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DES foodcrust protein extraction protocol

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Protocol status: Working
We have successfully used this protocol for the extraction of proteins from both experimental and archaeological foodcrusts. However, we acknowledge there may be room for optimisation.

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Abstract

This protocol is an adaptation of the DES protocol developed by Manasji Chowdhury for the extraction of proteins from ceramic. This version has been designed for the extraction of proteins from foodcrust.
The protocol consists of protein extraction from foodcrusts by incubating in a GuHCl and urea DES mixture, followed by reduction and alkylation, C18 StageTip desalting, sequential enzymatic digestion using trypsin and chymotrypsin. Lastly, the samples are cleaned up with Evotips in order to prepare them for loading on a Evosep LC system coupled to a mass spectrometer.

Materials

Equipment:

- Heating block
- Vortex
- Centrifuge
- Speedvac

Reagents:

- Urea
- Guanidine hydrochloride (GuHCl)
- Purified water
- TCEP (Tris-(2-carboxyethyl)-phosphine), concentration: 100 mM
- CAA (Chloroacetamide), concentration: 100 mM
- MeOH (methanol), 100%
- ACN (Acetonitrile)
- TFA (Trifluoroacetic acid), concentration: 0.1% v/v
- ABC (Ammonium bicarbonate), concentration: 50 mM.
- FA (Formic acid), concentration 0.1% v/v
- Trypsin Gold-Mass Spec Grade, 100ug **Promega Catalog #**V5280
- Chymotrypsin, Sequencing Grade, 25ug **Promega Catalog #**V1061

Consumables:

- C18 StageTips
- Evotips
- Eppendorf protein LoBind tubes, 1.5 mL

DES extraction

5h 35m

1 To make the DES solution mix urea and GuHCl in a 2:1 molar ratio.
Heat to 75-80°C until a clear liquid is obtained. This can take around an hour. Wait for additional 20 minutes until after it is molten to give it time to mix.

Note: the DES solution will crash out once it cools down. Once cooled, it can be reheated, but making the DES solution fresh is recommended.

1h 20m

2 Add 300 µL of the DES solution to 10-20 mg foodcrust.
Vortex to mix the DES and the foodcrust, then incubate in a heating block at 65°C for 4 hours.

Note: as the DES solution will rapidly cool down and crash out, it is recommended to preheat the pipette tips and work rapidly when pipetting.

4h

3 Take the samples off the heating block, add 500 µL of purified water to each sample and vortex to thoroughly homogenize the samples.
Centrifuge for a minimum of 15 minutes at 15000 rcf.

15m

Reduction and alkylation

10m

4 Add 15 µL of 100 mM TCEP and 100 mM CAA solution to each sample, then vortex to mix, centrifuge at 15000 rcf for 1 minute and incubate in a heating block at 99°C for 10 min.

10m

C18 StageTip cleaning

7m 30s

5 Desalting of the samples using C18 StageTips.

5.1 Conditions the StageTips by loading each tip with 150 µL 100% MeOH, then centrifuge until all methanol has flowed through. This can usually be achieved by centrifuging at 1000 rcf for 1 minute.

1m

5.2 Load 150 µL 80% ACN + 0.1% TFA on the tips and again centrifuge until complete flowthrough is achieved. Typically by centrifuging at 1000 rcf for 2 minutes.

2m

5.3 Load 150 µL 0.1% TFA. Centrifuge until completion, typically at 1000 rcf for 2 minutes.

2m

5.4 Load 150 µL of one sample, centrifuge at 1000 rcf until completion, then load another 150 µL of the sample and repeat until all the sample has been loaded on the StageTip. This typically requires 6 loadings.

Note: depending on the sample, it may take considerably longer for the sample to be pushed through the StageTip. A single loading can take up to 20 minutes, although a centrifugation time 5-10 frequently sufficed. Ensuring no leftover foodcrust pellet is transferred to the stagetip is imperative to ensure the tips do not block up and the time needed to spin in limited.

5.5 Wash the StageTips by loading 150 µL 0.1% TFA andd centrifuging until completion, usually 2:30 minute at 1000 rcf.
Repeat this for a total of 2 washes.

2m 30s

5.6 Elute the proteins by loading 50 µL of 50% ACN + 0.1% TFA and centrifuging at 700 rcf for 3 minutes. Ensure to capture the flowthrough as that fraction will contain the extracted proteins.

Digestion

1d 12h

6 Before digestion, dry down the samples to near dry, but still wet, by incubating the samples in a speedvac for 45 min at 45°C. Then, resolubilise the samples in 100 µL 50 mM ABC.

Note: a P1000 pipette tip placed with tip side facing up slots perfectly into a 1.5 mL protein loBind tube allowing for evaporation through the filter while limiting potential contamination during this step

7 If you wish to use a protein assay, such as a Bradford or BCA assay to quantify the amount of protein in your sample, take a 10 µL aliquot at this step.

8 For a sequential digestion, first add 1 µL if a 0.4 µg/µL chymotrypsin solution and incubate at 25°C for 18 hours.

18h

9 Following chymotryptic digestion, add 1 µL of a 0.4 µg/µL trypsin solution and incubate at 37°C for 18 hours.

18h

10 After finishing the tryptic digestion, centrifuge the samples for 1 minute at 13000 rcf. Then add 10 µL of 5% v/v TFA to acidify the samples.

Evotip loading

11 After digestion we loaded our samples on Evotips in accordance with the Evosep Evotip loading protocol to facilitate loading on the Evosep One LC system. However, this section can easily be replaced by other forms of C18 desalting, such as the earlier described StageTip cleaning, or ZipTips.

11.1 Load 20 µL of Solvent B consisting of 0.1% FA in ACN on the Evotips. Then centrifuge for 60 seconds at 700 rcf.

11.2 Place the filtered tips of the Evotips in isopropanol until the filters change from ivory white to a pale white, usually for a minimum of 10s.

Note: An easy way to do this is by filling the bottom of an empty pipette tip box with isopropanol and then transferring the insert with the tips.

11.3 Load 20 µL of Solvent A consisting of 0.1% FA in water on top of each Evotip, then centrifuge at 700 rcf for 60 seconds.

11.4 Load 20 µL of sample onto each Evotip and centrifuge again at 700 rcf for 60 seconds.

11.5 Wash the Evotips by loading 20 µL Solvent A consisting of 0.1% FA and centrifuging at 700 rcf for 60 seconds.
Repeat for a total of 3 washes.

Note: A larger volume of wash buffer can be used if the samples are very dirty, just ensure that no flowthrough touch the tips in the bottom of the box

11.6 Load 100-150 µL 0.1% FA onto the samples, then centrifuge at 700 rcf for 10 seconds. The solution should now sit directly on top of the filter. The samples are now ready for short term storage.

Note: For longer storage it is important that the filters of the Evotips remain wet. This can be achieved by filling the bottom of the tip box with 0.1% FA so that it touches the filters.

Protocol references

Our DES protocol for protein extraction from foodcrusts is based on the following DES protocol designed for the extraction of proteins from ceramic:

Citation

Pal Chowdhury M, Makarewicz C, Piezonka H, Buckley M (2022)
. Novel Deep Eutectic Solvent-Based Protein Extraction Method for Pottery Residues and Archeological Implications.

https://doi.org/10.1021/acs.jproteome.2c00340 LINK

For the use of StageTips in protein extraction:

Citation

Rappsilber J, Mann M, Ishihama Y (2006)
. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips.

https://doi.org/ LINK

The loading protocol for Evotips as described by their manufacturer can be found here:
https://www.evosep.com/wp-content/uploads/2020/03/Sample-loading-protocol.pdf

Citations

Pal Chowdhury M, Makarewicz C, Piezonka H, Buckley M. Novel Deep Eutectic Solvent-Based Protein Extraction Method for Pottery Residues and Archeological Implications.
https://doi.org/10.1021/acs.jproteome.2c00340

Rappsilber J, Mann M, Ishihama Y. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips.
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