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Suspension Trapping for Archaeological Bone Proteins

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

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Abstract

'Suspension Trapping for Archaeological Bone Proteins' is a suspension trapping-based protocol developed to prepare archaeological bone and dentine samples for analysis by LC-MS/MS. This straightforward protocol features a rapid digestion time and does not use any chemicals that are highly toxic. The authors use this protocol routinely to extract abundant collagen peptides for species identification by ZooMS/MS.

Guidelines

This protocol is designed to be used for powdered bone samples of 5-20 milligrams. You may wish to increase the reagent volumes if your sample is significantly larger.

Materials

- Invitrogen™ UltraPure™ SDS Solution, 10% **Invitrogen** **Catalog #**15-553-027
- Hydrochloric acid 1.2 mol/l **Chem-Lab Analytical bvba** **Catalog #**CL05.0312.1000
- Acetone **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**179124
- Triethylammonium bicarbonate buffer **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**90360-100ML
- DTT for Biochemistry 99+% C4H10O2S **Chem-Lab Analytical bvba** **Catalog #**CL00.0481
- S-Methyl methanethiosulfonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**64306-10ML
- Phosphoric acid 85% a.r. **Chem-Lab Analytical bvba** **Catalog #**CL00.0605.1000
- Methanol, LC-MS grade **Chem-Lab Analytical bvba** **Catalog #**CL00.1377.1000
- Trypsin/Lys-C Mix, Mass Spec Grade, 5 × 20ug **Promega** **Catalog #**V5073
- Formic acid 0.1% in Water HPLC **Biosolve** **Catalog #**232406
- Acetonitrile, LC-MS grade **Chem-Lab Analytical bvba** **Catalog #**CL00.0194.1000

Equipment
1.5mL Epps - Eppendorf Protein LoBind Tube (Safe-Lock, PCR clean)
Suspension trapping columns - Magen Biotechnology Co. Ltd. (China) HiPure Viral Mini Columns with 2mL collection tubes; Cat No. C13112
MS vials - Verex Vial, 9mm Screw, PP, 300uL, Clear, No Patch, 1000/pk; Part: AR0-39P2-13.
Centrifuge - Eppendorf Centrifuge 5417R
CentriVap - Thermo Scientific Savant SPD111V SpeedVac Concentrator
Thermomixer - Eppendorf Thermomixer Comfort
Vortexes - Scientific Industries Vortex-Genie 2
Sonicator - Elma Transsonic 460

Equipment

Verex Vial, 9mm Screw, PP, 300uL, Clear, No Patch, 1000/pk	NAME
Vial	TYPE
Verex	BRAND
AR0-39P2-13	SKU
https://www.phenomenex.com/part?partNo=AR0-39P2-13	LINK

Equipment

HiPure Viral Mini Column	NAME
Trapping Column	TYPE
Magen	BRAND
C13112	SKU
https://www.magen-tec.com/products/info.aspx?lcid=14&itemid=176	LINK

Safety warnings

Wear appropriate protective gear when performing this protocol, minimally a lab coat and gloves, and preferably goggles, closed shoes, and long leg coverings.

Before start

We advise you to prepare all reagents except the MMTS before starting your protocol. The lysis buffer in particular can take a long time to come into solution when preparing from solid SDS.

Lysis Buffer - 10% SDS in 100mM TEAB
Binding/Washing Buffer - 100mM TEAB in 90% MeOH

Demineralisation

1 Add 600µL of 0.6M HCl to each 1.5mL Protein Lo-Bind Eppendorf containing 5mg of bone powder. Vortex to suspend the powder in the acid.

> Note
>
> Bone powder can be highly static. To avoid sample loss, pipette the acid onto the side of the Eppendorf above the fluid.

2 Incubate overnight on a thermomixer at 650rpm.

3 Centrifuge at 15000g for 10 minutes

4 Pipette the supernatant off. You may discard this or separate into clean Eppendorfs for later analysis.

5 Wash the pellet by adding 500µL of ice-cold acetone, then centrifuge for 10 minutes at 15000g, and pipette the acetone off into a waste beaker. Leave the tubes open to dry out the pellet.

Solubilise, Reduce, Alkylate

6 Add 25.5µL lysis buffer solution (5% SDS + 50mM TEAB).

7 Resuspend the pellet by vortexing and sonicating the sample thoroughly.

8 Add DTT to a final concentration of 5mM.

9 Incubate in a thermo-mixer for 30 minutes at 37°C, at 600rpm.

10 Add MMTS to a final concentration of 10mM.

11 Incubate for 10 minutes in darkness while mixing at 600rpm.

Protein washing

12 Acidify the samples by adding 27.5% phosphoric acid until the pH is 1 or lower.

13 Add 165µL binding/washing buffer (100mM TEAB in 90% methanol) to the sample and gently vortex to mix.

Note

You may notice floculation of your sample at this point. This is normal. All of the fluid and floculated material should be transferred in the next step.

14 Transfer the sample to the suspension trapping column.

Note

Be very careful not to touch or disrupt the column filter with your pipette tip at any point. If this is significantly damaged the filter may perform poorly, resulting in peptide loss.

15 Centrifuge for 30 seconds at 4000g.

16 Add 150µL of washing/binding buffer to the column and centrifuge again for 30 seconds at 4000g.

17 Repeat the previous step once or twice (depending on the sample). You may need to empty the waste reservoir.

18 Dry the filter by centrifuging for 1 minute at 4000g.

Digestion

19 Transfer the trapping columns to fresh 1.5mL Protein lo-bind Eppendorfs.

20 Prepare your digestion solution: suspend your lyophilised Trypsin/Lys-C in 50mM TEAB at a concentration of 0.0125µg/µL [i.e. for a vial of 20µg protease, add 1.2mL TEAB].

21 Add 40µL of digestion solution (equal to 0.5µg Trypsin/Lys-C) to the column filters.

> Note
>
> You may modify the quantity of trypsin in your samples but the quantity of digestion solution added should be 40µL.

22 Incubate for 2 hours at 47°C.

Elution

23 Add 30µL of 50mM TEAB, incubate for 1 minute, then centrifuge for 1 minute at 4000g.

24 Add 30µL of 0.1% FA, incubate for 1 minute, then centrifuge for 1 minute at 4000g.

25 Add 30µL of 50% ACN, incubate for 1 minute, then centrifuge for 1 minute at 4000g.

26 Dry the sample in a SpeedVac. The dried sample may be stored at -20°C (or colder) until resuspension.

Resuspension

27 Add 20µL of 0.1% FA.

28 Vortex and sonicate the sample thoroughly to resuspend the peptides.

29 Centrifuge for 15 minutes at 15000g.

30 Transfer the sample to MS vials ready for analysis.

Protocol references

This protocol was developed by Ian Engels, drawing on existing protocols by Simon Daled & Alexandra Burnett (see reference), and Rachel Siân Dennis. RSD gave advice on the implementation of the suspension trapping portion of the protocol and AB contributed to minor protocol revisions.

Daled, S. & Burnett, A., (2023). Protein Extraction from Dental Enamel [protocol], 21 Aug 2023. protocols.io. **dx.doi.org/10.17504/protocols.io.8epv5j8n4l1b/v2**

This protocol is written as employed in the following publication:
Engels, I., Burnett, A., Robert, P., Pironneau, C., Abrams, G., Bouwmeester, R., Van der Plaetsen, P., Di Modica, K., Otte, M., Straus, L.G. and Fischer, V., (2025). Classification of Collagens via Peptide Ambiguation, in a Paleoproteomic LC-MS/MS-Based Taxonomic Pipeline. Journal of Proteome Research, 24(4), pp.1907-1925. https://doi.org/10.1021/acs.jproteome.4c00962