Apr 23, 2025 Version 2

On bead digest prior to mass spec V.2

DOI
https://dx.doi.org/10.17504/protocols.io.6qpvrkdy3lmk/v2

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Protocol Citation: Elias Adriaenssens 2025. On bead digest prior to mass spec. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrkdy3lmk/v2Version created by **Elias Adriaenssens**

Protocol status: Working

Created: April 23, 2025

Last Modified: April 23, 2025

Protocol Integer ID: 141122

Keywords: ASAPCRN, mass spec this protocol, mass spec, updated protocol, bead, experiment specific note, protocol, internal electronic lab notebook, experiment, process, automatic generation process

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

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 [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 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 [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

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