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On bead digest 1M urea LysC/Trypsin 90 min prior to Red/Alk V.1

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Abstract

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Materials

- urea
- ammonium bicarbonate
- LysC (mass spectrometry grade, FUJIFILM Wako chemicals)
- trypsin (Trypsin Gold, Promega)
- dithiothreitol
- iodoacetamide

On bead digest 1M urea LysC/Trypsin 90 min prior to Red/Alk

2h 49m

1 Add 50 µL of ABC and transfer beads to new 0.2 mL PCR tube and remove buffer Axygen.

2 Add appropriate volume of [M] 2 Mass Percent urea [M] 50 millimolar (mM) ABC. Here we added 30 µL .

3 Add 150 ng LysC/Trypsin (1:1 Mix) and incubate 01:30:00 at Room temperature . Here we added 1.5 µL . 1h 30m

4 Transfer supernatant to new 0.2 µL PCR tube. Here, the total volume becomes: 31.5 µL .

5 Rinse beads with 30 µL [M] 1 Mass Percent urea [M] 50 millimolar (mM) ABC, combine* 30 µL , sum: 61.5 µL .

> Note
>
> Optional: 2nd transfer to get rid of all beads.

6 Reduction: [M] 10 millimolar (mM) DTT (aliquot [M] 0.25 Mass Percent) 00:30:00 Room temperature . Here we added 2.4 µL . 30m

7 Alkylation: [M] 20 Mass Percent IAA (500mM stock = 0.092 g/mL) 00:30:00 Room temperature in the dark. Here, we added 2.4 µL . 30m

8 Quench IAA with half amount of DTT used in step 3 and incubate for 00:10:00 at Room temperature . We added 1.2 µL . 10m

9 Dilute to [M] 1 Mass Percent urea with [M] 50 millimolar (mM) ABC. We added 30 µL , which gave rise to a total sum of: 97.5 µL .

10 Add 150 ng LysC/Trypsin (1:1 Mix) and incubate at 37 °C Overnight . For us here, this meant 1.5 µL .

11 Acidify sample to final concentration of 0.5% TFA (from 10%). For us this was 4.5 µL .

12 Desalt: Load the peptides on a C18 STAGEtip.

Note

3-5 plugs depending on protein amount, 3 plugs for max. 20µg.

13 Equilibrate:

13.1 100 µL 100% MeOH, spin 2000 rpm, 00:03:00 . 3m

13.2 100 µL 80% ACN, 0.1% TFA, spin 2000 rpm, 00:03:00 . 3m

13.3 100 µL 0.1% TFA, spin 2000 rpm, 00:03:00 . 3m

14 Load sample: spin 1700 rpm .

15 Wash: 100 µL 0.1% TFA → keep flow through (FT) and wash together.

16 Elute: 2x 30 µL 60% ACN, 0.1% TFA in PCR tube.

17 Speedvac to remove ACN and take up sample in 20 µL 0.1% TFA 2% ACN.

Protocol references

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