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

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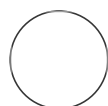
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Reverse-phase high pH fractionation, using the Pierce kit

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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 μ L 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 μ L 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 μ L 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% TEA)
- 16 Place the column into a new tube, and load  300 μ L 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