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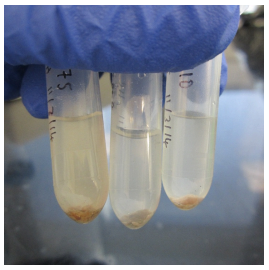
Ancient Proteins Extraction Protocol



In 1 collection

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Ashley Scott[1], Christina Warinner[1]

[1]Max Planck Institute for the Science of Human History

WarinnerGroup | MPI EVA Archaeogenetics

Ashley Scott
MPI

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Manuscript citation:
Wisniewski, J.R., Zougman, A., Nagaraj, N., & Mann, M. (2009). Universal sample preparation method for proteome analysis. Nature Methods, 6, 359-362.

Protocol status: Working
We use this protocol in our group and it is working.

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Abstract

This is a protocol for extracting total proteins from archaeological dental calculus. It is based on the filter-aided sample preparation (FASP) protocol first published by Wisniewski et al. 2009. Specific modifications have been made to enable protein extraction from calcified material and to ensure recovery of ancient proteins.

Guidelines

Working in an Ancient Protein Laboratory

- All steps of the protocol should take place in a dedicated clean room facility specifically designed for ancient proteins; do not extract or digest ancient proteins in a core facility laboratory where modern proteins are handled.
- Avoid introducing proteinaceous materials (e.g., latex, leather, silk, wool) into the lab. It is recommended that all laboratory clothing be made of cotton and shoes of synthetic materials.
- The researcher performing lab work should wear correspondingly suitable lab-wear, such as:
  - full-body suit with hood (e.g., Tyvek)
  - hairnet
  - face mask
  - two pairs of clean nitrile gloves
  - clean shoes
  - protective glasses
- Sample processing should be carried out in separated work benches (e.g. Dead Air PCR work bench)
- Surfaces and equipment should be regularly cleaned with water and/or ethanol or isopropanol and decontaminated with bleach solution.

Please see the following for more detailed guidance:

Hendy J, Welker F, Demarchi B, Speller C, Warinner C, Collins MJ. (2018) **A guide to ancient protein studies**. *Nature Ecology and Evolution*. DOI: 10.1038/s41559-018-0510-x

Materials

MATERIALS

NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014

Sequencing Grade Modified Trypsin, 100ug **Promega Catalog #**V5117

Trizma® hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T2319

Trifluoroacetic acid for HPLC > 99.0% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**302031-100ML

UltraPure 0.5M EDTA, pH 8.0 **Thermo Fisher Scientific Catalog #**15575-038

Iodoacetamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**I1149-5G

Urea **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**U5378

DL-Dithiothreitol (DTT) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43815

Triethylammonium bicarbonate (TEAB) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T7408

SDS, 20% Solution, RNase-free **Thermo Fisher Catalog #**AM9820

Solutions:

1M DTT (if from powder). Add 15.43mg of DTT powder to 100uL of water

Lysis buffer:
-In a 1.5 mL Eppendorf tube, combine 90 µL 20% SDS stock solution, 45 µL 1M DTT stock solution, 45 µL 1M Trizma (Tris/HCl) with 270 µL milliQ water.

Urea (8M):
-In a 15 mL Falcon tube, add 10 mL Tris/HCl (100 mM) to 5.76 g urea powder. Bring the final volume up to 12 mL with Tris/HCl.

IAA: (0.05M)
-In a black 1.5 mL Eppendorf tube, add 1.5 mL urea solution (8M) to 13.87 mg of IAA powder

NaCl (0.5M):
-Add 2.922 g of NaCl to 100 mL of MilliQ water

TEAB (0.05M)
-Add 5mL of 1M TEAB to 95mL of MilliQ water

Trypsin solution (only do immediately prior to digestion step)
-Make trypsin solution. Add 1.2 mL TEAB (0.05 M) to 20 µg of lyophilized trypsin and resuspend thoroughly.

Troubleshooting

Sample Prep

1 Weigh samples (aim for 5-10 mg per sample) and place within a 1.5 mL Safelock Eppendorf tube.

1.1 Also prepare empty tubes for blank extractions that should be processed alongside your samples to monitor for lab contamination

Demineralization

2 Add 500 µL of 0.5 M EDTA to each sample, including extraction blanks.

3 Close tubes tightly and set on a rotator until calculus becomes completely dissolved (invisible), or buoyant and feathery, typically between 2-5 days.

EXTRACTION DAY 1

4 Prepare solutions and label all tubes that will be needed for Day 1

5 Spin down decalcified samples at 14,000 rcf for 5 minutes

6 Remove 300uL of EDTA supernatant and place in a new labeled sample and store in -20˚C freezer as backup.

7 Add 50 µL of UA solution to a Microcon 30kDa filter unit. Avoiding the pellet, transfer the remaining 200 uL EDTA supernatant to the UA in the filter unit. Resuspend thoroughly to mix.

7.1 Spin at 14,000 rcf at 18˚C for 15-18 minutes.

Extraction Day 1: Lysis and Denaturation

8 To the remaining pellet, add 30 µL of SDS-lysis buffer and mix by resuspension. Incubate on heat block for 5 minutes at 95°C.

9 Centrifuge at 14,000 rcf at 18˚C for 10 minutes.

10 Add 200 µL of UA solution to the filter unit used in Step 7, followed by the pellet supernatant (~30 µL), avoiding any pellet debris, and resuspend to mix.

10.1 Centrifuge filter unit at 14,000 rcf at 18˚C for 15-18 min until all liquid has passed through

10.2 Discard flow-through

11 Add another 200 µL of UA solution to the filter unit

11.1 Centrifuge filter unit at 14,000 rcf at 18˚C for 15-18 min until all liquid has passed through

12 Place the remaining pellet tube in -20°C freezer for storage.

Extraction Day 1: Alkylation

13 Add 100 µL IAA solution (0.05 M) to the filter unit.

13.1 Mix at 300 rpm in the thermo-mixer for 1 min in the dark (cover with foil).

14 Incubate for 15-20 min in the dark

15 Centrifuge at 14,000 rcf at 18˚C for 12-15 min until all liquid has passed through.

15.1 Discard flow-through in halogenated waste

Extraction Day 1: Wash steps

16 Add 200 µL of urea solution to the Microcon unit and centrifuge at 14,000 rcf for 15-18 minutes.

16.1 Repeat for a total of three washes of urea solution. Discard flow-through.

17 Add 100 µL 0.05M TEAB to the Microcon unit and centrifuge at 14,000 rcf for 12-15 minutes.

17.1 Repeat twice for a total of three washes of TEAB solution.

Extraction Day 1: Digestion

18 Add 100uL of trypsin solution to each filter unit

18.1 Incubate overnight at 37C in the thermo-mixer at 300 rpm

Extraction Day 2

19 Transfer the Spin Filter to a new labeled collection tube.

20 Add 40 µL of TEAB Solution. Centrifuge the Spin Filter at 14,000 rcf for 10 min. DO NOT DISCARD FLOW-THROUGH.

20.1 Repeat this step once.

21 Add 50 µL 0.5 M Sodium Chloride Solution and centrifuge the Spin Filter at 14,000 rcf for 10 min.

22 The filtrate contains digested proteins. Acidify the filtrate with TFA to a pH below 3 (approximately 18 µL of TFA will be needed).

23 Desalt using method of choice (e.g., StageTips or ZipTips).