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Version 2

On bead digest prior to mass spec V.2

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Abstract

This protocol was generated from an internal electronic lab notebook and contains experiment specific notes, for example, sample-specific volumes and incubation times. Due to the automatic generation process it might also contain errors and should be interpreted cautiously. An updated protocols will still be uploaded.

Materials

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

Safety warnings

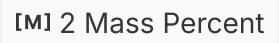
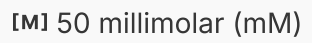
- ❗ This protocol was generated from an internal electronic lab notebook and contains experiment specific notes, for example, sample-specific volumes and incubation times. Due to the automatic generation process it might also contain errors and should be interpreted cautiously. An updated protocols will still be uploaded.



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

2h 49m

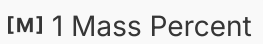
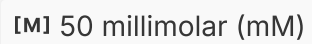
1 Add  50 μL of ABC and transfer beads to new 0.2 mL PCR tube and remove buffer Axygen. 

2 Add appropriate volume of  2 Mass Percent urea  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 . 

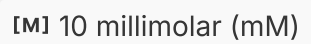
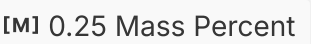
1h 30m

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

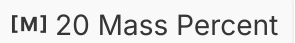
5 Rinse beads with  30 μL  1 Mass Percent urea  50 millimolar (mM) ABC, combine*  30 μL , sum:  61.5 μL . 

Note

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

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

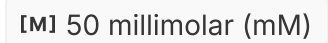
30m

7 Alkylation:  20 Mass Percent IAA (500mM stock =  0.092 μL)  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  1 Mass Percent urea with  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 $^{\circ}\text{C}$  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. 20 μg .

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 $\rightarrow$ 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

<https://doi.org/10.1038/nprot.2007.261>