Protocol example to remove lactic acid

Enriched phosphopeptides elute

pH control

-Add 100uL of 20% phosphoric acid solution or 20% TFA solution to 100uL enriched phosphopeptides elute which contains 5% ammonia and 5% pyrrolidine.
-Confirm that it is acidic pH by litmus paper.

Cleaning up

-To remove Lactic acid from the enriched Phosphopeptides using a reversed solid phase extraction such a SPE-C-TIP (Nikkyo Technos Co.). (How to use is shown at Figure.2)
-Evaporate a purified sample, prepared by a method at Figure 2, to dryness by such as a centrifugal evaporator.
-Dissolve it with 0.1% TFA in Water: CH_3CN=95:5

MS

Fig.2 Simplified protocol of clean up
SPE C-TIP Operation Manual

pH controlled sample

Conditioning and equilibration

-Add 20uL of solution Y and centrifuge (2,000*xg*, 2min)
Add 20uL of solution X and centrifuge (2,000*xg*, 2min)

Adsorption

-Add 100uL of solution X and sample
Centrifuge (2,000*xg*, 2min)

Rinsing

-Add 20uL of solution X
Centrifuge (2,000*xg*, 2min)

Elution

-Add 20uL of solution Y
Centrifuge (2,000 *xg*, 2min)

Solution X:
0.1%TFA in Water：CH_3CN＝95：5
Solution Y:
0.1%TFA in Water:CH_3CN=20:80

Purified sample

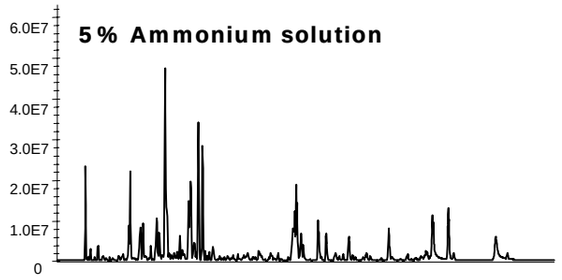
*Continue to evaporation at Fig.1

References

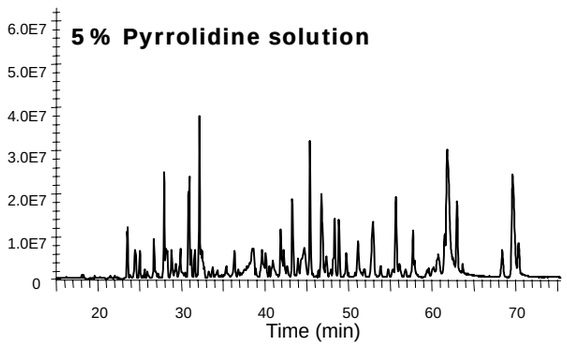
Titansphere Phos-TiO Kit was developed in reference to the following articles.

∗ Phosphopeptide enrichment by aliphatic hydroxy acid-modified metal oxide chromatography for nano-LC/MS/MS in proteomics applications Sugiyama N.et.al. Mol Cell Proteomics, 6, 1103-1109, 2007
∗ Successive and Selective Release of Phosphorylated Peptides Captured by Hydroxy Acid-Modified Metal Oxide Chromatography. Kyono Y. et.al. J Proteome Res., 2008

About elute
Hydrophilic phosphopeptides can be eluted more by using 5% ammonium aqueous solution.
Hydrophobic phosphopeptides can be eluted more by using 5% pyrrolidine aqueous solution.



The left figure is a base peak chromatogram of purified phosphopeptides from HeLa cell extract by LC/MS.
5% Ammonia aqueous solution tends to include more hydrophilic phosphopeptides.



5% Pyrrolidine aqueous solution tends to include more hydrophobic phosphopeptides.

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