Preciex™ Stage-tip Instructions for:

Peptide Fractionation

APPLICATIONS: Reduction of the high dynamic range of protein abundance is crucial for deep coverage of the proteome and especially the phosphoproteome, where the dynamic range is even more pronounced. This issue is usually addressed in two ways: either through introduction of more powerful and sensitive mass spectrometric instrumentation or by decreasing sample complexity via fractionation. Although great advances in instrument sensitivity and sequencing speed have been made, fractionation is still required for in-depth coverage of phosphoproteomes. High-pH RP-HPLC has a higher resolving power in comparison with SCX separations, decreasing peak broadening and peak multiplicity.

Conditioning procedure

a) Place a Centrifuge Adaptor in a 2 mL microcentrifuge tube and insert a Fractionation Stage-tip column into the adaptor.
b) Via a pipette tip inserted in the top of the Tip, add 100ul 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, discard the liquid.
c) Add 50ul of Equilibrating buffer(ii), centrifuge 1,000 g for 120 seconds, repeat this step once.

Suitable solutions are:

i. Conditioning Buffer: ACN
ii. Equilibrating Buffer: 0.1% TFA in water
iii. Loading Buffer: 0.1% TFA in water
iv. Wash Buffer: water

v. Fractionation Buffer list as below for unlabeled and native peptide,

Fraction No.	ACN(μl)	0.1% Triethylamine(μl)
1	5	95
2	7.5	92.5
3	10	90
4	12.5	87.5
5	15	85
6	17.5	82.5
7	20	80
8	50	50

vi. Fractionation Buffer list as below for labeled peptide,

Fraction No.	ACN(µl)	0.1% Triethylamine(µl)
1	10	90
2	12.5	87.5
3	15	85
4	17.5	82.5
5	20	80
6	22.5	77.5
7	25	75
8	50	50

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 150µl sample in loading buffer(iii) as above. Centrifuge 800 g for 300 seconds. Typical loading capacity(Part Number: 2104102) is 10µg of peptide 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.

Sample Washing

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

Wash the packed bed with 50µl volumes of the wash buffer(iv) for neutral balancing, centrifuge 1,000 g for 120 seconds.

** TMT-labeled samples require an additional column wash with 50µl of 5% ACN, 0.1% TEA to remove unreacted TMT reagent.*

Sample Fractionation

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

Proceed to elute the bound molecule from the packed bed with each 100µl of Factionation buffer(v) or Factionation buffer(vi) as table. Collect 8 fractions by centrifuging 1,000 g for 300 seconds in each step. Dry the fractions down using a vacuum centrifuge/lyophilizer, 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.

Preciex™ is trademarks of Fresh Bioscience (Shanghai) Co., Ltd.

Fresh Bioscience (Shanghai) Co., Ltd.
138 Xinjunhuan Road, Minhang District, Shanghai 201114, China
Tel. 21-3388-2916, Fax 21-3388-0027
www.freshbioscience.com
info@freshbioscience.com