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Reverse-phase high pH fractionation (using Thermo Fisher, Cat# 84868)

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol uses the Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (Thermo Fisher, Cat# 84868)

Conditioning the columns

4m

1 Remove the white cap on the end of the column and place the column in a 2 mL collection tube.

2 Centrifuge at 5000 rcf, 00:02:00 at Room temperature, discard the liquid. 2m

3 Remove the screw cap and add 300 µL acetonitrile (ACN) to the column (replacing the screw cap after).

4 Centrifuge at 5000 rcf, 00:02:00 at Room temperature, discard the liquid. 2m

5 Repeat steps 3 and 4 (for a total of 2 washes with ACN).

6 Repeat steps 3 – 5 with 0.1% TFA instead of ACN (total of 2x washes with 0.1% TFA).

7 The column is now ready to use.

Fractionating the samples

16m 20s

8 Add 300 µL of 0.1% v/v trifluoroacetic acid to each sample.

9 Vortex for ~ 00:00:10 10s

10 Leave to incubate for 00:05:00 at Room temperature 5m

11 Vortex for ~ 00:00:10 10s

12 Sonicate in a waterbath sonicator for 00:05:00 in an ice slurry. 5m

13 Load each sample into a fractionation column, replace the cap and centrifuge at 3000 rcf, 00:02:00 (keep eluate as ‘flow through’ fraction). 2m

14 Place the column into a new tube, and load 300 µL of water, and centrifuge at 3000 rcf, 00:02:00 (keep eluate as ‘wash’ fraction). 2m

15 Place the column into a new tube, and load the TMT wash solution (5% ACN, 0.1% triethylamine (TEA)) .

16 Place the column into a new tube, and load 300 µL of the appropriate elution solution (see table below), and centrifuge at 3000 rcf, 00:02:00 to collect the fraction. 2m

17 Repeat step 5 for each step of the gradient fraction.

18 If you are concatenating the fractions, combine the fractions into the desired combinations

19 Lyophilise all samples until there are only a few µL left in the tube