Preciex™ Stage-tip Instructions for:

Glycopeptide enrichment

APPLICATIONS: The Preciex® glycopeptide enrichment tip column uses a proprietary ZIK Glycocapture Resin to capture high-mannose, hybrid, and complex glycosylated peptides from complex protein mixtures for effective downstream analysis by MALDI and ESI-MS. In combination with the included optimized buffers, the ZIK Glycocapture Resin is highly selective for glycosylated peptides and enriches samples without bias towards or loss of particular glycan structures. The enrichment protocol is optimized for efficient removal of non-glycosylated peptides with close to 100% glycopeptide recovery (as quantitatively assessed by N-Glycan mapping) and offers the flexibility to perform both site-specific determination of the glycan structures and parallel analysis of the attached peptides.

Conditioning procedure

a) Place a Centrifuge Adaptor in a 2 mL collection tube and insert a glycopeptide 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 2,500 g, it may cost 100-300 seconds and then visually inspect that tips are pale white.
c) Add 20ul of Conditioning buffer(ii), centrifuge 1,000 g for 120 seconds to force the solution through the packed bed.
d) Add 20ul of Equilibrating buffer(iii), centrifuge 1,000 g for 120 seconds, repeat this procedure 2x.

Suitable solutions are:

i. Conditioning Buffer: isopropanol
ii. Conditioning Buffer: 0.1% Trifluoroacetic acid in water
iii. Equilibrating Buffer: 20:80:0.1%, water: acetonitrile: trifluoroacetic acid (TFA)
iv. Loading Buffer: 20:80:0.1%, water: acetonitrile: trifluoroacetic acid (TFA)
v. Wash Buffer: as loading buffer
vi. Elution Buffer: 0.1% Trifluoroacetic acid in water
vii. Elution Buffer: 25mM NH_4HCO_3 in water or 0.1%NH_4OH in water

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 20µl sample solutions(iv) as above. Centrifuge 800 g for 360 seconds. Typical loading capacity(Part Number: 2109022) is 1µg of glycopeptide mixture. The elute can be reloaded on the column if there is concern about the thoroughness of binding.

Sample Washing

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

Wash the packed bed 2x with 20µl volumes of the wash buffer(v) in order to wash out non retained proteins and peptides, centrifuge 1,000 g for 120 seconds.

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 120 seconds in each sample release step. Combine two elution together, evaporate and reconstitute for MS analysis.

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

REFERENCES:

Yong Zhang et al. Site-specific N-glycosylation Characterization of Recombinant SARS-CoV-2 Spike Proteins. Molecular & Cellular Proteomics. October 19, 2020

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