

Guide for cleaning up and desalting samples.

Phosphopeptides peaks may be detected with lactic acid peaks due to the remaining lactic acid in the eluate. Even after using an on-line desalting system by column switching, lactic acid peaks may appear. We recommend the following protocol as an example to remove lactic acid from enriched phosphopeptide eluate before using MS or LC-MS.

Solution A

It contains 2% TFA (Trifluoroacetic acid) in water.

TFA is volatile solution. The Change in TFA concentration might cause performance deterioration.

(* When TFA concentration is higher than 2%, the selectivity of phosphopeptides will be increased. However, it may also decrease the recovery rate of phosphopeptides.)

Volatilizing TFA in Solution A may cause performance deterioration.

We recommend preparing a solution of 2% TFA in 80% CH₃CN aqueous solution as the Buffer A when you suspect volatilizing TFA.

Solution B

It contains 100% lactic acid.

Lactic acid is used as an inhibitor of non-specific peptides adsorbing from crude sample such as a cell lysate to titanium dioxide.

Fig.1 -Protocol Example of Remove Lactic Acid

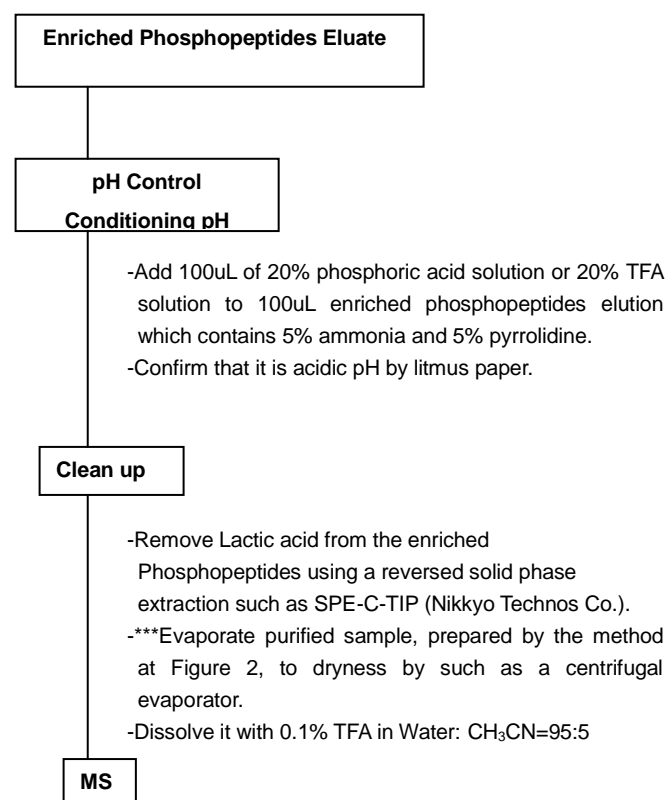
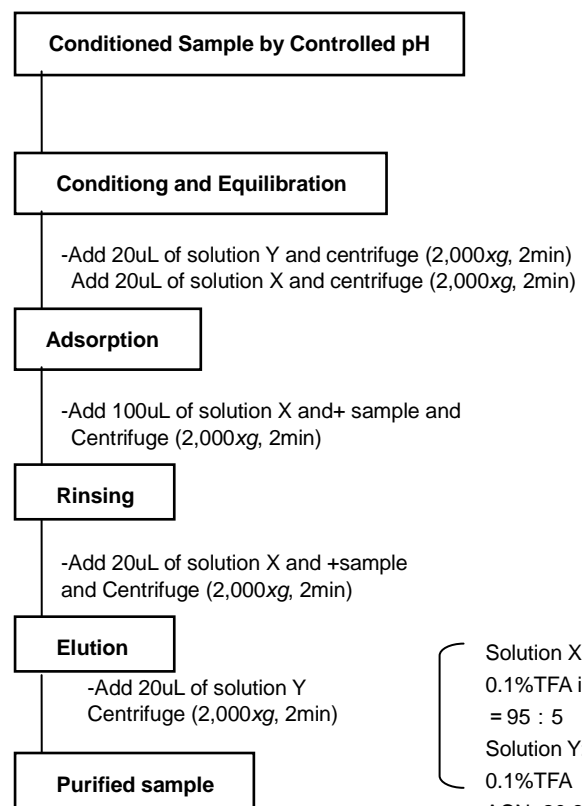


Fig.2 Simplified Protocol of Clean Up SPE C-TIP Operation Manual



Solution X:
0.1%TFA in Water : CH₃CN ACN = 95 : 5
Solution Y:
0.1%TFA in Water: CH₃CN ACN=20:80

*continue to evaporation process with

*** at Fig.1

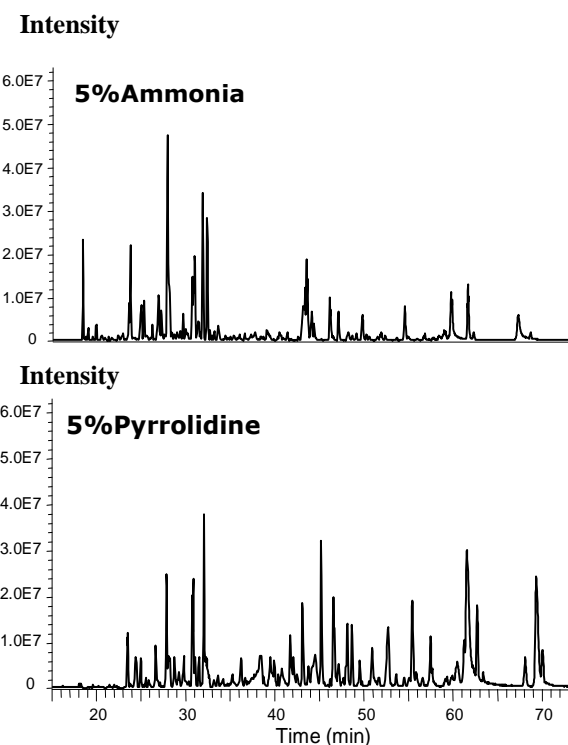
References

Titansphere Phos-TiO kit is referred in 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

• Eluate

5% ammonium aqueous solution tends to elute a hydrophilic phosphopeptides higher and 5% pyrrolidine aqueous solution tends to elute a hydrophobic phosphopeptides higher.



The left figure is a base peak chromatogram in an LC-MS to purify phosphopeptides from HeLa cell extract. The sample was measured with 5% ammonium solution and 5% pyrrolidine solution.

5% Ammonia eluate tends to extract more hydrophilic phosphopeptides.

5% Pyrrolidine eluate tends to extract more hydrophobic phosphopeptides compared with 5% ammonia eluate.