

Apr 21, 2025

Version 1

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

 Nature Cell Biology

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvrkdy3lmk/v1>

Markus Hartl¹

¹Max Perutz Labs – MS Facility



Elias Adriaenssens

Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.6qpvrkdy3lmk/v1>

External link: <https://doi.org/10.1038/s41556-025-01712-y>

Protocol Citation: Markus Hartl 2025. On bead digest 1M urea LysC/Trypsin 90 min prior to Red/Alk. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.6qpvrkdy3lmk/v1>

Manuscript citation:

Adriaenssens, E., Schaar, S., Cook, A.S.I. *et al.* Reconstitution of BNIP3/NIX-mitophagy initiation reveals hierarchical flexibility of the autophagy machinery. *Nat Cell Biol* **27**, 1272–1287 (2025). <https://doi.org/10.1038/s41556-025-01712-y>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: April 09, 2025

Last Modified: April 21, 2025

Protocol Integer ID: 126558

Keywords: ASAPCRN, bead digest, bead

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP)

Grant ID: ASAP-000350

Marie Skłodowska-Curie MSCA Postdoctoral fellowship

Grant ID: 101062916

Abstract

-

Materials

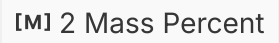
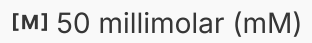
- urea
- ammonium bicarbonate
- LysC (mass spectrometry grade, FUJIFILM Wako chemicals)
- trypsin (Trypsin Gold, Promega)
- dithiothreitol
- iodoacetamide



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

2h 49m

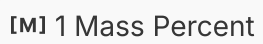
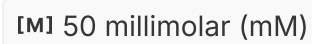
1 Add  50 μL of ABC and transfer beads to new 0.2 mL PCR tube and remove buffer Axygen. 

2 Add appropriate volume of  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 . 

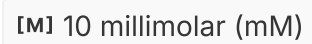
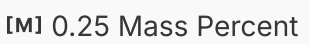
1h 30m

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

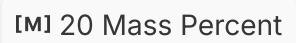
5 Rinse beads with  30 μL  1 Mass Percent urea  50 millimolar (mM) ABC, combine*  30 μL , sum:  61.5 μL . 

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 .

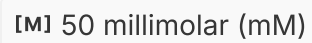
30m

7 Alkylation:  20 Mass Percent IAA (500mM stock =  0.092 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  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>