

Guide to clean up and desalting.

Phosphopeptides peak may be detected with a lactic acid peak caused by remaining a lactic acid in an eluate. Even if 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 eluate before using MS or LC-MS.

Solution A

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

TFA is a volatile solution. The change of TFA concentration may 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 phosphopeptide.)

Solution B

It contains 100% lactic acid.

Lactic acid is used to 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

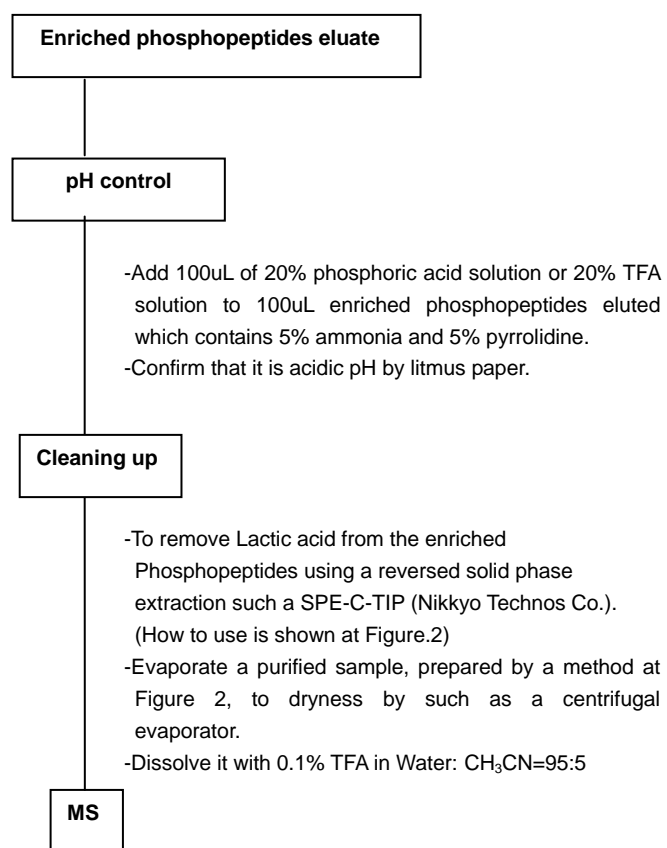
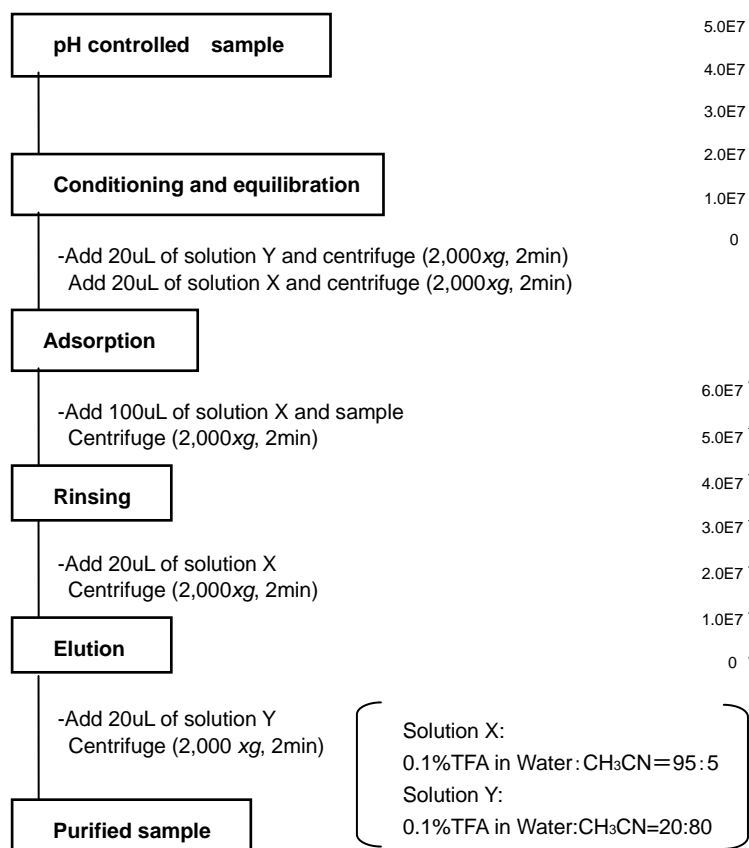


Fig.2 Simplified protocol of clean up
SPE C-TIP Operation Manual



*continue into evaporation at Fig.1

Reference

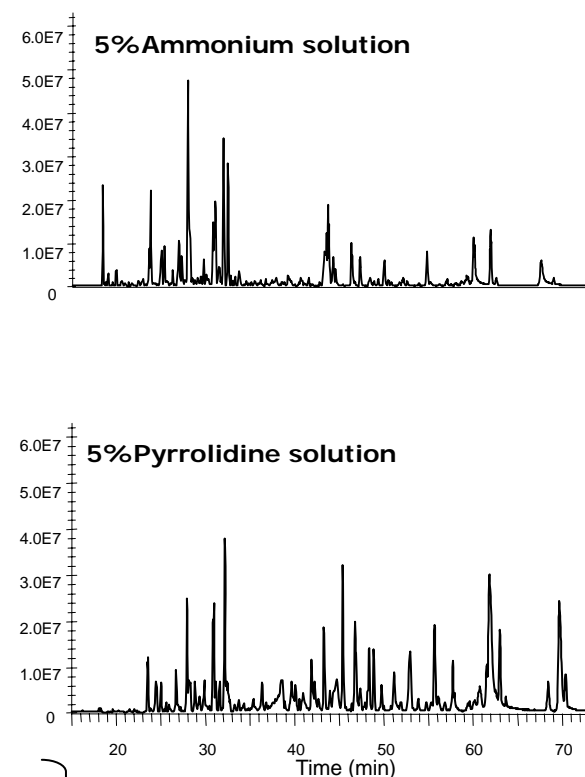
References

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 eluate

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



The left figure is a base peak chromatogram in LC-MS to purify phosphopeptides from HeLa cell extract.

5% Ammonia eluate tends to includes much hydrophilic phosphopeptides.

5% Pyrrolidine eluate tends to includes much hydrophobic phosphopeptides.