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Extraction buffer stock solution (w/o Proteinase K)

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Abstract

This document describes the preparation of extraction buffer stock solution for ancient DNA extraction from bones, teeth, sediments and other materials, following the method described by Dabney et al. 2013 and refined by Rohland et al. 2018. To obtain the final extraction buffer, proteinase K solution (see separate document) must be added on the day of extraction.

References

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. Proceedings of the National Academy of Sciences of the United States of America, 110(39), 15758-15763.

Rohland, N., Glocke, I., Ayinuer-Petri, A., & Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. Nature Protocols, 13, 2447-2461.

Note

The protocol provided here yields 243.75 ml stock solution (corresponding to 250 ml ready-to-use extraction buffer after addition of proteinase K), which suffices for 250 samples if 1 ml extraction buffer is used per sample.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Water	Sigma Aldrich/Merck	1153332500
	0.5 M EDTA, pH 8.0	AppliChem	A4577, 1000
	Tween-20	Thermo Fisher Scientific	11417160
	Consumables		
	Square media bottle, sterile 250 ml	VWR	391-0629
	50 ml serological pipette	Corning BV	357550

Equipment

- serological pipette controller (e.g. battery-powered pipetting aid ROTILABO, cat. no. TC16.1)

Protocol

1. Prepare the buffer in a 250 ml square media bottle by adding the following reagents. Use the glass pipette for transfer of large volumes (> 1 ml). Mix reagents by shaking the bottle.

	Reagent	Volume (ml)	Final Concentration
	0.5M EDTA, pH 8.0	225	0.462 M
	Water	18.625	
	100% Tween-20	0.125	0.051%

2. The buffer must be decontaminated using UV treatment. Instructions for UV-decontamination are provided in the Appendix.

Note

[Labeling]
Label the bottle with the buffer name, batch ID, date and the initials of the person who prepared the buffer.

Attention: Every single bottle prepared at the same day gets a new batch ID. Name the batches with Roman numerals (e.g. batch I, batch II, etc.)

3. Store the buffer at room temperature until used. Shelf life is at least one year from preparation.

Note

[Documentation]
Note the lot numbers and the date and initials of the reagents used for the preparation in Labfolder (orange fields).

Appendix

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

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Preview