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Propionylation and tryptic digestion

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We use this protocol and it's working

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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).

Troubleshooting



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) $\Rightarrow$ 1 μ g trypsin/20 μ g histones

12 Spin down & incubate overnight at 37°C

13 Vacuum dry sample

Second propionylation

14 Vacuum dried sample (20 μ g/sample)

15 Add 20 μ L 1M TEAB

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

17 Spin down & Incubate at room temperature for 30 minutes

18 Add 20 μ L H₂O

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

20 Vacuum dry sample

Reversing overpropionylation hydroxylamine mediated

21 Vacuum dried sample (20 μ g/sample)

22 Add 50 μ L 0.5 M hydroxylamine (NH₂OH)



- 23 Add 15 μ L ammonium hydroxide (NH_4OH) at pH 12
- 24 Spin down & Incubate at room temperature for 20 minutes
- 25 Adjust pH with formic acid (FA): 30 μ l 100% FA
- 26 Vacuum dry sample

Note

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

Protocol references

Meert P, Dierickx S, Govaert E, De Clerck L, Willems S, Dhaenens M, Deforce D. Tackling aspecific side reactions during histone propionylation: The promise of reversing overpropionylation. *Proteomics*. 2016 Jul;16(14):1970-4. doi: 10.1002/pmic.201600045. PMID: 27139031; PMCID: PMC5096241.