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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.

**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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## MATERIALS

Solutions: (steps to make them in the protocol)

- EDTA Buffer 0.5M
- $NH_4HCO_3$50mM
- 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

## Solutions to prepare before starting

2h

**1** EDTA 500 mL [M] 0.5 Molarity (M) pH 7.4 — 1h

**Safety information**

Under a fume hood

**Note**

pH meter in the solution and bar magnet

**2** $NH_4HCO_3$ 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.

3 Iodoacetamide solution 1 mL [M] 1 Molarity (M) 10m

Weigh 184.9 mg of iodoacetamide and add to 1 mL of milliQ water in a tube covered with foil or brown microtube. Shake manually until the crystals are dissolved. Can be stored at -20°C.

4 Dithiothreitol (DTT) 1 mL [M] 1 Mass Percent 10m

5 Trypsin 10m

According to manufacturer

5.1 MilliQ water + Acetic acid 100 µL

Trypsin 100 µg

**Note**

Mix in the tube by ups and downs

5.2 Divide into pellets 10 µL

Store -20 °C

**Safety information**

Do not thaw the trypsin more than 3 times

## Chemical preparation of samples 1d

6 Sampling

6.1 Weigh empty tubes

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 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 13400 x g, 00:01:00 1m

Collect the supernatants in a tube labelled with the sample code.

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 350 rpm, 67°C, 03:00:00

## 9 Reduction - Alkylation

9.1 For every 100µL of sample, add DTT 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 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 1 µL of trypsin prepared at [M] 1 µg/µL to 300 µL of solubilisation solution

**10.2** ThermoMixer 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 1 µL of prepared 10% formic acid.

**10.5** centrifuge , 00:10:00 10m

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