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Palaeoproteomics protocol - arid environment samples

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ABSTRACT

The analysis of the skeletal remains of vertebrates in archaeological contexts provides information about human-animal relationship and their environment. Their taxonomic identification based on macroscopic observation is not always possible due to fragmentation and poor preservation. In recent years, proteomics has emerged as an alternative but there is clearly a lack of data in arid environment where diagenesis rapidly affects the integrity of bone proteins. Here, we report the efficiency of three protocols for protein extraction. The protocols used harsh (1 M HCl and 0.6 M HCl) and soft (Tris-EDTA) decalcification agents and were tested on unidentified splinters from the 2000 years-old site of Toteng, Botswana. The preservation of the organic phase was first estimated using attenuated total reflectance Fourier transform infrared spectroscopy and a set of samples with contrasted collagen contents were selected for palaeoproteomics. The extracted proteins were submitted to a bottom-up proteomic approach involving trypsin digestion followed by ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS). Our results identify Tris-EDTA buffer as the most suitable decalcification protocol for poorly preserved bones and propose a collagen content threshold of ~3% weight content for successful detection of peptides. This approach, combined with biogeographical and chronological repartitions of mammals in Africa allows refining taxonomic attributions for four out of nine splinters, leading to species identification. Data are available via ProteomeXchange with identifier PXD010725.

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Protocol status: Working

We use this protocol and it's working. It has been developed as part of a palaeoproteomics study (Le Meillour et al. 2018) of African zooarchaeological remains.

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BCDiv

MATERIALS

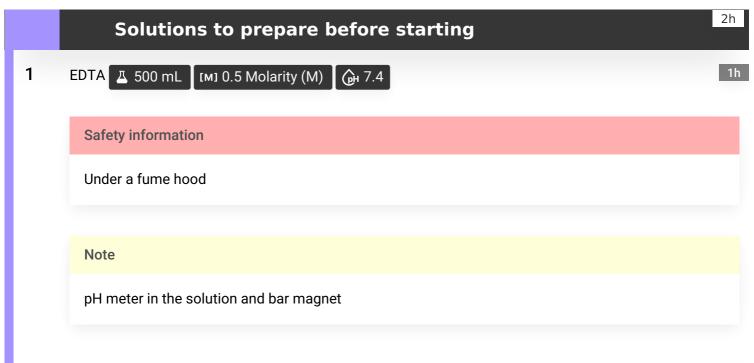
Solutions: (steps to make them in the protocol)

- EDTA Buffer 0.5M
- NH₄HCO₃50mM
- Iodoacetamide 1M (/!\ protect from light)
- DTT 1M (/!\ highly toxic)
- Trypsin 1μg/μL
- 10% Formic acid

SAFETY WARNINGS

 DTT Highly toxic, need to be under fume hood to handle it

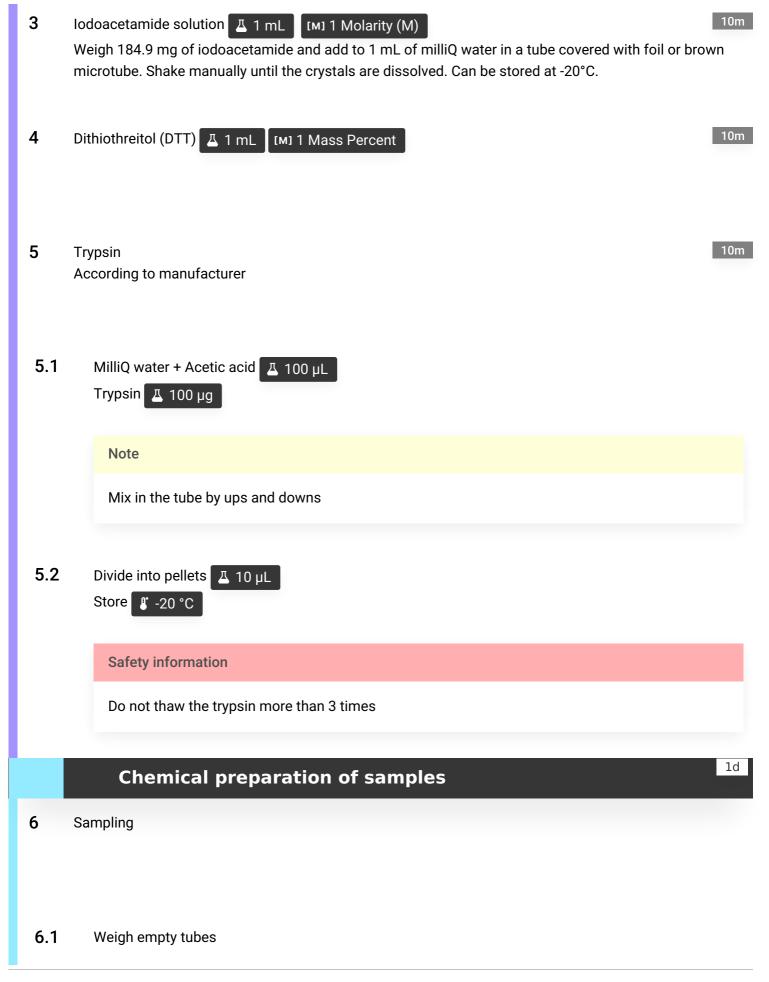
Iodoacetamide needs to be protected from light



2 NH₄HCO₃ \mathbb{Z} 200 mL [M] 50 millimolar (mM)

20m

Weigh 0.7906 g of ammonium bicarbonate (Sigma Aldrich). Add the weighed powder to 100 mL of milliQ water. Shake manually until the crystals are dissolved. Make up to 200 mL. Store at room temperature.



- **6.2** Prepare paper and EtOH for cleaning between samples
- **6.3** Use ultrasounds to clean diamond head of dremel in the end
- 7 Decalcification
- 7.1 Add EDTA to each sample 4 1 mL

Store 4 °C

Put on a mixing carousel to allow contact with every "particle" of bone/tooth

7.2 Change the solution once. Centrifuge 3 13400 x g, 00:01:00 Collect the supernatants in a tube labelled with the sample code.

1m

Homogenise the mixture (Vortex 1min)

Store 4 °C

Note

Decalcification is completed when only a bone phantom remains (should resemble wet cotton candy)

8 Solubilisation

- 8.1 Add ammonium bicarbonate to each sample 🚨 500-300 µL [M] 50 Molarity (M)
- 8.2 Thermomixer (5 350 rpm, 67°C, 03:00:00
- 9 Reduction Alkylation
- 9.1 For every 100μL of sample, add DTT Δ 1 μL [M] 1 Molarity (M) (Final concentration ~10mM)
- 9.2 Thermomixer \$\(\) 450 rpm, 50°C, 01:00:00

 Allow the samples to come to room temperature (on the bench, approx. 30 min)
- 9.3 Add Iodoacetamide solution Δ 1.6 μL [M] 1 Molarity (M) (final concentration approx. 15mM) Incubate for 30 min in the dark (protected by foil)
- 10 Enzymatic digestion
- 10.1 Add \underline{A} 1 μ L of trypsin prepared at \underline{M} 1 μ g/ μ L to 300 μ L of solubilisation solution

- ThermoMixer (5 350 rpm, 37°C, 18:00:00 Trypsin can act in only 3 to 5h
- 10.3 Prepare the formic acid which will be used to stop the digestion:
 - Pure formic acid 🗸 10 µL
 - MilliQ water 🗸 90 µL
 - Adjust the volume to be prepared according to the number of samples
- 10.4 Stop the digestion by adding $\boxed{\text{A}}$ 1 μL of prepared 10% formic acid.
- 10.5 centrifuge (, 00:10:00

10m

10.6 Place between 40 and 100 μ L of each sample in an insert dedicated to the LC-MS/MS analyses with an electrospray source.