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SP3 protocol optimised for foodcrust protein extraction from archaeological cooking vessels V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

A SP3-based protocol developed for the extraction of proteins from charred organic residues (foodcrust) found on cooking vessels. This protocol is to prepare samples for MALDI-ToF MS and LC-MS/MS analysis. We recommend starting with a batch size of 2-10 samples, and including flanking negative controls (blanks) to test for any downstream contamination.

Guidelines

Perform this protocol with the appropriate PPE and ideally in a clean lab setting.

Materials

Equipment

- Scale for laboratory use
- Magnetic tube rack
- Heat block/Thermoshaker
- Centrifuge
- Vortexer

Consumables

- Protein Low-Bind 1.5 mL tubes.
- Pipette tips: 1000, 200 and 10 µL
- Pipettes
- C18 ZipTips or equivalent (e.g. C18 StageTips)

Reagents

- GuHCl (Guanidine hydrochloride), concentration: 6 M
- TCEP (Tris-(2-carboxyethyl)-phosphine), concentration: 100 mM
- CAA (Chloroacetamide), concentration: 100 mM
- Water, molecular grade
- Ethanol, 100% v/v
- ABC (Ammonium bicarbonate), concentration: 50 mM
- TFA (Trifluoroacetic acid), concentration: 5% v/v and 0.1% v/v
- Sequencing Grade Modified Trypsin **Promega Catalog #**V5111
- ACN (Acetonitrile), concentration: 50%
- Sera-Mag Speedbead carboxylate-modified [E3] magnetic particles **Cytiva Life Sciences Catalog #**65152105050250
- Sera-Mag™ SpeedBead Carboxylate-Modified [E7] Magnetic Particles **Cytiva Life Sciences Catalog #**45152105050250

Protocol materials

Sequencing grade Trypsin **Promega Catalog #**V5111

Sera-Mag Speedbead carboxylate-modified [E3] magnetic particles **Cytiva Life Sciences Catalog #**65152105050250

Sera-Mag™ SpeedBead Carboxylate-Modified [E7] Magnetic Particles **Cytiva Life Sciences Catalog #**45152105050250

Sequencing Grade Modified Trypsin **Promega Catalog #**V5111

Troubleshooting

Safety warnings

Take care and adhere to all chemical safety data sheets.

Protein extraction

1 Prepare foodcrust samples by sampling into new Protein Low-Bind tubes. Suggested mass for each sample is 10-20 mg depending on sample availability.

2 Preheat a heat block to 65°C.

3 Add 150 µL 6 M GuHCl to each sample.

4 Briefly vortex to homogenise and centrifuge for 1 minute at 13k RPM.

5 Incubate samples at 65°C for 1 hour on the heat block to extract the proteins from the foodcrust into the GuHCl. Then remove the samples from the heat block.

Reduction & alkylation

6 Preheat a heat block to 99°C.

7 Add 15 µL of a solution containing 100 mM TCEP and 100 mM CAA.

8 Briefly vortex to homogenise and centrifuge for 1 minute at 13k RPM.

9 Incubate samples at 99°C for 10 minutes in a heat block, then remove the samples from the heat block and allow to cool down at room temperature for roughly 5 minutes.

Bead clean up

10 Preheat a Thermomixer or equivalent heating and shaking device to 24°C or room temperature.

11 Add 500 µg of beads (i.e. 10 µL of a 50 µg/µL bead solution in ultrapure water) to each sample. Homogenise through gentle aspirating and dispensing with a pipette in the sample.

Note: the ratio of protein to bead concentration should be 1:10 for optimal performance.

12 Add 175 µL 100% ethanol to each sample and homogenise through pipetting.

Note: added ethanol volume should be equal to volume currently in the sample tube.

13 Incubate samples in a Thermomixer at 24°C at 1000 RPM for 5 minutes.
The proteins will now bind to the magnetic beads.

14 Place the samples on the magnetic separation rack and allow the beads to fully migrate for at least 2 minutes.

15 With the samples still on the magnetic rack, remove the supernatant.

Note: the supernatant can be discarded or saved to test for bead extraction efficiency.

16 Remove the samples from the magnetic rack and add 500 µL of 80% ethanol. Resuspend the beads through gentle pipetting. Then place the beads back on the magnetic rack and allow the beads to migrate for at least 2 minutes.

17 With the beads on the magnetic rack, remove the supernatant and discard to waste.

18 Remove the samples from the magnetic rack and add 300 µL of 80% ethanol. Resuspend the beads through gentle pipetting. Then place the beads back on the magnetic rack and allow the beads to migrate for at least 2 minutes.

19 With the beads on the magnetic rack, remove the supernatant and discard to waste.

20 Remove the samples from the magnetic rack and add 200 µL of 80% ethanol. Resuspend the beads through gentle pipetting. Then place the beads back on the magnetic rack and allow the beads to migrate for at least 2 minutes.

21 With the beads on the magnetic rack, remove the supernatant and discard to waste.
Ensure that as much supernatant as possible is removed.

22 Resuspend the beads in 100 µL 50 mM ABC to elute the proteins from the beads and homogenise through gentle pipetting.

Separate aliquot for protein quantification (optional)

23 Place the samples on a thermomixer at 37°C and 750 RPM for 3 minutes.

24 Place the samples on the magnetic rack and allow the beads to migrate for at least 2 minutes.

25 Remove 10 µL and store separately for protein quantification (i.e. Bradford assay or BCA).

Tryptic digestion

18h

26 Resuspend 20 µg of Sequencing grade Trypsin **Promega Catalog #**V5111 with 100 µL of resuspension buffer to create a 0.2 µg/µL solution.
Add 1 µL of trypsin solution to each sample.

27 Incubate the samples at 37°C and 750 RPM for 18 hours to digest.

18h

28 Remove the samples from the mixer and centrifuge at 13k RPM for 1 minute.

29 Place the samples on the magnetic rack and allow the beads to migrate for at least 2 minutes.
Transfer all the supernatant to new Protein Low-Bind tubes.
The beads can now be discarded as all peptides are in the supernatant.

30 Halt tryptic activity by acidifying with 10 µL 5% v/v TFA.

Desalting clean up

31 Prime C18 ZipTips by aspirating 100 µL 50% ACN + 0.1% TFA. Discard solvent to waste.
Repeat this step for a total of 2 primings.

32 Wash the ZipTip by aspirating 100 µL 0.1% TFA and discarding to waste.
Repeat for a total of 2 washes.

33 Desalt samples by aspirating and dispensing 5-10 times in the sample.
The peptides should now sit on the C18 filter of the ZipTip.

34 Wash the ZipTip by aspirating 100 µL 0.1% TFA and discarding to waste.

Repeat for a total of 2 washes.

35 Elute the peptides in a new Low-Bind tube by taking up 100 µL 50% ACN + 0.1% TFA and dispensing.
The peptides have now been eluted off the C18 filter into the solution.

Reagent preparation

36 Preparing a 100 mM TCEP and 100 CAA solution:
Weigh out 28.66 mg TCEP and 9.35 mg CAA in a darkened tube (or one wrapped with foil).
Add 1 mL ultrapure water and vortex for 1 minute to homogenise.

37 Preparing a 50 µg/µL bead solution

37.1 Carefully invert the bottles of bead stock solutions and knock (!) them firmly on the counter to gain an equal distribution of the beads in the stock solution.

37.2 Pipette 500 µL of the

Sera-Mag Speedbead carboxylate-modified [E3] magnetic particles **Cytiva Life Sciences Catalog #**65152105050250

into a tube and combine with 500 µL of

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Place them on the magnetic rack and remove the supernatant.

37.3 Wash 3 times by removing the beads from the magnetic rack, resuspending in 1000 µL ultrapure water, homogenising through pipetting, placing back on the magnetic rack and removing the supernatant.

37.4 After the final wash resuspend the beads in 1000 µL ultrapure water and homogenise through gentle pipetting.

Protocol references

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