Preciex™ Stage-tip Instructions for:

Phosphopeptide enrichment

APPLICATIONS: The Preciex® Phosphopeptide enrichment tip column uses a TiO2 Resin with optimized protocol and buffers resulting in a higher yield of phosphopeptides ready for direct MS analysis without the need for additional clean up. It enhances the ability to routinely identify and characterize large numbers of phosphorylated species within complex protein mixtures.

Conditioning procedure

a) Place a Centrifuge Adaptor in a 2 mL collection tube and insert a phosphopeptide enrichment Stage-tip column into the adaptor.
b) Via a pipette tip inserted in the top of the Tip, add 20ul of Conditioning buffer(i) in order to wet the packing material. Centrifuge at 1,000 g, it may cost 100 seconds and then visually inspect that tips are pale white.
c) Add 20ul of Equilibrating buffer(ii), centrifuge 1,000 g for 120 seconds.

Suitable solutions are:

i. Conditioning Buffer: 0.5% Trifluoroacetic acid in Acetonitrile (20:80, v/v)
ii. Equilibrating Buffer: 300mg/mL lactic acid dissolved in 0.5% Trifluoroacetic acid in Acetonitrile (20:80, v/v)
iii. Loading Buffer: as equilibrating buffer
iv. Wash Buffer: as equilibrating buffer
v. Wash Buffer: as conditioning buffer
vi. Elution Buffer: 5% Ammonium hydroxide
vii. Elution Buffer: 10% Ammonium hydroxide in Acetonitrile (75:25, v/v)

We do not recommend any particular buffer, because each application has its own buffer and solutions. This products is in the method development stage and any application developed by you may be helpful.

Sample Loading

Transfer the equilibrated Stage-tip column and adaptor into a new 2 mL microcentrifuge tube.

Load 50µl sample in loading buffer(iii) as above. Centrifuge 800 g for 300 seconds. Typical loading capacity(Part Number: 2108042) is 2µg of phosphopeptide mixture. The eluate can be reloaded on the column if there is concern about the thoroughness of binding, then centrifuge 800 g for 120 seconds.

**Before loading, the digest sample was mixed 1:2 with loading buffer. This mixture was loaded onto the tip by taking 2.5µl aliquots and aspirating and expelling each aliquot ~20 times over several minutes. This was repeated until the desired amount of digest was loaded.*

Sample Washing

Place Stage-tip column and adaptor on a new collection tube.

Wash the packed bed with 20μl volumes of the wash buffer(iv) in order to wash out non retained phosphopeptides, centrifuge 1,000 g for 120 seconds. After that wash the packed bed with 20μl volumes of the wash buffer(v) to get rid of lactic acid. Repeat these two wash steps once more.

Sample Release

Elute the bound molecule from the packed bed with 20μl of Elution buffer(vi) to a new collection tube. In order to elute all of the adsorbed bound molecule, repeat to do elution with 20μl of Elution buffer(vii), centrifuge 1,000 g for 300 seconds in each sample release step. Combine two elution together, evaporate and reconstitute in 0.1% formic acid aqueous solution for MS analysis.

Please be sure of that tip column is not dried out in all steps.

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