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

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Propionylation and tryptic digestion

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ABSTRACT

Protocol to derivatize (propionylation) and digest histone proteins into peptides with the purpose of bottom-up label free LC-MS/MS analysis. In this method, aspecific overpropionylation at serine (S), threonine (T) and tyrosine (Y) is reversed by adding hydroxylamine (HA).

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First propionylation

1 Start with vacuum dried sample (20 µg/sample)

2 Add 20 µL 1M triethylammonium bicarbonate (TEAB)

3 Add 20 µL prop-reagent (Isopropylalcohol:propionic anhydride (79:1))

4 Spin down & Incubate at room temperature for 30 minutes

5 Add 20 µL H2O

6 Spin down & Incubate at 37°C for 30 minutes

7 Vacuum dry sample

Trypsin digest

8 Calculate the amount of 500 mM TEAB, Calciumchloride (CaCl2), acetonitrile (ACN) and trypsin necessary to result in a 1:20 ratio (w/w) (1 µg trypsin/20 µg histones) in a final volume of 50µL.

Note

Final conc CaCl2: 1mM
Final conc ACN: 5%

9 Add correct volume of 500 mM TEAB, CaCl2 and ACN

10 Resuspend trypsin in 500 mM TEAB

11 Add trypsin at a 1:20 ratio (w/w) => 1 µg trypsin/20 µg histones

12 Spin down & incubate overnight at 37°C

13 Vacuum dry sample

Second propionylation

14 Vacuum dried sample (20 µg/sample)

15 Add 20 µL 1M TEAB

16 Add 20 µL Prop-reagent (Isopropylalcohol:propionic anhydride (79:1))

17 Spin down & Incubate at room temperature for 30 minutes

18 Add 20 µL H2O

19 Spin down & Incubate at 37°C for 30 minutes

20 Vacuum dry sample

Reversing overpropionylation hydroxylamine mediated

21 Vacuum dried sample (20 µg/sample)

22 Add 50 µL 0.5 M hydroxylamine (NH2OH)

23 Add 15 µL ammonium hydroxide (NH4OH) at pH 12

24 Spin down & Incubate at room temperature for 20 minutes

25 Adjust pH with formic acid (FA): 30 µl 100% FA

26 Vacuum dry sample

Note

Store in -20°c or -80°C until further use