



VERSION 2
AUG 14, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.8epv5j8n4l1b/v2

Protocol Citation: simon.daled, Alexandra Burnett 2023. Protein Extraction from Dental Enamel. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.8epv5j8n4l1b/v2> Version created by Alexandra Burnett

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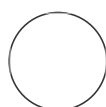
Protocol status: Working
We use this protocol and it's working.

🌐 Protein Extraction from Dental Enamel V.2

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ABSTRACT

This protocol details a method for extracting proteins from ~5mg of powdered dental enamel for proteomic analysis by LC-MS/MS. This procedure includes the use of alkylating and reducing agents, a sample cleanup step, and features the optional addition of synthetic peptide heavy standards for absolute quantification and quality control of the analysis.

Total protocol duration is dependent on the number of samples to be processed. Protein extraction will typically take a day-and-a-half, after which time the samples can be safely stored prior to instrumental analysis.

Created: Aug 14, 2023

Last Modified: Aug 14, 2023

PROTOCOL integer ID:
86456

Keywords: Enamel,
archaeology, dental,
proteomics, protein

GUIDELINES













Always process blank samples alongside your enamel samples to allow for the observation of laboratory contamination.

MATERIALS

Consumables

1.5mL protein lo-bind Eppendorf® tubes
Strata™ -X 33 µm Polymeric Reversed Phase 30mg/1mL tubes
Pipettes
Pipette tips
Tube racks
MS sampling vials
Ice

Chemicals


-  Ultrapure Water Contributed by users
-  Ammonium Bicarbonate BioUltra ≥99.5% (T) Merck MilliporeSigma (Sigma-Aldrich) Catalog #09830-1KG
-  Hydrochloric acid 37% a.r. 37+% HCl Chem-Lab NV Catalog #CL00.0310
-  Trichloroacetic acid a.r. 99.5+% C₂HCl₃O₂ Chem-Lab NV Catalog #CL00.2037
-  Urea 99.5-100.5% CH₄N₂O Chem-Lab NV Catalog #CL00.2101
-  2-propanol Biosolve Catalog #162641
-  Methanol for analysis EMSURE® ACSISOReag. Ph Eur Merck MilliporeSigma (Sigma-Aldrich) Catalog #1060092500
-  Acetone p. 99+% C₃H₆O Chem-Lab NV Catalog #CL00.0102
-  Formic acid 99% ULC/MS - CC/SFC Biosolve Catalog #069141
-  DTT for Biochemistry 99+% C₄H₁₀O₂S Chem-Lab Analytical bvba Catalog #CL00.0481
-  S-Methyl methanethiosulfonate 97% Merck MilliporeSigma (Sigma-Aldrich) Catalog #208795-10G
-  Trypsin/Lys-C Mix, Mass Spec Grade, 100ug Promega Catalog #V5072

Equipment

Centrifuge (Eppendorf Centrifuge 5417R)

Centri-Vap SpeedVac Concentrator (Thermo Scientific Savant SPD111V SpeedVac Concentrator)
Sonicator (Elma Transsonic 460)
Vortex (Scientific Industries Vortex-Genie 2)
Drill (Dremel; Proxxon)
Diamond-tipped drill bits (Dremel; Proxxon)
Thermo-Shaker
Solid-Phase Extraction vacuum pump and chamber
Analytical balance
Tube racks for 1.5mL Eppendorf® tubes

SAFETY WARNINGS

 This protocol includes the use of machinery and hazardous substances. Be sure to wear appropriate protective equipment, including a lab coat and gloves, at all times.

Sample acquisition

1

Note

Tip: label and weigh an Eppendorf tube for each sample prior to drilling. Use a fresh drill bit for each tooth and clean between uses to avoid transferral of enamel.

Optional: process additional, empty Eppendorfs alongside your samples to observe laboratory contaminants introduced throughout sample preparation (suggested 1:9).

Place your tooth on a fresh piece of weighing paper on a clean worktop.

Using a small drill bit, gently abrade the surface of the tooth crown to remove powdered enamel and collect it on the weighing paper.

2

Carefully tip the powdered enamel into a protein lo-bind Eppendorf tube. Weigh the tube and record the weight of the powdered enamel inside.

Protein Extraction

3

Safety information

This section includes the use of hazardous substances. Be sure to wear appropriate protective equipment including a lab coat and gloves.

Note

The following extraction protocol is predicated on archaeological enamel quantities of ~5mg. Smaller or larger solvent volumes (where listed) may be required for differing sample weights.

Tip: process extraction blanks alongside your samples as contamination controls.

4 **Demineralisation**

Add 200µL of 1.2M HCl to each sample tube. Vortex the tubes briefly to mix.
Incubate for two hours in a thermo-mixer, using moderate agitation at room temperature.

5 **Precipitation**

Add 100% TCA (in water) to a final concentration of 33% (V/V) (i.e. 100µL).
Incubate on ice for one hour.
Centrifuge for 10 minutes at 15,000G at 0°C.
Pipette off the supernatants and discard (or retain and freeze if desired).

6 **Wash the pellet**

Pipette 500µL of ice-cold acetone into each sample tube.
Centrifuge for 10 minutes at 15,000G.
Pipette off the supernatants and discard (or retain if desired).

7 **Resuspend in buffer**

Add 200µl of 8M urea in 1M ABC buffer (pH 8).
Vortex and sonicate until all pellets are resuspended.

8 **Reduction**

Add DTT to a final concentration of 5mM.
Incubate for 30 minutes at 37°C.

9 Alkylation

Create a fresh stock solution of MMTS in 2-propanol (200mM).
Add MMTS to a final concentration of 10mM in each sample.
Incubate at room temperature in the dark for 15 minutes.

Note

MMTS degrades in light and must be kept in darkness before use. Try to minimise exposure as much as possible and only prepare the alkylating solution immediately prior to use.

10 Buffer dilution

Dilute the samples to a final concentration of 2M urea with 1M ABC buffer.

11 Digestion

Prepare a solution of trypsin, or trypsin and lysine-C, in 1M ammonium bicarbonate.
Add enzyme to a 1:25 (w/w) enzyme/protein ratio (around 1-2µg per sample).
Incubate overnight at 37°C. If there is visible debris in the tubes following digestion, sonicate until this is broken up.

12h

12 Sample Cleanup via Solid-Phase Extraction [Optional]

Condition one Strata tube per sample with 1mL MeOH.
Equilibrate the Strata tubes twice with 1mL ultrapure water.

Note

Be careful not to let the filters inside the tubes dry out while conditioning, equilibrating, and loading the samples. It is best to stop draining the tubes ~1mm above the filter to avoid this. If a filter runs dry after conditioning, start over again.

Remove the waste MeOH and water and replace the waste collection tubes with fresh Eppendorf lo-bind tubes.

Slowly load 1mL of sample per tube.

Wash twice with 1 mL of 5% MeOH in ultrapure water. [The cartridge can be allowed to run dry at this stage.]

Elute the sample with 1 mL of 1% FA in MeOH; first let a small amount soak the cartridge for 2 minutes and then slowly elute the sample with the rest of the elution buffer.

13 Drying down

Vacuum-dry the samples. Drying at up to 40°C can accelerate the process without damaging the peptides.

Note

The samples can now be stored (refrigerated) in a dry state for transport or until it is time for LC-MS/MS. If storing for longer time periods (more than 2 days) it is recommended to freeze them at this point.

Resuspension for LC-MS/MS

- 14** [Optional] Prepare a solution containing amelogenin heavy labelled peptide standards in 0.1% formic acid. Add heavy peptides to a concentration of around 5fmol/ μ L, depending on the LOD and sensitivity of the LC-MS/MS system.

Add between 10-30 μ L of 0.1% formic acid [and heavy standards] to each sample tube.

Note

The volume of formic acid in which you resuspend your samples will be dependent on the desired injection volume, and the sensitivity of the analytical instrument to be used.

Sonicate and vortex the samples to ensure thorough solubilization.
Centrifuge at 16,000G for 10 minutes.

- 15** Transfer the samples to MS sampling vials ready for analysis.

Note

Tip: there may still be visible pellets in the bottom of the Eppendorf tubes. Avoid transferring any of this material into the MS vials. Should the pellet/s detach from the tube walls or begin to disintegrate into the supernatant, you may need to re-centrifuge the sample.